

**PHYTOCHEMICAL PROFILING AND PHARMACOLOGICAL
INVESTIGATION OF SELECTED SPECIES OF *LITSEA* LAM.
(LAURACEAE), SOUTHERN WESTERN GHATS, INDIA**

*Thesis
submitted to the University of Calicut
for the award of the Degree of*

DOCTOR OF PHILOSOPHY IN BOTANY

By
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Under the guidance of
Prof. (Dr.) RASMI A. R.



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DECLARATION

I, hereby declare that the work presented in the thesis entitled **“PHYTOCHEMICAL PROFILING AND PHARMACOLOGICAL INVESTIGATION OF SELECTED SPECIES OF *LITSEA* LAM. (LAURACEAE), SOUTHERN WESTERN GHATS, INDIA”** is based on the original work done by me under the guidance of Prof. (Dr.) Rasmi A.R, Professor and Head, Research Department of Botany, Govt. Victoria College, Palakkad and has not been included in any other thesis submitted previously for the award of any degree. The contents of the thesis are undergone plagiarism check using iThenticate software at C.H.M.K. Library, University of Calicut, and the similarity index found within the permissible limit. I also declare that the thesis is free from AI generated contents.

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This is to certify that the project **Proposal No: SLIMS/10/IAEC/2021-22** entitled "**Anti-inflammatory and wound healing activity of *Litsea wightiana* (Nees). Hook. f. in animal models**" submitted by **Mrs. K. Ummu Habeeba** has been approved by the IAEC of Sri Lakshmi Narayana Institute of Medical Sciences (932/Po/Pe/S/06/CPCSEA) in its meeting held on **21.06.2022** and **Wistar Albino Rats: 24** have been sanctioned under this.

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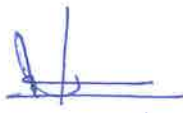


സംഗ്രഹം

ഇന്ത്യയിൽ പശ്ചിമ ഘട്ടത്തിലെ ലോറേസിയെ കടുംബത്തിലെ തിരഞ്ഞെടുക്കപ്പെട്ട ലിറ്റ്സിയിൽ സ്പെഷ്യലിസറ്റുകളുടെ ഫൈറ്റോകെമിക്കൽ പ്രൊഫൈലിങ്ങും ഫാർമക്കോളജിക്കൽ പഠനവും

ലോറേസിയെ കടുംബത്തിലെ ലിറ്റ്സിയിൽ ജനസ് പശ്ചിമ ഘട്ടത്തിൽ വ്യാപിച്ചു കിടക്കുന്നു. തെക്കു പശ്ചിമ ഘട്ടത്തിൽ മാത്രം കാണപ്പെടുന്ന ചില ലിറ്റ്സിയിൽ സ്പെഷ്യലിസറ്റുകളിൽ പഠനം വളരെ പരിമിതമാണ്. ഈ സാഹചര്യത്തിലാണ് ആദ്യമായി ലിറ്റ്സിയിൽ ബോർഷിളോണി, ലിറ്റ്സിയിൽ കൊറിയേഷ്യ, ലിറ്റ്സിയിൽ വൈറ്റിയായ എന്നിവയുടെ ഫൈറ്റോകെമിക്കൽ പ്രൊഫൈലിങ്ങും ഫാർമക്കോളജിക്കൽ പഠനവും നടത്തിയത്. മൂന്നു ഘട്ടങ്ങളിലായി നടത്തിയ ഈ പഠനത്തിന്റെ പ്രാരംഭ ഘട്ടത്തിൽ ഈ മൂന്ന് ചെടികളുടെ ഇല, കമ്പ് എന്നിവയുടെ ബാഹ്യ ആന്തരിക ഘടനകൾ, ഫിസ്സോകെമിക്കൽ, ഫിസിക്സോകെമിക്കൽ, പൗഡർ മൈക്രോസ്കോപ്പി പഠനങ്ങളും നടത്തി. ഈ മൂന്ന് ചെടികൾ തമ്മിൽ വളരെയധികം സാമ്യങ്ങളും അതോടൊപ്പം ചില വ്യത്യാസങ്ങളും കണ്ടെത്തി. രണ്ടാം ഘട്ടത്തിൽ ഈ ചെടികളുടെ മെത്തനോളിക് സത്തയുടെ ഗുണപരവും അളവുപരവുമായ കാര്യങ്ങൾ, എൽ സി എം എസ്, ജിസി എം എസ്, വിശകലനം നടത്തി അവയിലുടങ്ങിയ സംയുക്തങ്ങളും, അതോടൊപ്പം ലിറ്റ്സിയിൽ കൊറിയേഷ്യയിലെ അവശ്യ എണ്ണയുടെ വിശകലനവും നടത്തി സംയുക്തങ്ങളെ തിരിച്ചറിഞ്ഞു. മൂന്നാം ഘട്ടത്തിൽ തിരഞ്ഞെടുക്കപ്പെട്ട ലിറ്റ്സിയിൽ സ്പെഷ്യലിസറ്റുകളുടെ ആന്റിമൈക്രോബിയൽ, ആന്റിഓക്സിഡന്റ്, ആന്റിഡയാബറ്റിക്, സൈറ്റോടോക്സിസിറ്റി പഠനങ്ങൾ നടത്തി. ആന്റിഓക്സിഡന്റ്, പഠനത്തിന്റെ ഫലമായി ലിറ്റ്സിയിൽ വൈറ്റിയായ മറ്റുള്ളവയെ അപേക്ഷിച്ച് ഗുണങ്ങളിൽ മികച്ചതായി കണ്ടെത്തി. അതേ സമയം ആന്റിഫങ്കൽ, സൈയേറ്റോക്സിക് ഗുണങ്ങൾ ഒന്നിലും വ്യക്തമായി കണ്ടെത്തിയില്ല. തുടർന്നുള്ള ആന്റിഇൻഫ്ളമേറ്ററി, മുറിവ് ഉണക്കൽ പഠനങ്ങൾക്ക് ലിറ്റ്സിയിൽ വൈറ്റിയായ ഇലകളിലെ സത്ത മാത്രം ഉപയോഗിച്ചു. ആന്റിഇൻഫ്ളമേറ്ററി, മുറിവ് ഉണക്കൽ പഠനങ്ങളിൽ ഇൻവിട്രോ, ഇൻവിവോ രീതികൾ പരീക്ഷിച്ചു. ഇൻവിട്രോ പഠനത്തോടൊപ്പം എലികളിൽ നടത്തിയ ഇൻവിവോ പഠനങ്ങൾ വളരെ മികച്ച ഫലം നൽകി ഈ ചെടിയുടെ പ്രാധാന്യം വെളിപ്പെടുത്തി. ഇതു കൂടാതെ ലിറ്റ്സിയിൽ കൊറിയേഷ്യയുടെ അവശ്യ എണ്ണയുടെ ഷഡ്പദങ്ങളെ തുരത്താനുള്ള കഴിവും കണ്ടെത്തി.

സൂചകപദങ്ങൾ : ലിറ്റ്സിയി, ലിറ്റ്സിയി ബോർഡിപ്ലോണി, ലിറ്റ്സിയി കൊറിയേഷ്യ,
ലിറ്റ്സിയി വൈറ്റിയാന, ജി സി എം എസ്, എൽ സി എം എസ്, ഫൈറ്റോകെമിക്കൽ,
ഫാർമക്കോളജിക്കൽ ആന്റിഇൻഫ്ളമേറ്ററി, മുറിവ് ഉണക്കൽ.


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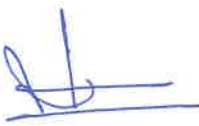
ABSTRACT

Phytochemical Profiling and Pharmacological Investigation of Selected Species of *Litsea* Lam. (Lauraceae), Southern Western Ghats, India.

The genus *Litsea* (Lauraceae) includes several medicinally important species distributed in the Western Ghats. Limited information exists on certain endemic taxa of the Southern Western Ghats, India. The present study represents the first systematic phytochemical and pharmacological investigation of *L. bourdillonii*, *L. coriacea*, and *L. wightiana*. In the initial phase, pharmacognostic characterization of leaf and bark samples was carried out through morphological, anatomical, histochemical physicochemical and powder analyses, which revealed close similarities with subtle distinguishing features. The second phase involved qualitative and quantitative phytochemical screening, which included LC/MS and GC/MS profiling of methanolic extracts, and essential oil analysis. Several bioactive compounds were identified, and a higher quantity of phenols, flavonoids, and tannins was estimated in *L. wightiana* leaves methanolic extracts. Essential oil extraction and analysis of *L. coriacea* leaves were conducted and revealed unique chemical constituents. In the third phase, pharmacological evaluation of leaf and bark extracts of three *Litsea* species was conducted, demonstrating that *L. wightiana* leaves extract possessed promising antibacterial, antioxidant, and antidiabetic activities, while antifungal activity was negligible, and cytotoxicity was low to moderate. Further *in vitro* and *in vivo* studies confirmed the anti-inflammatory potential of *L. wightiana* leaves via enzyme inhibition, gene expression, molecular docking analyses, together with studies by the carrageenan-induced paw edema method in model animals. Wound healing efficacy was validated by *in vitro* scratch wound healing assay and *in vivo* excision wound models in Wistar albino rats, along with histopathological and docking studies. Additionally, insecticidal activity of *L. coriacea* leaf essential oil was studied and found significant activity against *S. oryzae* and *T. castaneum* through contact, fumigant, and repellency assays. Overall, the findings highlight that the selected *Litsea* species possess significant bioactivities, and among all, *L. wightiana* leaves showed the highest activity with

profound anti-inflammatory and wound healing applications, and insecticidal potential of *L. coriacea* leaves essential oil.

Key words: *Litsea*, *L. bourdillonii*, *L. coriacea*, *L. wightiana*, GC/MS, LC/MS analysis, pharmacognostic, phytochemical, anti-inflammatory, gene expression, molecular docking, wound healing, insecticidal activity.


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Ummu Habeeba K.

CONTENTS

<i>Chapters</i>	<i>Title</i>	<i>Page No.</i>
I	INTRODUCTION	1-9
II	REVIEW OF LITERATURE	11-33
	I. Medicinal uses of <i>Litsea</i>	11
	II. Pharmacognostic studies	15
	III. Phytochemical analysis	17
	IV. Pharmacological studies	20
III	MATERIALS AND METHODS	35-73
	I. Plant materials	35
	a. Collection and authentication	35
	II. Methods	38
	PHASE I- PHARMACOGNOSTIC CHARACTERIZATION	39
	a. Morphological study	39
	b. Anatomical characterization	39
	c. Histochemical study	39
	d. Physicochemical study	40
	e. Powder microscopic study	42
	PHASE II- PHYTOCHEMICAL PROFILING	43
	a. Extraction of plant materials	43
	b. Qualitative phytochemical screening	44
	c. Quantitative phytochemical analysis	46
	d. Liquid Chromatography and mass spectrometric analysis	47
	e. Gas Chromatography and mass spectrometric analysis	48
	f. Essential oil extraction and GCMS/GC-FID analysis	48
	PHASE III. PHARMACOLOGICAL STUDY	50
	A. Antimicrobial activity	50
	i. Antibacterial study by agar well diffusion method	50
	ii. Determination of minimum inhibitory concentration	50
	iii. Antifungal assay by agar well diffusion method	51

	B. Antioxidant assay	52
	i. DPPH radical scavenging assay	52
	ii. Hydroxyl radical scavenging assay	53
	iii. Superoxide radical scavenging assay	53
	iv. Ferrous reducing antioxidant power assay	54
	C. Antidiabetic assay	54
	i. Alpha amylase inhibition assay	54
	ii. Alpha glucosidase inhibition assay	55
	D. <i>In vitro</i> cytotoxicity assay	56
	E. Anti-inflammatory study	57
	i. <i>In vitro</i> anti-inflammatory activity	58
	ii. Gene expression studies	60
	iii. Docking study	64
	iv. <i>In vivo</i> anti-inflammatory study	64
	F. Wound healing study	66
	i. <i>In vitro</i> wound healing study by scratch wound method	66
	ii. <i>In vivo</i> wound healing study	67
	iii. Docking study	68
	G. Study of insecticidal activity	69
	i. Contact toxicity toxicity	71
	ii. Fumigant toxicity assay	71
	iii. Repellent activity assay	72
IV	RESULTS	75-144
	Phase I. Pharmacognostic Characterization	75
	a. Morphological study	75
	b. Anatomical study	76
	c. Histochemical study	85
	d. Physicochemical study	87
	e. Powder microscopic study	89
	Phase II- Phytochemical Profiling	89
	a. Determination of extractive value	89
	b. Qualitative analysis of phytochemicals	90
	c. Quantitative analysis of phytochemicals	90
	d. Liquid Chromatography and mass spectroscopic analyses	94

	e. Gas Chromatography and mass spectrometric analyses	102
	f. Analysis of essential oil	104
	Phase III. Pharmacological Study	106
	A. Antimicrobial activity	106
	a. Antibacterial study	106
	b. Antifungal study	108
	B. Antioxidant assay	108
	i. DPPH Radical scavenging activity	108
	ii. Hydroxyl radical scavenging activity	110
	iii. Superoxide radical scavenging assay	111
	iv. Ferrous reducing antioxidant power assay	112
	C. Antidiabetic study	114
	D. <i>In vitro</i> cytotoxicity	117
	E. Anti-inflammatory activity	120
	i. <i>In vitro</i> anti-inflammatory activity	120
	ii. Gene expression studies	126
	iii. Docking study	126
	iv. <i>In vivo</i> anti-inflammatory study	127
	F. Wound healing study	129
	i. <i>In vitro</i> scratch wound healing assay	129
	ii. <i>In vivo</i> wound healing study	129
	iii. Docking study	130
	G. Study of insecticidal activity	131
	i. Contact toxicity	132
	ii. Fumigant toxicity	132
	iii. Repellency	132
V	DISCUSSION	145-174
	Phase I. Pharmacognostic Characterization	146
	Phase II- Phytochemical Profiling	150
	Phase III. Pharmacological Study	156
VI	SUMMARY& CONCLUSIONS	175-181
VII	RECOMMENDATIONS	183-185
	REFERENCES	187-216
	LIST OF PUBLICATIONS AND PRESENTATIONS	217

LIST OF TABLES

Table No.	Title	Page No.
1	Ethnopharmacological uses of different species of <i>Litsea</i>	13
2	Phytochemical compounds isolated from different species of <i>Litsea</i>	18
3	Inoculum used in antibacterial assay	50
4	Inoculum used in antifungal assay	51
5	Reagents used in TRIzol method	61
6	Instruments used in qRT-PCR analysis	62
7	Various steps involved in qRT-PCR analysis	63
8	List of primers used in qRT-PCR study	63
9	Comparative morphological characterization of three <i>Litsea</i> spp.	76
10	Comparative anatomical characterization of the stem of three <i>Litsea</i> spp.	78
11	Comparative anatomical characterization of the leaves of three <i>Litsea</i> spp.	81
12	Comparative anatomical characterization of the petiole of three <i>Litsea</i> spp.	84
13	Comparative histochemical characterization of the stem of three <i>Litsea</i> spp.	86
14	Comparative histochemical characterization of the leaves of three <i>Litsea</i> spp.	87
15	Comparative physicochemical characterization of the leaves of three <i>Litsea</i> spp.	88
16	Comparative physicochemical characterization of the bark of three <i>Litsea</i> spp.	88
17	Extractive values of the leaf and bark samples of three <i>Litsea</i> spp.	89
18	Qualitative phytochemical screening of the methanolic extracts of three <i>Litsea</i> spp.	90
19	Quantitative phytochemical analysis of the methanolic extracts of three <i>Litsea</i> spp.	81
20	LC/MS analysis data of the leaves extract of <i>L. bourdillonii</i>	94
21	LC/MS analysis data of the leaves extract of <i>L. coriacea</i>	98
22	LC/MS analysis data of the leaves extract of <i>L. wightiana</i>	101

23	GC/MS analysis data of methanolic extract of leaves of <i>L. wightiana</i>	103
24	GC/MS with GC-FID analysis data of <i>L. coriacea</i> leaves essential oil	105
25	Antibacterial activity of the leaves and bark extracts of three <i>Litsea</i> spp.	107
26	Antibacterial activity expressed by minimum inhibitory concentration (MIC) of the leaf and bark extracts of three <i>Litsea</i> spp.	108
27	DPPH free radical scavenging activity of the leaves of three <i>Litsea</i> spp.	109
28	Hydroxyl radical scavenging activity of the leaves extract of <i>L. wightiana</i>	111
29	Superoxide radical scavenging activity of the leaves extract of <i>L. wightiana</i>	112
30	Ferrous reducing antioxidant power assay of the leaves extract of <i>L. wightiana</i>	114
31	Antidiabetic activity of the leaves and bark extracts of three <i>Litsea</i> spp. by α -amylase inhibition method	115
32	Antidiabetic activity of leaf and bark extracts of the three <i>Litsea</i> spp. by α -glucosidase inhibition method	117
33	Cytotoxicity effect of leaf and bark extracts of the three <i>Litsea</i> spp. in SK-MEL cell lines	118
34	<i>In vitro</i> anti-inflammatory activity study by Enzyme inhibition assay	125
35	<i>In vitro</i> anti-inflammatory activity study by myeloperoxidase inhibition assay	125
36	Binding energy of docked proteins with target anti-inflammatory compounds	127
37	<i>In vivo</i> anti-inflammatory activity of LWME by carrageenan induced paw edema method (Paw thickness)	128
38	<i>In vivo</i> anti-inflammatory activity of LWME by carrageenan induced paw edema method (% of inhibition)	128
39	Effect of LMWE on excision wounds in rats (Reduction in wound area)	129
40	Effect of LMWE on excision wounds in rats (Percentage of reduction in wound size)	130
41	Binding energy of docked proteins with target wound healing compounds	131
42	Contact toxicity effect of <i>L. coriacea</i> leaves essential oil against <i>S. oryzae</i> and <i>T. castaneum</i>	133

43	Contact toxicity effect of <i>L. coriacea</i> leaves essential oil against <i>S. oryzae</i> and <i>T. castaneum</i> represented by Probit analysis	134
44	Fumigant effect of <i>L. coriacea</i> leaves essential oil against <i>S. oryzae</i> and <i>T. castaneum</i>	135
45	Fumigant effect of <i>L. coriacea</i> leaves essential oil against <i>S.oryzae</i> and <i>T. castaneum</i> represented by Probit analysis	136
46	Repellency effect of <i>L. coriacea</i> leaves essential oil against <i>S. oryzae</i> and <i>T. castaneum</i>	137
47	Repellency Effect of <i>L. coriacea</i> leaves essential oil against <i>S. oryzae</i> and <i>T. castaneum</i> represented by Probit analysis	138

LIST OF FIGURES

<i>Figure No.</i>	<i>Title</i>
1	Targets of Anti-inflammatory agents in control of inflammation
2	Wound healing mechanism
3	Extraction by Soxhlet apparatus
4	Essential oil extraction by Clevenger apparatus
5	Macromorphological characters of <i>L. bourdillonii</i>
6	Macromorphological characters of <i>L. coriacea</i>
7	Macromorphological characters of <i>L. wightiana</i>
8	Microscopic characters of <i>L. bourdillonii</i> stem
9	Microscopic characters of <i>L. coriacea</i> stem
10	Microscopic characters of <i>L. wightiana</i> stem
11	Microscopic characters of <i>L. bourdillonii</i> leaf
12	Microscopic characters <i>L. coriacea</i> leaf
13	Microscopic characters <i>L. wightiana</i> leaf
14	Microscopic characters of <i>L. bourdillonii</i> petiole
15	Microscopic characters of <i>L. coriacea</i> petiole
16	Microscopic characters of <i>L. wightiana</i> petiole
17	Histochemical localization of <i>L. bourdillonii</i> stem
18	Histochemical localization of <i>L. coriacea</i> stem
19	Histochemical localization of <i>L. wightiana</i> stem
20	Histochemical localization of leaf of <i>L. bourdillonii</i> , <i>L. coriacea</i> and <i>L. wightiana</i>
21	Powder microscopic characterization of stem bark of <i>L. bourdillonii</i> , <i>L. coriacea</i> and <i>L. wightiana</i>
22	Standard graph of quercetin
23	Standard graph of gallic acid
24	Standard graph of tannic acid
25	Quantitative analysis of phytochemicals
26	LC/MS chromatogram of <i>L. bourdillonii</i> leaves methanolic extract
27 (i-iv)	Mass spectra of compounds detected by LC/MS analysis of methanolic extract of <i>L. bourdillonii</i> leaves
28	LC/MS chromatogram of <i>L. coriacea</i> leaves methanolic extract

29 (i-v)	Mass spectra of compounds detected by LC/MS analysis of methanolic extracts of <i>L. coriacea</i> leaves
30	LC/MS chromatogram of <i>L. wightiana</i> leaves methanolic extract
31 (i-iv)	Mass spectra of compounds detected by LC/MS analysis of <i>L. wightiana</i> methanolic leaves extract
32	GC/MS chromatogram and list of compounds of <i>L. wightiana</i> methanolic leaf extract
33 (i-iv)	Mass spectra of compounds detected by GC/MS analysis of the methanolic extract of <i>L. wightiana</i> leaves
34	GC/MS chromatogram and compounds of <i>L. coriacea</i> leaves essential oil
35	Mass spectra of compounds detected by GC/MS analysis of essential oil of <i>L. coriacea</i> leaves
36	Antibacterial activity of leaf and bark extracts against <i>E. coli</i>
37	Antibacterial activity of leaf and bark extracts against <i>P. aeruginosa</i>
38	Antibacterial activity of bark extracts against <i>S. aureus</i>
39	Antifungal activity of leaf and bark extracts of <i>L. bourdillonii</i> , <i>L. coriacea</i> & <i>L. wightiana</i> methanolic extracts against <i>A. niger</i>
40	Antifungal activity of leaf and bark extracts of <i>L. bourdillonii</i> , <i>L. coriacea</i> & <i>L. wightiana</i> methanolic extracts against <i>C. albicans</i>
41	Antioxidant activity of the leaf extracts of the three <i>Litsea</i> spp. using DPPH method
42	Antioxidant activity of <i>L. wightiana</i> leaves extract using hydroxyl radical scavenging assay
43	Antioxidant activity of <i>L. wightiana</i> leaves extract by superoxide radical scavenging activity
44	Antioxidant assay of <i>L. wightiana</i> leaves extract by FRAP method
45	Antidiabetic assay of leaf and bark extracts of <i>Litsea</i> spp. using alpha amylase inhibition method
46	Antidiabetic assay of leaf and bark extracts of <i>Litsea</i> spp. by alpha glucosidase inhibition method
47	Cytotoxicity of leaf extracts of three <i>Litsea</i> spp.
48	Cytotoxicity of bark extracts of three <i>Litsea</i> spp.
49	<i>In vitro</i> anti-inflammatory activity of LWME by COX inhibition
50	<i>In vitro</i> anti-inflammatory activity of LWME by LOX inhibition
51	<i>In vitro</i> anti-inflammatory activity of LWME by inducible nitric oxide synthase inhibition
52	<i>In vitro</i> anti-inflammatory activity of LWME by Myeloperoxidase inhibition
53	Cellular nitrite level after the treatment with LWME

54	Gene expression analysis of LWME
55 (i-ii)	Docking of anti-inflammatory compounds with target proteins
56 (i-ii)	Docking of anti-inflammatory compounds with target proteins
57	<i>In vivo</i> anti-inflammatory study by carrageenan induced paw edema in Wistar albino rats
58	<i>In vitro</i> scratch wound healing assay
59	<i>In vivo</i> wound healing assay by LWME
60	Histopathological study-Histological sections of granuloma tissue
61 (i-ii)	Docking of wound healing compounds with target proteins
62 (i-ii)	Docking of wound healing compounds with target proteins
63	Contact toxicity of <i>L. coriacea</i> leaves essential oil against <i>S. oryzae</i>
64	Contact toxicity of <i>L. coriacea</i> leaves essential oil against <i>T. castaneum</i>
65	Fumigant toxicity of <i>L. coriacea</i> leaves essential oil against <i>S. oryzae</i>
66	Fumigant toxicity of <i>L. coriacea</i> leaves essential oil against <i>T. castaneum</i>
67	Repellency effect of <i>L. coriacea</i> leaves essential oil against <i>S. oryzae</i>
68	Repellency effect of <i>L. coriacea</i> leaves essential against <i>T. castaneum</i>

ABBREVIATIONS

°C	:	Degree Celsius
µg	:	Microgram
µg/mL	:	Microgram per millilitre
µL	:	Microlitre
µm	:	Micrometer
µM	:	Micromolar
(-OH)	:	Hydroxyl radical
ATCC	:	American Type Culture Collection
BSA	:	Bovine Serine Albumin
CALI	:	Calicut University Herbarium
cDNA	:	Complementary DNA
cm	:	Centimeter
CO ₂	:	Carbon dioxide
COX	:	Cyclooxygenase
C _t value	:	Cycle threshold value
DMEM	:	Dulbecco's Modified Eagle's Medium
DMSO	:	Dimethyl Sulfoxide
DFS	:	Diclofenac Sodium
DNSA	:	3,5-Dinitrosalicylic acid
dNTP	:	deoxy Nucleotide Tri Phosphate
DPPH	:	2,2-diphenyl-1-picrylhydrazyl
EBr	:	Ethidium Bromide
EDTA	:	Ethylene Diamine Tetra Acetic acid
eV	:	Electron volt
FAA	:	Formalin Acetic acid Alcohol
FCI	:	Food corporation of India
FeCl ₃	:	Ferric Chloride
GAE	:	Gallic Acid Equivalent
GAPDH	:	Glyceraldehyde 3- Phosphate Dehydrogenase
GC/MS	:	Gas Chromatography and Mass Spectrometry
GSK	:	Glycogen synthase kinase
IC ₅₀	:	Inhibitory Concentration 50%
IL	:	Interleukin
iNOS	:	Inducible Nitric oxide synthase
IPNI	:	International Plant Names Index

Kb	:	Kilo base pair
Kg	:	Kilogram
L	:	Litre
L929	:	Normal Fibroblast cell line
LC/MS	:	Liquid Chromatography and Mass Spectrometry
LC ₅₀	:	Lethal Concentration 50%
LD ₅₀	:	Lethal Dose 50%
LOX	:	Lipoxygenase
LPS	:	Lipopolysaccharide
M	:	Molar
m/z	:	Mass-to- charge
mg	:	Milligram
mM	:	Millimolar
mmol/l	:	Millimole per litre
MIC	:	Minimum Inhibitory Concentration
MPO	:	Myeloperoxidase
MS-Q-TOF	:	Mass spectrometry- Quadrupole Time Of Flight
MTT	:	3-(4,5-Dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide
MTCC	:	Microbial Type Culture Collection
NaOH	:	Sodium hydroxide
NCCS	:	National Centre for Cell Sciences
NF-KB	:	Nuclear factor Kappa light chain enhancer of activated B cells
NIST	:	National Institute of Standards and technology
NO	:	Nitric oxide
NSAIDs	:	Non-Steroidal Anti-inflammatory Drugs
OD	:	Optical Density
PBS	:	Phosphate Buffered Saline
PDB	:	Protein Data Bank
PR	:	Percentage Repellency
qRT-PCR	:	Quantitative Reverse Transcriptase-Polymerase Chain Reaction
RAW 264.7	:	Mouse leukemic monophyte macrophage cell
ROS	:	Reactive Oxygen Species
rpm	:	Rotations per minute
RT	:	Retention time
s	:	Seconds
SD	:	Standard Deviation

SE	:	Standard Error
SEM	:	Standard Error of Mean
SPSS	:	Statistical Package for Social Sciences
TBA	:	Tertiary butyl alcohol
TCA	:	Trichloro acetic acid
TGF	:	Transforming growth factor
T _m	:	Annealing temperature
TNF	:	Tumor necrosis factor
U/ml	:	Units per millilitre
UV	:	Ultraviolet
V	:	Volt
w/v	:	Weight per volume
WHO	:	World Health Organization

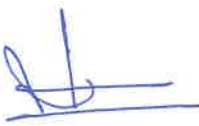
ABSTRACT

Phytochemical Profiling and Pharmacological Investigation of Selected Species of *Litsea* Lam. (Lauraceae), Southern Western Ghats, India.

The genus *Litsea* (Lauraceae) includes several medicinally important species distributed in the Western Ghats. Limited information exists on certain endemic taxa of the Southern Western Ghats, India. The present study represents the first systematic phytochemical and pharmacological investigation of *L. bourdillonii*, *L. coriacea*, and *L. wightiana*. In the initial phase, pharmacognostic characterization of leaf and bark samples was carried out through morphological, anatomical, histochemical physicochemical and powder analyses, which revealed close similarities with subtle distinguishing features. The second phase involved qualitative and quantitative phytochemical screening, which included LC/MS and GC/MS profiling of methanolic extracts, and essential oil analysis. Several bioactive compounds were identified, and a higher quantity of phenols, flavonoids, and tannins was estimated in *L. wightiana* leaves methanolic extracts. Essential oil extraction and analysis of *L. coriacea* leaves were conducted and revealed unique chemical constituents. In the third phase, pharmacological evaluation of leaf and bark extracts of three *Litsea* species was conducted, demonstrating that *L. wightiana* leaves extract possessed promising antibacterial, antioxidant, and antidiabetic activities, while antifungal activity was negligible, and cytotoxicity was low to moderate. Further *in vitro* and *in vivo* studies confirmed the anti-inflammatory potential of *L. wightiana* leaves via enzyme inhibition, gene expression, molecular docking analyses, together with studies by the carrageenan-induced paw edema method in model animals. Wound healing efficacy was validated by *in vitro* scratch wound healing assay and *in vivo* excision wound models in Wistar albino rats, along with histopathological and docking studies. Additionally, insecticidal activity of *L. coriacea* leaf essential oil was studied and found significant activity against *S. oryzae* and *T. castaneum* through contact, fumigant, and repellency assays. Overall, the findings highlight that the selected *Litsea* species possess significant bioactivities, and among all, *L. wightiana* leaves showed the highest activity with

profound anti-inflammatory and wound healing applications, and insecticidal potential of *L. coriacea* leaves essential oil.

Key words: *Litsea*, *L. bourdillonii*, *L. coriacea*, *L. wightiana*, GC/MS, LC/MS analysis, pharmacognostic, phytochemical, anti-inflammatory, gene expression, molecular docking, wound healing, insecticidal activity.


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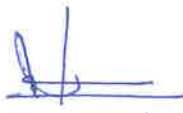

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സംഗ്രഹം

ഇന്ത്യയിൽ പശ്ചിമ ഘട്ടത്തിലെ ലോറേസിയെ കടുംബത്തിലെ തിരഞ്ഞെടുക്കപ്പെട്ട ലിറ്റ്സിയിൽ സ്പെഷ്യലിസറ്റുകളുടെ ഫൈറ്റോകെമിക്കൽ പ്രൊഫൈലിങ്ങും ഫാർമക്കോളജിക്കൽ പഠനവും

ലോറേസിയെ കടുംബത്തിലെ ലിറ്റ്സിയിൽ ജനസ് പശ്ചിമ ഘട്ടത്തിൽ വ്യാപിച്ചു കിടക്കുന്നു. തെക്കു പശ്ചിമ ഘട്ടത്തിൽ മാത്രം കാണപ്പെടുന്ന ചില ലിറ്റ്സിയിൽ സ്പെഷ്യലിസറ്റുകളിൽ പഠനം വളരെ പരിമിതമാണ്. ഈ സാഹചര്യത്തിലാണ് ആദ്യമായി ലിറ്റ്സിയിൽ ബോർഷിളോണി, ലിറ്റ്സിയിൽ കൊറിയേഷ്യ, ലിറ്റ്സിയിൽ വൈറ്റിയായ എന്നിവയുടെ ഫൈറ്റോകെമിക്കൽ പ്രൊഫൈലിങ്ങും ഫാർമക്കോളജിക്കൽ പഠനവും നടത്തിയത്. മൂന്നു ഘട്ടങ്ങളിലായി നടത്തിയ ഈ പഠനത്തിന്റെ പ്രാരംഭ ഘട്ടത്തിൽ ഈ മൂന്ന് ചെടികളുടെ ഇല, കമ്പ് എന്നിവയുടെ ബാഹ്യ ആന്തരിക ഘടനകൾ, ഫിസ്സോകെമിക്കൽ, ഫിസിക്സോകെമിക്കൽ, പൗഡർ മൈക്രോസ്കോപ്പി പഠനങ്ങളും നടത്തി. ഈ മൂന്ന് ചെടികൾ തമ്മിൽ വളരെയധികം സാമ്യങ്ങളും അതോടൊപ്പം ചില വ്യത്യാസങ്ങളും കണ്ടെത്തി. രണ്ടാം ഘട്ടത്തിൽ ഈ ചെടികളുടെ മെത്തനോളിക് സത്തയുടെ ഗുണപരവും അളവുപരവുമായ കാര്യങ്ങൾ, എൽ സി എം എസ്, ജിസി എം എസ്, വിശകലനം നടത്തി അവയിലുടങ്ങിയ സംയുക്തങ്ങളും, അതോടൊപ്പം ലിറ്റ്സിയിൽ കൊറിയേഷ്യയിലെ അവശ്യ എണ്ണയുടെ വിശകലനവും നടത്തി സംയുക്തങ്ങളെ തിരിച്ചറിഞ്ഞു. മൂന്നാം ഘട്ടത്തിൽ തിരഞ്ഞെടുക്കപ്പെട്ട ലിറ്റ്സിയിൽ സ്പെഷ്യലിസറ്റുകളുടെ ആന്റിമൈക്രോബിയൽ, ആന്റിഓക്സിഡന്റ്, ആന്റിഡയാബറ്റിക്, സൈറ്റോടോക്സിസിറ്റി പഠനങ്ങൾ നടത്തി. ആന്റിഓക്സിഡന്റ്, പഠനത്തിന്റെ ഫലമായി ലിറ്റ്സിയിൽ വൈറ്റിയായ മറ്റുള്ളവയെ അപേക്ഷിച്ച് ഗുണങ്ങളിൽ മികച്ചതായി കണ്ടെത്തി. അതേ സമയം ആന്റിഫങ്കൽ, സൈയേറ്റോക്സിക് ഗുണങ്ങൾ ഒന്നിലും വ്യക്തമായി കണ്ടെത്തിയില്ല. തുടർന്നുള്ള ആന്റിഇൻഫ്ളമേറ്ററി, മുറിവ് ഉണക്കൽ പഠനങ്ങൾക്ക് ലിറ്റ്സിയിൽ വൈറ്റിയായ ഇലകളിലെ സത്ത മാത്രം ഉപയോഗിച്ചു. ആന്റിഇൻഫ്ളമേറ്ററി, മുറിവ് ഉണക്കൽ പഠനങ്ങളിൽ ഇൻവിട്രോ, ഇൻവിവോ രീതികൾ പരീക്ഷിച്ചു. ഇൻവിട്രോ പഠനത്തോടൊപ്പം എലികളിൽ നടത്തിയ ഇൻവിവോ പഠനങ്ങൾ വളരെ മികച്ച ഫലം നൽകി ഈ ചെടിയുടെ പ്രാധാന്യം വെളിപ്പെടുത്തി. ഇതു കൂടാതെ ലിറ്റ്സിയിൽ കൊറിയേഷ്യയുടെ അവശ്യ എണ്ണയുടെ ഷഡ്പദങ്ങളെ തുരത്താനുള്ള കഴിവും കണ്ടെത്തി.

സൂചകപദങ്ങൾ : ലിറ്റ്സിയി, ലിറ്റ്സിയി ബോർഡിപ്ലോണി, ലിറ്റ്സിയി കൊറിയേഷ്യ,
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ഫാർമക്കോളജിക്കൽ ആന്റിഇൻഫ്ളമേറ്ററി, മുറിവ് ഉണക്കൽ.


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CERTIFICATE ON PLAGIARISM CHECK

1.	Name of the Research Scholar	UMMU HABEEBA K	
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3.	Name of the Supervisor	Prof.(Dr.) RASMI A.R.	
4.	Department/Institution	RESEARCH DEPARTMENT OF BOTANY, GOVT.VICTORIA COLLEGE,PALAKKAD-678001	
5.	Similar content (%) identified	Non Core	Core
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CONTENTS

<i>Chapters</i>	<i>Title</i>	<i>Page No.</i>
I	INTRODUCTION	1-9
II	REVIEW OF LITERATURE	11-33
	I. Medicinal uses of <i>Litsea</i>	11
	II. Pharmacognostic studies	15
	III. Phytochemical analysis	17
	IV. Pharmacological studies	20
III	MATERIALS AND METHODS	35-73
	I. Plant materials	35
	a. Collection and authentication	35
	II. Methods	38
	PHASE I- PHARMACOGNOSTIC CHARACTERIZATION	39
	a. Morphological study	39
	b. Anatomical characterization	39
	c. Histochemical study	39
	d. Physicochemical study	40
	e. Powder microscopic study	42
	PHASE II- PHYTOCHEMICAL PROFILING	43
	a. Extraction of plant materials	43
	b. Qualitative phytochemical screening	44
	c. Quantitative phytochemical analysis	46
	d. Liquid Chromatography and mass spectrometric analysis	47
	e. Gas Chromatography and mass spectrometric analysis	48
	f. Essential oil extraction and GCMS/GC-FID analysis	48
	PHASE III. PHARMACOLOGICAL STUDY	50
	A. Antimicrobial activity	50
	i. Antibacterial study by agar well diffusion method	50
	ii. Determination of minimum inhibitory concentration	50
	iii. Antifungal assay by agar well diffusion method	51

	B. Antioxidant assay	52
	i. DPPH radical scavenging assay	52
	ii. Hydroxyl radical scavenging assay	53
	iii. Superoxide radical scavenging assay	53
	iv. Ferrous reducing antioxidant power assay	54
	C. Antidiabetic assay	54
	i. Alpha amylase inhibition assay	54
	ii. Alpha glucosidase inhibition assay	55
	D. <i>In vitro</i> cytotoxicity assay	56
	E. Anti-inflammatory study	57
	i. <i>In vitro</i> anti-inflammatory activity	58
	ii. Gene expression studies	60
	iii. Docking study	64
	iv. <i>In vivo</i> anti-inflammatory study	64
	F. Wound healing study	66
	i. <i>In vitro</i> wound healing study by scratch wound method	66
	ii. <i>In vivo</i> wound healing study	67
	iii. Docking study	68
	G. Study of insecticidal activity	69
	i. Contact toxicity toxicity	71
	ii. Fumigant toxicity assay	71
	iii. Repellent activity assay	72
IV	RESULTS	75-144
	Phase I. Pharmacognostic Characterization	75
	a. Morphological study	75
	b. Anatomical study	76
	c. Histochemical study	85
	d. Physicochemical study	87
	e. Powder microscopic study	89
	Phase II- Phytochemical Profiling	89
	a. Determination of extractive value	89
	b. Qualitative analysis of phytochemicals	90
	c. Quantitative analysis of phytochemicals	90
	d. Liquid Chromatography and mass spectroscopic analyses	94

	e. Gas Chromatography and mass spectrometric analyses	102
	f. Analysis of essential oil	104
	Phase III. Pharmacological Study	106
	A. Antimicrobial activity	106
	a. Antibacterial study	106
	b. Antifungal study	108
	B. Antioxidant assay	108
	i. DPPH Radical scavenging activity	108
	ii. Hydroxyl radical scavenging activity	110
	iii. Superoxide radical scavenging assay	111
	iv. Ferrous reducing antioxidant power assay	112
	C. Antidiabetic study	114
	D. <i>In vitro</i> cytotoxicity	117
	E. Anti-inflammatory activity	120
	i. <i>In vitro</i> anti-inflammatory activity	120
	ii. Gene expression studies	126
	iii. Docking study	126
	iv. <i>In vivo</i> anti-inflammatory study	127
	F. Wound healing study	129
	i. <i>In vitro</i> scratch wound healing assay	129
	ii. <i>In vivo</i> wound healing study	129
	iii. Docking study	130
	G. Study of insecticidal activity	131
	i. Contact toxicity	132
	ii. Fumigant toxicity	132
	iii. Repellency	132
V	DISCUSSION	145-174
	Phase I. Pharmacognostic Characterization	146
	Phase II- Phytochemical Profiling	150
	Phase III. Pharmacological Study	156
VI	SUMMARY& CONCLUSIONS	175-181
VII	RECOMMENDATIONS	183-185
	REFERENCES	187-216
	LIST OF PUBLICATIONS AND PRESENTATIONS	217

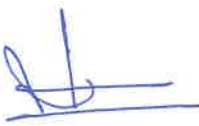
ABSTRACT

Phytochemical Profiling and Pharmacological Investigation of Selected Species of *Litsea* Lam. (Lauraceae), Southern Western Ghats, India.

The genus *Litsea* (Lauraceae) includes several medicinally important species distributed in the Western Ghats. Limited information exists on certain endemic taxa of the Southern Western Ghats, India. The present study represents the first systematic phytochemical and pharmacological investigation of *L. bourdillonii*, *L. coriacea*, and *L. wightiana*. In the initial phase, pharmacognostic characterization of leaf and bark samples was carried out through morphological, anatomical, histochemical physicochemical and powder analyses, which revealed close similarities with subtle distinguishing features. The second phase involved qualitative and quantitative phytochemical screening, which included LC/MS and GC/MS profiling of methanolic extracts, and essential oil analysis. Several bioactive compounds were identified, and a higher quantity of phenols, flavonoids, and tannins was estimated in *L. wightiana* leaves methanolic extracts. Essential oil extraction and analysis of *L. coriacea* leaves were conducted and revealed unique chemical constituents. In the third phase, pharmacological evaluation of leaf and bark extracts of three *Litsea* species was conducted, demonstrating that *L. wightiana* leaves extract possessed promising antibacterial, antioxidant, and antidiabetic activities, while antifungal activity was negligible, and cytotoxicity was low to moderate. Further *in vitro* and *in vivo* studies confirmed the anti-inflammatory potential of *L. wightiana* leaves via enzyme inhibition, gene expression, molecular docking analyses, together with studies by the carrageenan-induced paw edema method in model animals. Wound healing efficacy was validated by *in vitro* scratch wound healing assay and *in vivo* excision wound models in Wistar albino rats, along with histopathological and docking studies. Additionally, insecticidal activity of *L. coriacea* leaf essential oil was studied and found significant activity against *S. oryzae* and *T. castaneum* through contact, fumigant, and repellency assays. Overall, the findings highlight that the selected *Litsea* species possess significant bioactivities, and among all, *L. wightiana* leaves showed the highest activity with

profound anti-inflammatory and wound healing applications, and insecticidal potential of *L. coriacea* leaves essential oil.

Key words: *Litsea*, *L. bourdillonii*, *L. coriacea*, *L. wightiana*, GC/MS, LC/MS analysis, pharmacognostic, phytochemical, anti-inflammatory, gene expression, molecular docking, wound healing, insecticidal activity.


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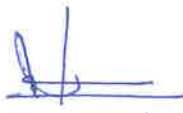

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സംഗ്രഹം

ഇന്ത്യയിൽ പശ്ചിമ ഘട്ടത്തിലെ ലോറേസിയെ കടുംബത്തിലെ തിരഞ്ഞെടുക്കപ്പെട്ട ലിറ്റ്സിയിൽ സ്പെഷ്യലിസറ്റുകളുടെ ഫൈറ്റോകെമിക്കൽ പ്രൊഫൈലിങ്ങും ഫാർമക്കോളജിക്കൽ പഠനവും

ലോറേസിയെ കടുംബത്തിലെ ലിറ്റ്സിയിൽ ജനസ് പശ്ചിമ ഘട്ടത്തിൽ വ്യാപിച്ചു കിടക്കുന്നു. തെക്കു പശ്ചിമ ഘട്ടത്തിൽ മാത്രം കാണപ്പെടുന്ന ചില ലിറ്റ്സിയിൽ സ്പെഷ്യലിസറ്റുകളിൽ പഠനം വളരെ പരിമിതമാണ്. ഈ സാഹചര്യത്തിലാണ് ആദ്യമായി ലിറ്റ്സിയിൽ ബോർഷിപ്പോണി, ലിറ്റ്സിയിൽ കൊറിയേഷ്യ, ലിറ്റ്സിയിൽ വൈറ്റിയായ എന്നിവയുടെ ഫൈറ്റോകെമിക്കൽ പ്രൊഫൈലിങ്ങും ഫാർമക്കോളജിക്കൽ പഠനവും നടത്തിയത്. മൂന്നു ഘട്ടങ്ങളിലായി നടത്തിയ ഈ പഠനത്തിന്റെ പ്രാരംഭ ഘട്ടത്തിൽ ഈ മൂന്ന് ചെടികളുടെ ഇല, കമ്പ് എന്നിവയുടെ ബാഹ്യ ആന്തരിക ഘടനകൾ, ഫിസ്സോകെമിക്കൽ, ഫിസിക്സോകെമിക്കൽ, പൗഡർ മൈക്രോസ്കോപ്പി പഠനങ്ങളും നടത്തി. ഈ മൂന്ന് ചെടികൾ തമ്മിൽ വളരെയധികം സാമ്യങ്ങളും അതോടൊപ്പം ചില വ്യത്യാസങ്ങളും കണ്ടെത്തി. രണ്ടാം ഘട്ടത്തിൽ ഈ ചെടികളുടെ മെത്തനോളിക് സത്തയുടെ ഗുണപരവും അളവുപരവുമായ കാര്യങ്ങൾ, എൽ സി എം എസ്, ജിസി എം എസ്, വിശകലനം നടത്തി അവയിലടങ്ങിയ സംയുക്തങ്ങളും, അതോടൊപ്പം ലിറ്റ്സിയിൽ കൊറിയേഷ്യയിലെ അവശ്യ എണ്ണയുടെ വിശകലനവും നടത്തി സംയുക്തങ്ങളെ തിരിച്ചറിഞ്ഞു. മൂന്നാം ഘട്ടത്തിൽ തിരഞ്ഞെടുക്കപ്പെട്ട ലിറ്റ്സിയിൽ സ്പെഷ്യലിസറ്റുകളുടെ ആന്റിമൈക്രോബിയൽ, ആന്റിഓക്സിഡന്റ്, ആന്റിഡയാബറ്റിക്, സൈറ്റോടോക്സിസിറ്റി പഠനങ്ങൾ നടത്തി. ആന്റിഓക്സിഡന്റ്, പഠനത്തിന്റെ ഫലമായി ലിറ്റ്സിയിൽ വൈറ്റിയായ മറ്റുള്ളവയെ അപേക്ഷിച്ച് ഗുണങ്ങളിൽ മികച്ചതായി കണ്ടെത്തി. അതേ സമയം ആന്റിഫങ്കൽ, സൈയേറ്റോക്സിക് ഗുണങ്ങൾ ഒന്നിലും വ്യക്തമായി കണ്ടെത്തിയില്ല. തുടർന്നുള്ള ആന്റിഇൻഫ്ളമേറ്ററി, മുറിവ് ഉണക്കൽ പഠനങ്ങൾക്ക് ലിറ്റ്സിയിൽ വൈറ്റിയായ ഇലകളിലെ സത്ത മാത്രം ഉപയോഗിച്ചു. ആന്റിഇൻഫ്ളമേറ്ററി, മുറിവ് ഉണക്കൽ പഠനങ്ങളിൽ ഇൻവിട്രോ, ഇൻവിവോ രീതികൾ പരീക്ഷിച്ചു. ഇൻവിട്രോ പഠനത്തോടൊപ്പം എലികളിൽ നടത്തിയ ഇൻവിവോ പഠനങ്ങൾ വളരെ മികച്ച ഫലം നൽകി ഈ ചെടിയുടെ പ്രാധാന്യം വെളിപ്പെടുത്തി. ഇതു കൂടാതെ ലിറ്റ്സിയിൽ കൊറിയേഷ്യയുടെ അവശ്യ എണ്ണയുടെ ഷഡ്പദങ്ങളെ തുരത്താനുള്ള കഴിവും കണ്ടെത്തി.

സൂചകപദങ്ങൾ : ലിറ്റ്സിയി, ലിറ്റ്സിയി ബോർഡിപ്ലോണി, ലിറ്റ്സിയി കൊറിയേഷ്യ,
ലിറ്റ്സിയി വൈറ്റിയാന, ജി സി എം എസ്, എൽ സി എം എസ്, ഫൈറ്റോകെമിക്കൽ,
ഫാർമക്കോളജിക്കൽ ആന്റിഇൻഫ്ളമേറ്ററി, മുറിവ് ഉണക്കൽ.


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CHAPTER 1

INTRODUCTION

Human existence has been associated with plants in multiple ways. Plants play a crucial role in the treatment of many diseases affecting humans and in maintaining overall health. India, with its rich heritage of indigenous medicinal plants, has long been utilizing traditional treatments such as ayurveda and ethnomedicine. The evergreen forests of the Western Ghats are incredible sources of medicinal and other economically significant plants, characterized by high endemism and diversity. About 35 % of plant species in the Western Ghats are endemic (Suja, 2005). The extreme environmental heterogeneity of the Western Ghats' biogeography makes it more diverse and unique (Gadgil, 1996). According to the World Health Organization, “nearly 80% of the earth’s population relies on traditional medicine for their primary healthcare requirements, which employs plant extracts or their active ingredients” (Mushtaq et al., 2014).

Medicinal plants possess immense therapeutic value owing to their natural compounds' healing properties. These plants have been utilized for many years in traditional healing practices and continue to play a vital role in modern health care. Bioactive compounds such as alkaloids, flavonoids, terpenoids, polyphenols, tannins, glycosides, *etc.*, provide a wide range of curative benefits. Medicinal plants can alleviate symptoms and treat ailments like pain, inflammation, infections, and chronic illness. As research advances, the therapeutic potential of medicinal plants is being further explored, leading to the development of new drugs and treatments, offering hope for improved health and well-being.

The Lauraceae family, well known as the Laurel family, consists of several medicinally valuable plants. The members of this family have been extensively utilized in traditional medicinal practices all over the world. *Litsea* is a medicinal and dominant genus belonging to the family Lauraceae. There are about 400 tree species distributed in tropical and subtropical regions of the world. Out of these, 27 are endemic to the megadiversity hotspots, including the Western Ghats, India (Goh

et al., 2024). The therapeutic effects of different species of *Litsea*, like *L. glutinosa*, *L. cubeba*, and *L. monopetala*, have been revealed and are used for various medical ailments. Around 400 secondary metabolites have been investigated from different *Litsea* species including crucial chemicals like lactones, alkaloids, sesquiterpenes, flavonoids *etc.* (Wang et al., 2016).

Several species of *Litsea* were reported to be significant in traditional Chinese medicine (Xie & Yu, 1996). Various decoctions have been prepared from leaves, bark, roots, flowers, and fruits of *Litsea* plants and are administered for various diseases. The ethnomedicinal and therapeutic values of *L. glutinosa* have been extensively studied (Jamaddar et al., 2022). The ethnomedicinal importance of 11 *Litsea* species among five ethnic groups in the Northern Thailand region is explored (Chaisoung et al., 2023). *L. coreana* roots and leaves are used to treat edema, stomach distension, diarrhoea *etc.* (Jia et al., 2017).

The ethnomedicinal and pharmacological values of twenty *Litsea* species have been reported to be used to cure diarrhea, stomachache, dyspepsia, diabetes, gastroenteritis, arthritis, asthma, pain, *etc.* Around 200 phytochemicals are isolated, including bioactive ingredients like flavonoids, terpenoids, and alkaloids, which possess significant pharmacological effects (Kong et al., 2015).

Xie & Lu (1996) reported the ethnomedicinal uses of leaves of *L. auriculata*, bark of *L. coreana* var. *sinensis* and *L. coreana* var. *lanuginosa*, fruits of *L. cubeba*, *L. euosma*, bark and roots of *L. glutinosa*, leaves of *L. hupehana*, fruits and roots of *L. ichangensis*, roots and leaves of *L. monopetala*, fruits of *L. moupinensis*, roots of *L. populifolia*, fruits and leaves of *L. pungens*, roots of *L. rotundifolia*, *L. rubescens*, and *L. verticillata*, and leaves, bark and fruits of *L. mollis*.

Bhuinya & Mukherjee (2010) studied the fruit characteristics of 13 *Litsea* species, including infructescence, the nature of perianth, berry shape, and the nature and diameter of hypanthium. Based on the identified features, the authors created an artificial key of specimens studied. The morphological features of *L. glutinosa* (Lour.) C. B. Rob from Central India were analyzed and leaf, bark, and crown characteristics were studied and documented (Mohammed et al., 2020). A revision

study of *Litsea* found in Peninsular Malaysia and Singapore was conducted. Taxonomic characters, along with morphological characterization, were carried out (de Kok, 2021).

Histochemistry is a microscopic technique that enables the chemical examination of cells and tissues with respect to their structural arrangement (Coleman, 2000). Histochemical studies in three endangered medicinal plants in Gujarat revealed that starch deposition is ubiquitous throughout the plant body, it is most frequently found in secondary vascular tissues particularly in parenchyma tissue of stem, root tubers *etc.* (Kadam, 1999). The diverse class of phenol derivatives known as tannins is typically associated with glucosides. Many plants have a high concentration of tannin in their leaves, mainly in xylem cells (Kadam et al., 1996). Histochemical studies in *L. glaucescens* Kunth revealed a substantial presence of phenolic compounds, lipids and non-structural carbohydrates in both leaves and woody tissues (Tapia-Torres et al., 2014).

Phytochemicals are naturally occurring chemical constituents occurs in plants that provide health benefits along with macronutrients and micronutrients (Saxena et al., 2013). These phytochemicals play a significant role in treating chronic diseases like cancer, diabetes, coronary heart diseases, *etc.* A diverse array of phytochemicals are distributed in fruits, vegetables, grains, nuts, legumes *etc.* and belong to different categories such as phenols, flavonoids, alkaloids, terpenoids, saponins, tannins *etc.* Phenolic compounds constitute the most extensive class of phytochemicals and are ubiquitously distributed throughout the plant kingdom. Phenolics are generally considered as defensive compounds and possess multiple bioactive properties beneficial to human health. Flavonoids are known to exhibit a wide range of bioactivities in which antioxidant capacity is most extensively documented (Koche et al., 2016). Alkaloids and terpenoids represent the pharmacological activities including antimicrobial, antidiabetic, anti-inflammatory and anti-proliferative properties (Rodriguez-Garcia et al., 2017). Saponins demonstrated several biological properties including anti-tumor, anti-HIV, antimicrobial, anti-inflammatory, antileishmanial effects (Yendo et al., 2010).

Tannins are phytochemicals which specifically possess anti diabetic properties (Ajebli & Eddouks, 2019).

To uncover the diverse range of plant chemicals, researchers employ several techniques in phytochemistry. One fundamental technique is solvent extraction, where plant material is macerated or treated with various solvents to separate different classes of compounds based on their solubility. This process yields crude extracts that can be further purified by commonly used chromatographic techniques such as thin-layer chromatography (TLC) or high-performance liquid chromatography (HPLC). TLC separates compounds based on their mobility in a thin-layer adsorbent material, while HPLC uses liquid chromatography for precise separation and quantification. Spectroscopic techniques such as nuclear magnetic resonance (NMR) and mass spectrometry (MS) are essential for elucidating the structures of isolated compounds, enabling the identification and characterization of the phytochemicals present in plant extracts.

The extracts of leaves, bark, roots, fruits, flowers, *etc.*, are subjected for various phytochemical studies. The extraction can be carried out by various methods, such as Soxhlet method, microwave-assisted type of extraction, ultrasound extraction (sonication), and supercritical fluid extraction (Velaven, 2015). Gas chromatography and mass spectrometry (GC/MS) can be utilized for the separation and identification of volatile components in a sample extract. At the same time, liquid chromatography and mass spectrometric (LC/MS) studies are used for the separation and analysis of non-volatile components. In LC/MS, the variant mass analyzers such as quadrupole (Q), time-of-flight (TOF), ion trap (IT), and Fourier transform ion cyclotron resonance (FT-ICR) can be adopted (Wu et al., 2013).

The inappropriate consumption of antibiotics leads to antibiotic resistance and makes medications ineffective. Compounds derived from medicinal plants can offer potent and innovative solutions against pathogenic bacteria (Vaou et al., 2021). Medicinal plants provide an unlimited source of bioactive compounds, which have been utilized as antimicrobial agents (Hostettmann, 1999). A diverse array of techniques are currently employed to assess the antimicrobial properties of natural

extracts. Various antibacterial susceptibility tests are available to determine bacterial resistance to antimicrobials. The disc diffusion method, agar well diffusion method, broth dilution method, and determination of minimum inhibitory concentration and minimum bactericidal concentration are the common methodologies adopted to determine antimicrobial activity.

Antioxidants are chemicals that can neutralize the free radicals produced during metabolism. They can stop radical chain reactions of lipid peroxidation at cellular and molecular levels, where they inactivate the ROS (Reactive oxygen species) under certain low concentration limits or delay oxidative activities. Natural antioxidants such as carotenoids, tocopherols, lipoic acids and polyphenols scavenge the free radicals. Superoxide dismutase (SOD), catalase, glutathione peroxidase, and glutathione reductase are some of the examples of endogenous enzymes to work with antioxidants (Prakash & Gupta, 2009). Flavonoids and phenols are widespread in plants and have been discovered to possess diverse pharmacological functions, mainly including antioxidant activity (Priya et al., 2011).

Diabetes is a rapidly increasing disease worldwide. The World Health Organization predicts that the diabetic population could rise to 300 million or more by 2025 (Sy et al., 2005). Untreated diabetes may lead to multiple organ failures and complications such as neurodegenerative diseases, kidney diseases and eye-related ailments. Multiple plant-derived compounds with hypoglycemic properties are effective in treating diabetes by insulin-sensitizing activities. Reducing diet-dependent blood glucose levels by utilizing these compounds is a practical treatment strategy for the management and prevention of diseases.

Alpha-amylase and alpha-glucosidase are the two enzymes which restrict the gut's ability to break down dietary carbohydrates (Kim et al., 2005; Ogunyemi et al., 2022). Alpha-amylase breaks the α -D (1-4) glycosidic linkages in complex carbohydrates to yield oligosaccharides, which are then split in to monosaccharide, glucose by α -glucosidase. The activity of these enzymes can be reduced by inhibitors can control the increased level of glucose in blood in individuals who

intake more carbohydrate containing foods, helping to keep postprandial hyperglycemia (PPHG) under control (Poovitha & Parani, 2016). Alpha-glucosidase enzymes in the membrane of small intestine can hydrolyze di, tri, and oligosaccharides and convert them to glucose. Inhibiting these enzyme systems slows down the digestion of carbohydrates (Ahmad et al., 2011). Alpha-amylase is an essential enzyme in the conversion of starch to oligosaccharides. In animals, alpha-amylase inhibitors help to reduce post-meal high glucose levels by slowing down the conversion of starch to simple sugars (Nair et al., 2013).

Cytotoxicity studies are commonly employed to check the toxicity effects of plant extracts and to develop drugs. These drugs can be used to cure cancer. Plant extracts with less toxicity effect can be recommended for the development of biologically promising drugs (Mc Gaw et al., 2014). Cell lines are employed in *in vitro* cytotoxicity assays, for the determination of cytotoxicity (Sowa et al., 2020). Cell viability can be determined by various methods like MTT and WST methods, crystal violet and colony formation methods, tritium labelled thymidine uptake method *etc.* (Thangaraj, 2016).

Inflammation is a biological response to tissue injury. At the site, increased permeability of blood vessel walls, followed by the migration of immune cells, results in the formation of edema during inflammation (Perwez- Hussain & Harris, 2007). The inflammatory response is distinguished by redness, heat, swelling, loss of function, pain against an infection, damage or irritation. Many pro inflammatory factors including kinins, platelet-activating factors (PAF), prostaglandins, leukotrienes, amines, purines, cytokines, *etc.* act on targets, causing the production of additional inflammatory factors from leukocytes locally and more neutrophils to the site of inflammation (Bellik et al., 2012). Nitric oxide (NO) is released in large amounts during inflammation, particularly in lipopolysaccharide-induced cells. Nitric oxide synthase enzymes present in macrophages and hepatocytes are responsible for the synthesis of nitric oxide. Nitric oxide is also synthesized by L-arginine metabolism. Increased nitric oxide causes cytotoxicity. Moreover, nitric oxide is a pro-oxidant molecule and induces oxidative stress (Vigo et al., 2004).

Prostaglandin E2 (PGE₂) is a pro-inflammatory factor produced by the action of cyclooxygenase (COX). A high amount of PGE₂ synthesis is induced by pro-inflammatory mediators like TNF- α (Tumor necrosis factor alpha) and interleukins β (IL- β). NF KB (Nuclear Factor kappa B) is a transcription factor, and its activation leads to the production of cytokine and several genes associated with inflammation (Park et al., 2005). Lipid mediators like leukotrienes and prostaglandins, which are produced by lipoxygenases, can cause several inflammatory conditions, including bronchial asthma, allergic rhinitis, cardiovascular disorders, rheumatoid arthritis and some types of cancers (Schneider & Bucar, 2005). During the past ten years, research using *in vivo* and *in vitro* models of inflammation has discovered several natural extracts with established anti-inflammatory properties. Non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids are being utilized extensively in modern medicine to treat inflammatory conditions, but they cause serious side effects (Wang et al., 2013). Plant-derived phenolic compounds are effective anti-inflammatory agents and are useful in the treatment of skin conditions. The most prevalent kind of natural antioxidants are phenols, which have anti-inflammatory and antioxidant properties (Rahman et al., 2021).

Herbal medicines have a unique role in the healing of wounds in humans and animals. Ancient Egyptians, the native peoples of Africa, Asia, Rome, and America have used medicinal herbs as the first line of treatment for inflammation, burns, ulcers, and surgical wounds for more than 5000 years. They contain a variety of biologically active compounds that can cause immediate wound healing and promote tissue regeneration in the vicinity of the wound (Warrier, 1993). Many investigations have highlighted the wound recovery properties of various phytocompounds, such as flavonoids, alkaloids, terpenes *etc.* (Manzoureh & Farahpour, 2021). Multiple kinds of cells, the extracellular matrix, and immune-soluble mediators, including growth promoting substances and cytokines, are involved in wound healing. Researchers are looking into new mixtures, bandages, and medicinal plant components to create more effective, affordable, stable, and long-lasting delivery systems for the management and treatment of wounds (Sharma et al., 2021).

Numerous researchers have documented how various plant extracts and isolated compounds improve the rate of wound healing in animal models. Factors such as epithelialization, assessment of hydroxyproline content in the granulation tissue and associated biochemical changes are involved in wound healing (Sasidharan et al., 2011). Effective wound-healing treatments with better wound repair mechanisms can be emerge from novel wound-healing techniques utilizing medicinal plants in conjunction with nanotechnology (Tyavambiza et al., 2021). Numerous plant-derived substances with strong wound-healing abilities are employed to create topical formulations and wound dressings. Several wound-healing chemicals, including polyphenols, terpenoids, flavonoids and alkaloids, have already been identified and isolated.

Essential oils are produced from various plant organs and are concentrated liquids of intricate combinations of volatile substances. They are usually aromatic with a characteristic odor. Many bioactive substances with antibacterial and antioxidant effects can be found in essential oils. Due to the risk involved in using synthetic preservatives, essential oil usage has gained more attention as natural remedies for prolonging the shelf life of goods (Tongnuanchan & Benjakul, 2014). Major compounds present in essential oils are terpenes (mono and sesquiterpenes), aromatic chemicals (aldehyde, phenols, alcohols), and terpenoids (isoprenoids) (Bakkali et al., 2008). Many of the essential oil components provide them the insecticidal potential and act as natural insecticides that can replace synthetic chemical formulations.

The utilization of medicinal plants for their curative effect is an old practice since the start of human civilization. Ayurveda has been one of the main focuses of medicinal practitioners since ancient times. With the development of ayurvedic knowledge, the information about indigenous medicinal plants also expanded. The ethnomedicinal field has also significantly impacted the medical field. With these traditional systems, modern medicine also developed. New drugs and therapeutic products have been developed from medicinal plants to cure various ailments.

Western Ghats are a principal repository of a wide variety of medicinal plants in which the position of *Litsea* plants of Lauraceae are prominent. Most of the *Litsea* species are ethno medicinally important, and the therapeutic properties of the major species, such as *L. glutinosa*, *L. cubeba*, *L. salicifolia*, *L. monopetala*, etc., are well known. In this scenario, it is decided to conduct studies on the pharmaceutical potential of selected *Litsea* species, viz., *L. bourdillonii*, *L. coriacea*, and *L. wightiana*. These plants are found in the evergreen forests of Western Ghats. No previous investigation has been carried out regarding the said species. This is the first attempt intended to reveal the phytochemicals and their pharmacological values. The discovery and characterization of novel medicinal plants with potential therapeutic properties is still an exciting frontier in the field of phytochemical research. Knowledge in phytochemistry paves the way for future research into the health benefits and therapeutic applications of these plants.

The objectives of the study include,

- Collection and authentication of selected species of *Litsea*
- Morphological and anatomical characterization
- Characterization of physicochemical features
- Qualitative and quantitative phytochemical profiling
- Characterization of biological activities of plant materials
- To isolate the essential oils and characterize the components in the oil qualitatively and quantitatively
- Evaluation of biological activities of essential oils

CHAPTER II

REVIEW OF LITERATURE

Phytochemicals are natural compounds produced through secondary metabolism in plants, and they provide health benefits that go beyond the basic nutritional value of macronutrients and micronutrients (Hasler & Blumberg, 1999). More than 4000 phytochemicals have been identified and reported from various plants. Key classes of these phytochemicals include dietary fiber, antioxidants, anticancer agents, detoxifying agents, immunity boosters, and neuropharmacological agents. These classes contain a variety of chemicals with varying levels of effectiveness, and some phytochemicals serve multiple functions (Saxena et al., 2013).

Litsea is the predominant genus in the family Lauraceae and comprises several medicinally important species distributed in tropical and subtropical regions of the world. Western Ghats are enriched with endemic *Litsea* species and most of them are part of India. Eighteen indigenous *Litsea* species were reported from India (Srinivas & Krishnamurthy, 2019). Recently there are some new reports of *Litsea* species from Kerala. *Litsea indoverticillata* Robi & Udayan (Robi et al., 2017), *Litsea gorayana* Udayan & Robi *sp.nov.* (Udayan & Robi, 2017), *Litsea manilaliana* Robi & Udayan (Robi & Udayan, 2021), *Litsea udayanii* Robi (Jose et al., 2015) and *Litsea robiana* K.M.P Kumar (Prabhukumar et al., 2025).

I. MEDICINAL USES OF LITSEA

Most species in the genus *Litsea* are of medicinal importance. Different plant parts like leaves, stems, roots, and seeds are used for medicinal purposes. *L. stocksii* and *L. glutinosa* are utilized for their bark. The essential oils, like citral and lauric acid, are extracted from *Litsea* species. Twenty plant species from the genus *Litsea* have been identified as significant traditional medicines in China. These plants have a long history of use as treatments for various ailments, including traumatic injury, arthritis, diabetes, edema, cold, diarrhea, and dyspepsia (Kong et al., 2015).

L. cubeba essential oil exhibiting various biological activities like antioxidant, antimicrobial, antiproliferative, insect repellent, pesticide effects and can also be used as fragrance agent in cosmetics (Sivamaruthi et al., 2020). The antitumor, anti-anaphylactic, anti-inflammatory, and antidiabetic properties of *L. cubeba* essential oil were also reported (Kamle et al., 2019). Research on *L. coreana* has revealed that it contains unique polysaccharides, polyphenols, essential oils, and numerous flavonoids. These compounds exhibit significant bioactivities through various molecular mechanisms such as hepatoprotection, hyperglycemia management, anti-inflammatory effects, antioxidation and antibacterial properties (Jia et al., 2017). The *in vitro* thrombolytic, anti-inflammatory, analgesic, and antipyretic potentials of *L. glutinosa* used in folk medicine were studied, and the results obtained support their above-mentioned properties (Bhowmick et al., 2014).

In India there are reports on phytochemical and pharmacological studies on some *Litsea* species. An assessment of antioxidant properties of *L. floribunda*, endemic to Southern Western Ghats, India was carried out. The authors affirmed that the plant contains a high amount of phenols which contributed to the high antioxidant activity of *L. floribunda* (Devika et al., 2016). Phytochemical screening of leaves of *L. glutinosa* revealed the occurrence of phytochemicals such as flavonoids, tannins, steroids, cardiac glycosides, phenols *etc.* The plant constitutes antimicrobial property due to these phytochemicals. Leaf extracts exhibited antibacterial activity against bacterial strains such as *Bacillus*, *Enterococcus*, *Staphylococcus* and *Pseudomonas* species (Lagudu & Owk, 2018). Shafiq et al. (2022) reported the antioxidant and anti-cancer potentials of *L. glutinosa* methanolic extracts by *in vitro* and *in vivo* studies. Chawra et al. (2021) conducted a review study on the ethnomedicinal applications of *L. glutinosa* and documented the same.

Table 1. Ethnopharmacological uses of different species of *Litsea*

Name of <i>Litsea</i> species	Part used	Ethnopharmacological activity	Article cited
<i>L. graciae</i> S. vidal	Fruits	Antioxidant, antifungal properties	Hassan et al., 2013
<i>L. polyantha</i> Juss.	Bark and roots	Active against Pain, fractures, diarrhea, and bruises	Ghosh & Sinha, 2010
<i>L. chinensis</i> (Gaertn.) Sonner	Stem, leaves, and fruits	Aphrodisiac activity	Ageel et al., 1994
<i>L. turfusa</i> Kosterm	Bark	Antifungal, antitumor properties	Holloway & Scheinmann, 1973
<i>L. cubeba</i> (Lour.) Pers	Fruit Root, and Leaves	Used for pain relief, promotes blood circulation, treats asthma, diarrhea, traumatic injury, cold, stomachache, headache, dermatophytosis treats insect and snake bites	Kong et al., 2015; Normile, 2003; Kumar et al., 2011
<i>L. japonica</i> (Thunb.) Juss	Leaves	Antioxidant, anti-inflammatory effects	Min et al., 2003
<i>L. monopetala</i> (Roxb.) Pers	Bark, roots, leaves, trunk	Used to treat gonorrhea, skin disease, diarrhea, and have antimicrobial properties	Hua et al., 2014
<i>L. akoensis</i> Hayata	Stem bark	Cytotoxicity, antimicrobial effects	Ho et al., 2011
<i>L. odorifera</i> Val.	Leaves	Anti HSV-1 agent	Nawawi et al., 1999
<i>L. salicifolia</i> (J.Roxb.ex Nees) Hook.f.	Fruits	Used to treat bone fractures, stomach pain	Kala, 2005

<i>L. glutinosa</i> (Lour.) C.B. Rob.	Leaves, bark	Active against boils, traumatic injury	Ramana & Raju, 2017
<i>L. mollis</i> Hemsl	Fruits	Used to treat chronic eczema	Xie & Yu, 1996
<i>L. rubescens</i> Lecomte	Fruits	Active against enterogastritis, stomachache, dyspepsia	Xie & Yu, 1996
<i>L. verticillata</i> Hance	Root, leaves, bark	Promotes blood circulation, relieves pain, and treats traumatic injury	Xie & Yu, 1996
<i>L. glutinosa</i> (Lour.) C. B Rob.	Leaves, berries oil	Antiseptic, emollient properties and used in the treatment of diarrhea, dysentery, wounds, bruises and rheumatism	Chowdhury et al., 2008; Uddin et al., 2014
<i>L. glutinosa</i> (Lour.) C. B Rob.	Seed	Active against skin boils	Mishra et al., 2010

II. PHARMACOGNOSTIC STUDIES

- **Morphology**

The morphological features of *L. glutinosa* like tree height, bole height, crown diameter, number of primary branches, leaf area, leaf weight and bark weight/unit area as well as their relationships were studied and found variations. It is found that there is high variation in bole height, crown diameter *etc.* (Mohammed et al., 2020). Bhuinya et al. (2010) studied the fruit morphology of 17 *Litsea* species collected from Sikkim and created an identification key, which can be used in field studies. Morphological and micromorphological features including qualitative and quantitative traits of *L. cubeba* were studied and documented. The micromorphological features such as trichome, configuration of epidermal cells, pollen morphology, shape and spatial pattern of exine ornamentation are included (Aini & Wisanti, 2023).

- **Anatomy**

According to WHO guidelines, microscopic and macroscopic studies are the primary steps in the standardization and identification of any plant material (WHO, 2002; Kokate, 2008). Microscopic studies of the leaf, stem, and petiole of *L. quinqueflora* (Dennst.) were carried out (Hari et al., 2021). The prominent anatomical features such as cutinized epidermis, paracytic stomata, fibres, and vessels were listed. Scanning electron microscopic studies of *L. chinensis* were done by Bhatt & Pandya (2012), who observed the abundance of mucilage, well-developed vascular bundles, hypodermis, and ground tissues, which were quoted as the best identifying features of this *Litsea* species. Microscopic, macroscopic, and organoleptic characters of *L. glutinosa* were analyzed and documented the anatomical details of periderm, cortex, secondary xylem, and secondary phloem (Rao et al., 2012).

- **Histochemistry**

Histochemical studies are conducted to find out the presence of chemical components in cells with the aid of stains, reagents and light and electron

microscopy (Wick, 2012). Histochemical experiments are also utilized to study plant secretions in tissues and localization of phytochemicals using specific reagents (Demarco, 2022). Histochemical staining has gained attention in the field of plant research for the localization of proteins, minerals, cell wall components, oxidative stress markers *etc.* (Yadav et al., 2021). The basic idea behind histochemical detection is that when the tissue or cell is exposed to a staining solution, it will specifically react by producing a colored insoluble product that can be seen under the microscope (Ruifrok & Johnston, 2001). Nowadays, many fast-coloring reactions are utilized for the localization of secondary metabolites (Giuliani et al., 2018). For conducting an exact chemical analysis of cells concerning the structural organization, histochemical analytical methods are adopted (Fahn, 1988). The anatomical studies, together with histolocalization, support the quality control of plant drugs (Souza et al., 2017). Kadam et al. (2013) investigated the histochemicals, like starch, protein, tannin, saponin, fat, glucoside, and alkaloid in *Mimusops elengi* Linn. and *Syzygium cumini* Linn. Histochemical investigation of some medicinal plants in Ericales revealed the occurrence of chemicals in idioblasts and epidermal tissues (Sabila et al., 2022).

- **Physicochemical studies and powder characterization**

Physicochemical evaluation and studies of fluorescent characters were carried out in *Acacia ferruginea*, and it was concluded that the results obtained help to standardize drugs by determining the purity and virtue of the plant (Jeevitha et al., 2021). In this study, it is discovered that the total ash in the bark is significantly higher than that of the leaves, and the water content is opposite to the quantity of ash, which is higher in leaves. This indicates the presence of biological residue in leaves. Some physicochemical properties of *Moringa oleifera*, like moisture content, total ash value, and saponification value, were analyzed together with qualitative and quantitative phytochemical screening (Belay & Sisay, 2014). The physicochemical parameters like loss on drying (85.5%w/w), total ash value (15.625%), acid-insoluble ash (26.984%), and water-soluble ash (29.32%) in *Eclipta alba* were determined (Kumar et al., 2020). Physicochemical characteristics of leaf powder, including its foreign matter content, extractive values, and ash content of *Wedelia trilobata* L. were studied. Significant results were obtained from the study, which are crucial in the characterization of crude drug (Karthika & Manivannan, 2018).

The standardization of the drug from *Woodfordia fruticosa* was done by physicochemical evaluation, phytochemical profiling, and chromatographic separations (Grover et al., 2014).

The pharmacognostic, phytochemical, and physicochemical standards assessment of *Aralia racemosa* was conducted, and the results supported the standardization and identification of drugs (Prasanth et al., 2016). The physicochemical features, phytochemical constituents, and chromatographic studies of leaves of *Schleichera oleosa* (Lour.) Oken was performed and obtained promising findings (Muthukrishnan & Sivakumar, 2018). *Kalanchoe pinnata* Lam. was subjected to physicochemical, qualitative, and quantitative analysis of phytochemicals and found that the plant possesses various biologically important phytochemicals such as phenols, flavonoids, tannins, alkaloids etc. Physicochemical studies proved the therapeutic potential of the plant (Singh et al., 2019). The pharmacological, physicochemical, and phytochemical studies of *Huberantha senjiana* provided ground information for the identification and standardization of drug preparation from the plant (Pandiyan & Ilango, 2022). The physicochemical analysis of *L. unguolata* leaves essential oil fractions was carried out. The parameters like chemical composition, color and refractive index were noted (Putri et al., 2025).

III. PHYTOCHEMICAL ANALYSIS

The phytochemical analysis of *L. angulata* revealed phytochemicals like alkaloids, flavonoids, terpenoids, and coumarins. The antioxidant activity study findings indicated that all plant extracts demonstrated the capability to inhibit the free radicals (Kuspradini et al., 2018). The GC/MS analysis of essential oil in *L. cubeba* identified 59 compounds with monoterpenes (94.4-98.4%) as the predominant components, primarily consisting of neral and geranial (78.7-87.4%). D-limonene was found to be a minor component (0.7-5.3%) in fruits (Si et al., 2012). The main constituents of the oils derived from stem, leaves and fruits of *L. cubeba* were beta phellandrene, beta-terpinene and neral (Wang & Liu, 2010).

Table 2. Phytochemical compounds isolated from different species of *Litsea*

SL.No	<i>Litsea</i> sp.	Compounds identified/isolated	Plant part	Article cited
1.	<i>L. lancilimba</i>	Lancilimboid C, Lancilimboid D, Lancilimboid E, Shiluone B	Fruits	Muhammad et al., 2022
2.	<i>L. cubeba</i>	LCA-Dibenzyl butane lignan	Root	Yu et al., 2020
3.	<i>L. glutinosa</i>	Nicotiflorin, rutin, afzelin, quercitrin, magnocurarine, oblongine chloride, boldine methochloride, pallidine, predicentrine, aripuanin, blumenol A, phloroglucinol, 2-phyten-1-ol, cryptodorine, reticuline	Leaves and bark	Thao et al., 2019
4.	<i>L. cubeba</i>	Corydne, corytuberine, laurolitsine, isocorydine, decentrine, dicentrinone, xanthoplanine	Leaves and fruits	Kamle et al., 2019
5.	<i>L. japonica</i>	Litsenolide F ₁ , lisealactone H ₁ , akolactone D, akolactone E	Fruit pericarp	Ngo et al., 2019
6.	<i>L. pungens</i>	Pinocembrin, pinocembrin chalcone, apigenin, quercetin, luteoline, kaempferitrin, apigenin-7- <i>O</i> - β -D-glucoside, luteoline-7- <i>O</i> - β -D-glucoside, isoquercetin and quercetin-3- <i>O</i> -rhamnoside.	Leaves	Duan et al., 2017
7	<i>L. verticillata</i>	Litseaverticillols, litseasesquibutenolide	Bark	Guan et al., 2016
8.	<i>L. helferi</i>	limonene, β -caryophyllene, Bicyclogermacrene, bicycloelemene, α -phyllandrene	Leaf oil	Son et al., 2014
9	<i>L. ferruginea</i>	Sabinene, α -pinene, γ -terpinene, limonene, terpen-4-ol	Leaf oil	Son et al., 2014
10	<i>L. coreana</i>	5-(2-phenylethyl)-3 hydroxyphenol-1- <i>O</i> - β -D-glucopyranoside, 6-(2-phenylethyl)-2,4-dihydroxy benzoic acid 2- <i>O</i> - β -D-glucopyranoside	Leaves	Tang et al., 2013
11.	<i>L. costalis</i>	4,4'-dialyl-5,5'-dimethoxy-[1,1'-biphenyl]-2,2'-diol,		Hosseinzadeh et al.,

		biseuginol A, 2'2-oxybis (4-allyl-1-methoxybenzene) biseuginol B,	Bark	2013
12.	<i>L. fruticosa</i>	Pinostrobin, pinocembrin, chalcone, apigenin, kaempferol, astragalin, isoquercetin, kaempferol 3,4'-di-O-L- rhamnopyranoside, boldine, laurilitsine, actinodaphnine.	Leaves	Liu et al., 2013
		(+)-N-(methoxycarbonyl)-N-nordicentrine, (+)-N- (methoxycarbonyl)-N-norpredicentrine, (+)-N- (methoxycarbonyl)-N-norbulbodione,	Bark	Zhang et al., 2012
13.	<i>L. cubeba</i>	(+)methoxyisolaurenine-N-norglaucine litseacubebic acid, lactone (6R)-3,7-dimethyl-7-hydroxy-2- octen-6-olide	Fruit oil	Yang et al., 2010

IV. PHARMACOLOGICAL STUDIES

- **Antimicrobial activity**

The principal advantage of plant-based antimicrobials is that they possess effective and immense therapeutic potential. Antimicrobial studies of methanolic extracts from *Mucuna pruriens* leaves using agar well diffusion method against various microorganisms, including gram-positive, gram-negative, spore-forming bacteria and fungi revealed that the extract exhibited broad-spectrum antimicrobial activity against all tested microorganisms (Salau & Odeleye, 2007). Organic solvent extraction, especially with methanol, acts as a suitable method to demonstrate antimicrobial activity in plants (Girish & Satish, 2008). Plant extracts and plant chemicals are gaining interest as antiseptics and antimicrobial agents in dermatology in the context of bacteria's increasing resistance to antibiotics (Weckesser, 2007).

Kalaivani et al. (2012) reported that the volatile essential oils of *Ocimum sanctum*, *Ocimum basilicum*, and *Coleus aromaticus* have significant antibacterial capability against human pathogens like *Bacillus*, *E. coli*, *Klebsiella*, *Shigella*, *Proteus*, *Streptococcus*, and *Staphylococcus* species. Shan et al. (2007) studied the antibacterial effects of 46 plant extracts using the agar well diffusion method against five food poisoning bacteria. The study suggested that the high phenolic content in plants is the main factor behind the antibacterial effect. The antibacterial activity is checked by utilizing the extracts of selected medicinal plants and found a synergetic relationship between plant extracts and antibiotic-resistant bacteria (Nascimento et al., 2000).

The ethanolic extracts of *L. glutinosa* leaf and bark confirmed the antibacterial potential of the plant against the selected bacterial strains and increased inhibitory effect of leaves extract towards *Klebsiella pneumoniae* (Pradeepa et al., 2011). The inhibition against *Propionibacterium acnes* was done using the 2, 3, 5 triphenyl tetrazolium chloride method, and found that leaf and bark extracts of *L. graciae* possess antioxidant and antibacterial properties due to a high content of phenol and flavonoids (Wulandari et al., 2018). The antibacterial study of *L. cubeba* fruit essential oil revealed that the oil can inhibit the bacteria, *E. coli* by 100% at

0.125% concentration (Li et al., 2014). The antibacterial efficacy of *L. petiolata* leaves was examined against the bacterial genera such as *Bacillus*, *Staphylococcus*, *E.coli*, *Salmonella*, *Vibrio* etc. and showed significant effect towards *E. coli* and *staphylococcus* (Thongthip et al., 2017). The antifungal effect of *L. cubeba* essential oil against *Botrytis cinerea* was studied and found effective. The Scanning electron microscopic analysis showed the damage of the hyphal wall caused by *L.cubeba* essential oil (Yu et al., 2024).

● Antioxidant activity

The *in vitro* antioxidant property of *L. martabanica* root extract was tested using the DPPH method, the ferric reducing antioxidant power (FRAP) assay, the 2,2'azino-bis (3 ethylbenzothiazoline-6-sufonic acid) assay, the superoxide radical scavenging assay and ABTS methods. All fractions showed higher antioxidant activity (Kunnaja et al., 2021). The *in vitro* antioxidant investigation of *L. glutinosa* leaf extract revealed a concentration-dependent antioxidant activity. The DPPH assay confirmed that the bark extract has potential antioxidant properties (Shafiq et al., 2022). The alkaloid fractions, isolated from ethanolic extracts *L. cubeba* (Lour.) Pers. subjected to DPPH and ABTS assays and revealed the antioxidant potential with IC₅₀ values, 23.8±0.01µg/mL and 8.62±µg/mL, respectively (Dalimunthe et al., 2018). The individual efficacy of substances would be determined by isolating the bioactive components from extracts (Hwang et al., 2005). The antioxidant activity of *L. petiolata* HK.f. is determined using DPPH and FRAP assay and found better effect with IC₅₀ values 27.36 µg/mL for DPPH assay against control quercetin 3.96 µg/mL (Ambarwati et al., 2021). The antioxidant activity due to the occurrence of phenolic compounds identified from *L. glaucescens* was evaluated by various antioxidant assays (Cesar et al., 2022). The DPPH assay of *L. unguolata* leaves, bark and branches revealed the antioxidant potential of extracts (Kuspradini et al., 2019). The *in vitro* antioxidant studies in *L. martabanica* root extract showed the efficacy of extracts to scavenge free radicals formed in liver cells (Kunnaja, 2021).

The chemical constituents of *L. cubeba* essential oil were analyzed by GC/MS, and the main compounds reported were limonene (26.5%), citral (25.97%), and

pinene (6.20%). Citral is the key component of antioxidant activity and scavenging of free radicals (Wang et al., 2012). The root and stem extracts of *L. elliptica* and *L. resinosa* possess good-quality antioxidant activity with free radical scavenging potentials of EC50 values of 23.99 mg/L and 41.69mg/L for *L. elliptica* and 11.22 mg/L and 35.48 mg/L for *L. resinosa*, respectively (Wong et al., 2014). The antioxidant and antimicrobial efficacy of essential oil content in *L. cubeba* fruits from different seasons were studied and found to vary. Antibacterial activity was found to be higher in July against *S. aureus*, *E. coli* and *S. typhimurium* (She et al., 2020). The interrelationship between polyphenols and flavonoids was discovered in *L. coreana* (Xu et al., 2020). GC/MS analysis results of the methanolic extracts of *L. deccanensis* revealed the presence of 11 compounds, mainly quassin, squalene, stigmasterol, vitamin E, and oleic acid, which contribute to its antioxidant property (Kumar et al., 2011). *L. coreana* is rich in various bioactive compounds, including polysaccharides, polyphenols, essential oils and numerous flavonoids. These compounds are associated with significant health benefits such as hepatoprotection, hyperglycemia, reduced inflammation, antioxidation and antibacterial properties (Jia et al., 2017).

● Antidiabetic activity

Diabetes is treated with insulin or other medications like sulfonylureas, biguanides, glinides, *etc.* Most of them have detrimental side effects. In search of more effective drugs with fewer or no side effects, drugs have been stuck on medical preparations derived from plants. Studies have attempted to prove the antidiabetic effect of certain plants. *In vitro* studies have gained more attention in this field. The methanolic leaf extracts of *L. lancifolia* were subjected to antidiabetic studies with 3T3L1 cell lines. The treated 60.22% and 86.26% of cells exhibited antidiabetic activity with effective glucose uptake, and the same was proved using a GLUT4 expression study. Alpha amylase and alpha glucosidase enzymes showed an inhibition by the leaf extracts (Alsawalha et al., 2019). The antidiabetic effect of total flavonoids in *L. coreana* leaf extracts experimented on diabetic rats resulted in a remarkable insulin-sensitive effect in them and caused a reduction in

hyperglycemia, hyperlipidemia, and inflammation (Lu et al., 2010). The antidiabetic potential of *L. cubeba* (Lour.), which has already been used as a traditional medicine to treat diabetes, was determined and found that methanolic leaf extracts were analyzed for the inhibition of α - amylase and α - glucosidase enzymes and realized that the *L. cubeba* leaf extracts performed significant antidiabetic activity. The researcher affirmed that the presence of polyphenolic compounds can contribute to the antidiabetic activity of the plant (Chakraborty et al., 2022).

- **Cytotoxicity**

The cytotoxicity studies of *L. monopetala* using brine shrimp lethality bioassay showed minimum level of cytotoxicity together with antioxidant, anthelmintic and thrombolytic properties and concluded that this may be due to the active presence of major phytochemicals like glycosides, saponins, flavonoids, and alkaloids which make the plant medicinally important (Mohammad et al., 2021). The methanolic leaf extract of *L. elliptica* showed significant cytotoxicity against A549 lung cancer cells. The leaves created induced apoptotic deaths in treated cells with signs of apoptotic bodies, nuclear compactions and membrane blebbings (Goh et al., 2022). The isoquinol alkaloids isolated from *L. cubeba* were evaluated for cytotoxicity in six experimental tumor cell lines and revealed significant cytotoxicity potential of plants (Zhang et al., 2012). The cytotoxicity activity of *L. cubeba* heartwood alkaloid fractions was studied to find out cell cycle arrest activity and inhibition of metastasis in cervical cancer cells. The results indicated that the alkaloid fractions effectively inhibited the spread of cancer (Dalimunthe et al., 2018). The studies conducted by Ho et al. (2010) concluded that *L. cubeba* fruit oil exhibits cytotoxicity and leaf oil has no cytotoxicity effect. Lactones from the pericarp of *L. japonica* showed cytotoxicity against HeLa, HL-60 and MCF-7 cells. Compounds with 2-alkylidene-3-hydroxy-4 methylbutanolids exhibited anticancer activity (Ngo et al., 2019). In a study, the cytotoxicity of fourteen extracts from various parts of eleven medicinal plants from Bangladesh, which have been historically used to treat various types of cancer, tumors, leprosy, and disorders related to cancers, were assessed. The plants with the highest levels of cytotoxicity were those that have been traditionally employed in various cancer and microbial therapies (Khatun et al., 2014). The cytotoxicity and anti-inflammatory effects of *L.*

graciae bark and fruit extracts were evaluated and found promising results. Cytotoxicity was assessed using the MTT assay, and anti-inflammatory experiments were conducted using an enzyme inhibition assay (Kutoi et al., 2012).

- **Anti-inflammatory activity**

Inflammation is a natural response to any infection by pathogenic invasion or a foreign body within the cell of an organism. A series of pro-inflammatory mediators and activators are involved in the inflammatory response. It is a complex and coordinated response of the immune system to tissue injury, infection or other harmful stimuli. It involves a series of events aimed at containing and eliminating the initial trigger, promoting tissue repair and restoring homeostasis. Inflammation is orchestrated by a variety of inflammatory mediators, which are signaling molecules that help to regulate the various stages of inflammatory response. Cytokines are signaling proteins that regulate the immune cell communication and function. Cytokines like interleukins (IL), tumor necrosis factor (TNF), and interferons (IFNs) are involved in immune cell recruitment promotion, activation and proliferation. Chemokines and lipid mediators also engage with cytokines in inflammatory processes.

Enzyme inhibition assay is one of the major tools to identify the anti-inflammatory activity of plant derived extracts. Enzymes such as Cyclooxygenase-2 (COX-2), Lipoxygenase (LOX), Myeloperoxidase (MPO) and inducible Nitric oxide synthase (iNOS) are commonly utilized in various studies. The inhibition of these enzymes is considered as an indication of anti-inflammatory efficiency of a particular bio extract. The dual activity of COX/LOX enzymes was studied in 16 medicinal plant extracts from Kerala and found that the plant extracts containing saponins showed better inhibition of combined action of COX and LOX enzymes (Jacob,2015). The anti-inflammatory activity studies in seven selected medicinal plants were confirmed by the inhibitory activity of COX and LOX enzymes (Lebeloane et al., 2024).

DAMPs (Damage-associated molecular patterns) are produced in response to cellular stress or damage. Metabolites, including nucleic acids, glycans, and proteins, can act as DAMPs, and normally, these molecules cause no harm to cells. Under physical or chemical stimulus, these molecules can trigger an immune

response and induce inflammatory diseases (Ma et al., 2024). It is also found that DAMPs can associate with pattern recognition receptors (PRRs) and activate the innate immune system and lead to chronic illnesses like cancer, Parkinson's, Alzheimer's, etc. Hence, DAMPs can be utilized as biomarkers and molecular targets in inflammatory diseases (Roh & Sohn, 2018). DAMPs-associated inflammation is called sterile inflammation as it is a pathogen-free type and progresses with aging. DAMPs can activate major immune effectors such as TLRs (Toll-like receptors), NLRs (NOD-like receptors) and pyrin domain-containing inflammasomes and play a major role in inflammation (Feldman et al., 2015).

The anti-inflammatory activity in cells is significant as it helps maintain immune balance and prevent excess tissue damage. Chronic inflammation, if left unchecked, can lead to a range of diseases, including autoimmune disorders, cardiovascular diseases, neurodegenerative conditions, and cancer. Anti-inflammatory drugs play a crucial role in managing and alleviating inflammatory responses within cells and tissues. These drugs are designed to target and modulate the inflammatory process by inhibiting specific molecules or pathways involved in inflammation. But synthetic drugs have some ill effects. Nowadays, plant-derived anti-inflammatory drugs are widely recommended to reduce side effects and increased effectiveness.

The *in vitro* anti-inflammatory study of *L. cubeba* fruit and leaf essential oils using egg albumin denaturation assay demonstrated that the oil can effectively denature proteins. This research also confirmed the antioxidant potential of the plant essential oil (Gogoi et al., 2018). The superficial fluid extraction of *L. japonica* leaves showed anti-inflammatory properties by inhibiting the inflammatory cytokine production in Lipopolysaccharide-stimulated RAW 264.7 cells (Song et al., 2016). The geranial and neral extracted from *L. cubeba* fruits were found to possess potential anti-inflammatory compounds, which can inhibit inflammatory mediators. When compared to geraniol, neral showed superior effectiveness in preventing the generation of pro-IL-1 β , iNOS, COX-2 and NLRP-3, which are inflammatory mediators (Liao et al., 2015). It is reported that the compound 5,7,3'-tetrahydroxy-isoflavone isolated from *L. guatemalensis* Mez. possess anti-inflammatory properties (da Silva et al., 2012). The anti-inflammatory effects of compounds isolated from *L. japonica* fruits were studied, and it was noted that litsenolide (LN)

B2 caused the release of pro-inflammatory mediators like nitric oxide, Prostaglandin-2, and LNC2. It was also found that the mechanism for LNB2 inhibition is based on the level of NF-KB and MAPKs phosphorylation (Ham et al., 2015). Methanolic extracts of *L. quinqueflora* (Dennst.) Suresh were proved to hold anti-inflammatory capability using protein denaturation assay. The antioxidant activity of the extract against free radicals also proved significant, with IC_{50} value of 97.8 $\mu\text{g/mL}$ (Jose & Anilkumar, 2021). The methanolic fractions of *L. cubeba* leaves inhibit NO and PEG2 production in lipopolysaccharide induced RAW264.7 cells and reduce the synthesis of myeloperoxidase enzyme (Choi & Hwang, 2004). Wang et al. (2016) reviewed the pharmacological uses of *Litsea* plants including anti-inflammatory activity. The inhibitory effect on pro-inflammatory cytokines using *L. japonica* leaf extract was evaluated and found to be effective in reducing inflammation in the periodontal ligament during periodontal disease. The effect of LJLE was proved to be dose-dependent in LPS-induced cells (Yun et al., 2018).

- **Gene expression studies**

The anti-inflammatory and gene expression studies of *Terminalia chebula* extracts against interleukins showed that the extract effectively inhibited the production of NO and the expression of COX-2, IL-6, IL-1 β and TNF- α . By 100 $\mu\text{g/mL}$ of extract, COX-2 and TNF- α were downregulated by 12 and 17 fold, respectively ($p < 0.005$) and dramatically decreased the levels of IL-6 to $45.67 \pm 3.31 \text{ pg/ml}$ and IL-1 β to $21.23 \pm 0.21 \text{ pg/ml}$ at 100 $\mu\text{g/mL}$ concentration (Farhan et al., 2020). Tunon et al. (2009) conducted a review study and cited several pieces of evidence in support of the fact that flavonoids are potential anti-inflammatory agents and can be involved in cascading the inhibition of the pro-inflammatory mediators. The gene expression analysis of *L. japonica* leaf extract explored the down regulation of iNOS and COX-2 genes and inhibition of Nitric oxide and Prostaglandin-2 (Yoon et al., 2010).

The antioxidant efficacy along with anti-inflammatory potential of *L. salicifolia* was evidenced by gene expression studies in which the extract effectively inhibited pro-inflammatory cytokines and associated genes and protein expression levels. Major genes include TNF- α , IL-6, IL-1 β , Prostaglandin-2 and proteins such as NF-KB, iNOS, TLR-4 and COX-2 were selected for the experiment and obtained

significant results (Puppala et al., 2023). The analgesic and anti-inflammatory activity of *L. japonica* fruits was confirmed with the support of gene expression studies. The study revealed that the fruit extracts can inhibit the NF-KB and accelerate the JNK/P₃₈ MAPK protein signaling pathway (Koo et al., 2014). Hence these pro-inflammatory cytokines proteins and enzymes are commonly utilized as major targets in anti-inflammatory experiments (Fig 1).

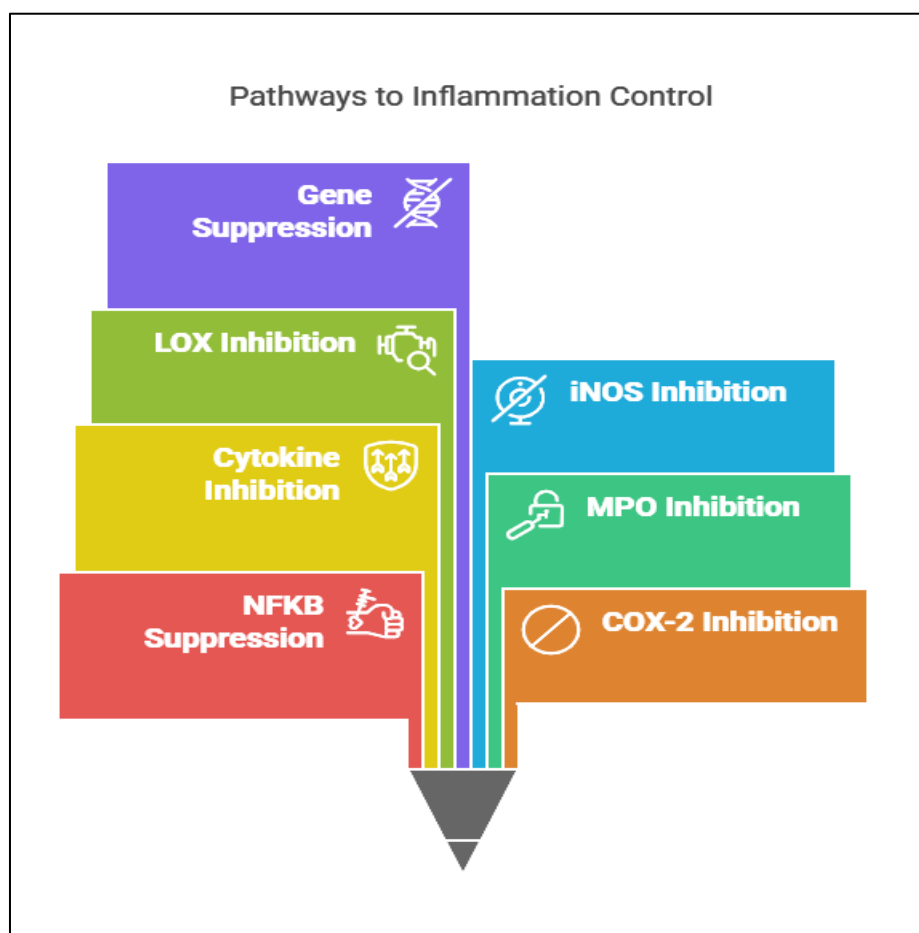


Fig 1 Targets of anti-inflammatory agents in control of inflammation

- **Wound healing activity**

Wound healing is a multifaceted process that encompasses distinct but interconnected phases include inflammatory response, proliferation and remodeling leading to tissue integrity (Fig 2). The inflammatory process triggers a proliferative wound repair response characterized by vascular events such as blood coagulation and hemostasis. Leukocyte infiltration with cytokine production is the main cellular

activity during this period. During the proliferative phase, epithelization covers the wound surface, followed by the development of granulation tissue to fill the wound cavity. Granulation tissue formation involves the proliferation of fibroblasts, deposition of collagen and the formation of new blood vessels (Li et al., 2007; Sharma et al., 2021).

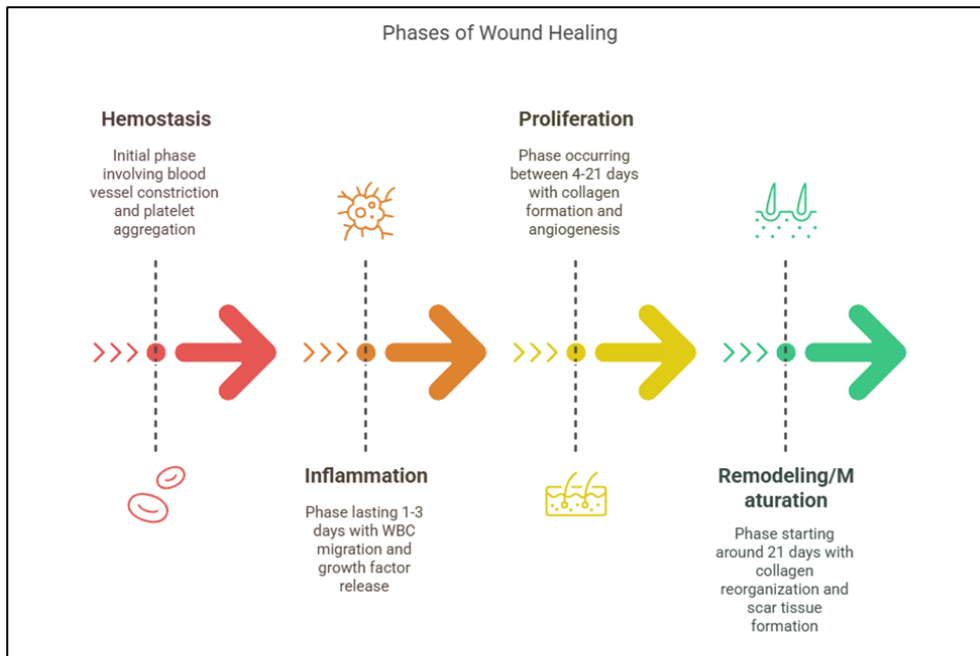


Fig 2 Wound healing mechanism

Wound healing is a complex biological process that aims to restore the structural and functional integrity of damaged tissues. While the human body possesses remarkable regenerative capabilities, certain wounds, such as chronic or severe injuries, may pose significant challenges to the natural healing process. In recent years, researchers have been exploring alternative therapeutic approaches to expedite wound healing. Among these alternatives, plant extracts have emerged as promising candidates due to their rich reservoir of bioactive compounds with potential wound-healing properties. Plant extracts contain a plethora of biologically active compounds, including polyphenols, flavonoids, terpenoids, alkaloids, and essential oils, which possess pharmacological properties like wound healing, anti-inflammatory, *etc.* Numerous pharmacological studies have been conducted on plants, utilizing diverse wound healing models and investigating their underlying

molecular mechanisms. These studies aimed to validate the traditional use of these plants and develop safe, effective and globally accepted herbal drugs for wound treatment (Thakur et al., 2011).

The wound-healing efficacy of *L. glutinosa* leaves was studied by excision and incision wound models. The extract was applied as ointment and found to be an active wound-healer in both the wound models (Devi & Meera, 2010). In excision-wound healing studies with *Siegesbeckia pubescens*, the methanol extracts at 0.5 to 100g/mL significantly stimulated the proliferation of mouse fibroblast NIH3T3. Animals given 4 and 5% (w/w) SP showed a substantial increase in the rate of wound contraction, duration of epithelialization and hydroxyproline content (Wang et al., 2011).

- **Docking studies**

In drug discovery programs, molecular docking serves as a valuable tool for predicting interactions between small molecules and drug targets, thereby informing critical decisions in the synthesis of drugs. Utilizing a molecular docking approach in natural product-based drug discovery programs offers a means to elucidate traditional applications and reveal novel medicinal uses for plant-based remedies. Docking studies rely on software applications with algorithms, scoring functions, and several novel features. Proteins or enzymes are used as drug targets, and the interaction of these drug targets with phytochemicals provides the knowledge on the drug to be synthesized. The structure prediction and visualization of proteins can be performed by employing protein databases like PDB, SWISS PROT *etc.* (Asiamah et al., 2023).

Anti-inflammatory and *in silico* docking studies in *Ficus religiosa* revealed that among the compounds, campesterol, COX inhibitor showed the highest affinity with firm binding to TYR385 and SER530 of the receptor. The same was visualized by Discovery Studio software (Utami et al., 2020). A compound, namely hesperetin, flavonoid compound isolated from the *Cordia sebestena* flower, with anticancer activity, was subjected to *in silico* molecular docking, and it exhibited better affinity with the E6 protein of HPV16 cervical carcinoma (Prakash et al., 2020). Wound

healing and *in silico* docking studies in *Convolvulus arvensis* L. revealed that the binding manner of selected compounds against targets like glycogen synthase kinase, transforming growth factor-beta, c-myc, and beta catenin were studied and validated the wound-healing potential using molecular simulation studies (Saleem et al., 2022).

The docking studies of sesquiterpenes isolated from *Curcuma zedoaria* rhizomes against Human serum albumin indicated good binding efficiency with binding energies -5.72 and -5.74 kcal /mol. Hydrophobic interactions and hydrogen bonding are well exhibited (Hamdi et al., 2015). Molecular docking studies of Thai mango kernel extracts confirmed the ability of extracts to bind strongly in the active site of enzyme phospholipase A2 and can play a major role in anti-hemorrhagic and anti-dermonecrotic effects (Leanpolchareanchai., 2009). The antioxidant activity of phenolic compounds from *Thymus migricus* was validated by docking studies and found that the extract can effectively bind with enzymes such as acetylcholine esterase, glutathione transferase, and α -glucosidase (Aras et al., 2021). The inhibition of isoforms of cyclooxygenase-2(COX-2) enzymes was examined using docking studies and obtained positive results supporting the inhibitory effect (Oniga et al., 2017).

- **Essential oil analysis**

Essential oils are complex, aromatic compounds produced in specialized glandular trichomes and ducts in plants. These oils are typically found in the leaves, stems, flowers, bark, fruits and seeds of plants. These oils are used in aromatherapy, natural medicine, skincare, and other applications. These oils possess therapeutic properties, like antimicrobial, anti-inflammatory, analgesic, and antioxidant activities. Many essential oils possess insecticidal properties, making them effective natural alternatives for repelling and controlling various insects.

The analysis of essential oil from *L. glutinosa* leaves and fruits using the GC/MS method identified 83 and 33 compounds, respectively. The major compounds present in leaves are phytol, caryophyllene, thujone, and β myrcene, and fruits contain lauric acid (Chowdhury et al., 2008). Hussain (2009) isolated the

essential oils of some Lamiaceae plants, subjected them to GC/MS analysis and found the presence of monoterpene hydrocarbons and oxygenated monoterpenes. Biological activities, like antioxidant, antimicrobial, and cytotoxicity potentials of plants essential oils are widely studied.

- **Insecticidal activity**

The *L. salicifolia* Roxb ex Walls fruits essential oil containing compounds with insecticidal properties against *S. zeamis* and *T. castaneum* were identified. The major compounds identified are (E)-citral and (Z)-citral. The plant oil showed repellent effects on both insect species at the lowest application rate (0.6µg/cm²). Furthermore, it displayed fumigant toxicity towards *S. zeamis*, contact toxicity towards both the species, and high antifeedant toxicity towards *T. castaneum* (Ko et al., 2010). The essential oil extracted from rosemary and basil plants of Lamiaceae contain active compounds which can control stored pests like *S. granarius* L and *T. castaneum* (Teke & Mutlu, 2021). The insecticidal activity of *L. cubebea* (Lour.) essential oil is evaluated against the pests *S. zeamis* and *T. castaneum*. The fumigant and contact toxicity assays proved that *S. zeamis* were more sensitive to essential oil than *T. castaneum* (Ko et al., 2010). For the management of pests in stored foods, essential oils should be considered as a potential substitute for chemical insecticides (Isman, 2000). Haouas et al. (2012) studied the chemical composition and insecticidal activity of three *Chrysanthemum* species, and the three species showed activity with variations in accordance with their chemical composition.

Crude extracts and essential oils from plant resources have repellent effects on insects, including stored grain pests. The essential oil isolated from *L. cubeba* has profound repellency against cigarette beetles (*Lasioderma serricone*) and *Liposcelis bostrychophila*. Essential oil also showed fumigant toxicity. Numerous compounds were identified by GC/MS analysis of essential oil in which citral and linalool were found to be more responsible for fumigant toxicity against two tested insects (Yang et al., 2014). Repellent activity of essential oils of 41 plants against mosquito species such as *Anophilous*, *Aedes* and *Culex* have been reported. Out of which, five plants' essential oil including *L. cubeba*, *Leucadendron*, *Viola odorata*, *Malaeleuca*

quinquenervia and *Nepeta cataria* caused 100% repellency of mosquitos (Amer & Mehlhorn, 2006). Elgizawy et al. (2019) found that the chemical ingredients derived from essential oil of *L. cubeba* fruits like citral and D-limonene hold potential insect repellent ability against *S. oryzae* and *T. castaneum*. The contact toxicity represented by LD 50 values, 253.6 µg/L and 3803 µg /L, respectively. In terms of fumigation toxicity, the respective IC₅₀ values were 204.34, 296.64 and 592.08 µg/L. Cosimi et al. (2009) reported the repellency of essential oils from some Mediterranean plants against *S. zeamis*, *Cryptolestes ferrugineus* and *Tenebrio molitor*. The essential oil components, like monoterpenes, limonene, linalool, and linalyl acetate, are responsible for repellency. It is described that the plants grown in Columbia, like *Cananga odorata* and *Lipia alba*, are the sources of repellents and found more effective against the stored pest *T. castaneum* than commercial insecticides (Caballero-Gallardo et al., 2011).

The insecticidal and fumigant activity of the compound calamusenone isolated from the rhizome of *Acorus calamus* against *S. zeamis* and *Rhizopertha dominica* was proved with LC₅₀ values of 125.71µL/L and 93.64µL/L, respectively (Huang et al., 2011). Garlic emulsion (sirinol) was found to be a good biopesticide in the management of cigarette beetles and red flour beetles (Jahromi et al., 2012). Kim et al. (2003) tested the insecticidal properties of five essential plant oils against *L. serricornis* (F.) adults using fumigant and contact toxicity studies and obtained encouraging results. Fumigant, contact and repellent efficacy of *Evodia rutaecarpa* Hook.f.et Thomas against *S. zeamis* and *T. castaneum* was analyzed and concluded that *T. castaneum* and *S. zeamis* adults and *T. castaneum* larvae are sensitive to the contact of essential oil of the selected plant and *T. castaneum* larvae found tolerant than adults (Liu & Ho, 1999).

The chemical composition and toxicity effects of essential oils from *Murraya exotica* on *T. castaneum* and *S. zeamis* were tested. The major compounds identified were spathulenol, caryophyllene oxide, α-pinene, and α-caryophyllene. The authors assumed that the plant showed contact toxicity against the tested insects may be due to these phytocompounds (Li et al., 2010). The insecticidal potential of

Trachyspermum ammi, *Anethum graveolens* and *Nigella sativa* essential oils against *T. castaneum* was studied using the fumigant toxicity method and obtained positive results (Chaubey, 2007). Fumigant and contact toxicities of some monoterpenes were evaluated by conducting experiments on *S. oryzae* and *T. castaneum*. The inhibitory activity of monoterpenes on acetylcholinesterase activity of *S. oryzae* showed maximum as compared to *T. castaneum* (Abdelgaleil et al., 2009). The essential oils isolated from *Alpinia conchigera*, *Zingiber zerumbet*, *Curcuma zedoaria* and the major phytochemicals of the oil were tested in the adults of *S. zeamis*, *T. castaneum*, *Anisopteromalus calandrae*, and *Trichogramma deion* larvae. Differential effects were exhibited by various insects to different oil compounds (Suthisut et al., 2011). *L. cubeba* fruit essential oil exhibited insecticidal properties, like fumigant, contact toxicities and repellency against *S. zeamis* and can be suggested as a natural insecticide (Zhang et al., 2017).

CHAPTER III

MATERIALS AND METHODS

The present study aimed to conduct a phytochemical profiling and pharmacological analysis of three species of the genus *Litsea*, belonging to the Lauraceae, namely *Litsea bourdillonii* Gamble, *Litsea coriacea* (Heyne ex Meisner), and *Litsea wightiana* Nees. (Hook). f, distributed in the evergreen and semi-evergreen forests of the Southern Western Ghats, India.

1. PLANT MATERIALS

a. Collection and authentication

Plant materials for the study were collected from the evergreen forests of Nelliampathy hills of Nemmara Forest division, Palakkad. *L. bourdillonii* Gamble was collected from Kaikati (N10°32.075'E076°40.736'), *L. coriacea* (Heyne ex Meisner) from Nelliampathy viewpoint (N10°32.233'E076°40.019') and *L. wightiana* Nees. (Hook).f. from Minnampara (N10°31.883'E076°43.132'). The collection was on the ground of permission (KFDHO-29980/2018-CWW/WL/10) granted by Kerala Forest and Wildlife Department, Government of Kerala, Thiruvananthapuram. Vegetative and floral parts of the three species were collected in different seasons during 2019-2021. The three species viz., *L. bourdillonii* Gamble., *L. coriacea* (Heyne ex Meisner) and *L. wightiana* Nees. (Hook). f. were authenticated by Dr. Robi. A.J, Assistant Professor, BAM College, Pathanamthitta, Kerala, India. The nomenclature validation was done with IPNI (www.ipni.org) and the plant list (www.theplantlist.org). The Herbaria of plant specimens were prepared by standard herbarium techniques (Jain & Rao, 1977) and kept at Calicut University Herbarium (CALI) with accession numbers, *L. bourdillonii* Gamble (CU7091, CU7072), *L. coriacea* (Hyene ex Meisner) Hook.f. (CU7093, CU7094) and *L. wightiana* (Nees). Hook.f. (CU7095, CU7096). The details of the plants are given below.

1. Botanical name: *Litsea bourdillonii* Gamble.

Family: Lauraceae

Common name: Bourdillon's *Litsea*

Habitat: Evergreen Forests between 750-1800m

Habit: Tree

Distribution: Endemic to the Southern Western Ghats

Flowering and fruiting: August-March

Conservation status: Nearly Threatened (NT) category of IUCN (de Kok & Geethakumari, 2020).

Plant descriptions:

The tree is dioecious, reaching up to 20 m in height, with brownish black tomentose branchlets. Leaves are simple alternate and exstipulate with stout, dark-brown tomentose petioles. Flowers are unisexual and grouped in axillary or lateral clusters of 2-5, with 4-6 male flowers featuring 12 stamens arranged in 4 rows. Filaments are glabrous, with the first two rows typically eglandular, while the latter have two glands. Anthers are 4-celled and introrse. Female flowers have staminodes resembling male stamens, but outer row staminodes are clavate or linear and inner rows are subulate and 2-glandular. The ovary is half inferior and ovoid with a thick style and dilated stigma. The fruit is a berry 10-12mm long and oblong, situated on a cup-shaped perianth tube.

2. Botanical name: *Litsea coriacea* (Heyne ex Meisner).

Family: Lauraceae

Common names: Leather-leaved *Litsea*, Kanayooram, Maravettithali, Pannithali, Vettithali

Habitat: Semi-evergreen forests between 100-1500m

Habit: Tree

Distribution: Peninsular India

Flowering and fruiting: December- January

Conservation status: Nearly Threatened (NT) category of IUCN (de Kok & Geethakumari, 2020).

Plant descriptions:

These dioecious trees can grow up to 18m tall and feature pale brown, thin, scaly bark. The leaves are simple, alternate and exstipulate with petioles with elliptical or lanceolate lamina with coriaceous texture. The flowers are unisexual, white, and are arranged in crowded 4-flowered axillary, sub-sessile umbels. There are four silky bracts. The perianth tube is funnel shaped finely silky, with six lobes. Each male flower has 12 stamens arranged in four rows, and the first two rows are typically eglandular while inner rows with glands and opposite to the tepals are quite large. In female flowers, the staminodes resemble the male stamens, but the inner rows are subulate and have two glands. The ovary is half inferior and ovoid with a slender style and a small erect stigma. The fruit is a berry measuring 10-12 mm in length, ovoid in shape, greenish white in colour when young and seated in a thickened perianth tube.

3. Botanical name: *L. wightiana* Nees. (Hook). f.

Common names: Wight's *Litsea*, Manjakuddala

Habitat: Evergreen Forests between 1000-2400m

Habit: Tree

Distribution: Endemic to Southern Western Ghats

Flowering and fruiting: October-May

Conservation status: Nearly Threatened (NT) category of IUCN (de Kok & Geethakumari, 2020).

Plant descriptions:

Trees have a height of 10-15m; the bark is brown. The leaves are simple, alternate and exstipulate with rusty tomentose petioles. The lamina is long, obovate, and elliptic. The inflorescence is an umbel bearing a raceme in the axils of leaves. Each umbel contains 5 white flowers. There are 6 tepals measuring 3x1.5mm, oblong, acute, and densely fulvous hairy and white. The male flowers with 12 stamens arranged in 3 whorls of unequal length. Filaments are slender, 5 mm long and villous, with those in the first and second whorls usually eglandular. In female flowers, the number of staminodes is like the male stamens, but those of the outer whorls are clavate or linear, while the inner whorls are subulate and 2 glandular. The fruit is a berry, ellipsoid, greenish, glabrous and glossy, turning reddish when mature. The perianth tube is cup-shaped, greenish to orange-yellow and sparsely pubescent.

3. METHODS

Methodology is divided into three phases

Phase 1. Pharmacognostic characterization, including morphological, anatomical, histochemical, physicochemical, and powder microscopic studies of selected *Litsea* species.

Phase II. Phytochemical Profiling by quantitative and qualitative phytochemical screening, GC/MS and LC/MS analysis of extracts of selected species of *Litsea* and essential oil analysis of *L. coriacea*.

Phase III. Pharmacological study involving the antibacterial, antioxidant, antidiabetic and cytotoxicity studies of leaf and bark extracts of three *Litsea* species and anti-inflammatory and wound healing studies of leaf extracts of *L. wightiana*. Insecticidal activity studies of essential oil from *L. coriacea* leaves.

PHASE I. PHARMACOGNOSTIC CHARACTERIZATION

a. Morphological study

The macromorphological features of the plant parts were studied by considering the parameters, like plant habit, height, and leaf characteristics like shape, length, tip, margin, lamina, petiole and phyllotaxy. The stem features like nature, bark color and floral features like inflorescence, number of flowers, male and female flowers, size, color, fruit features like type, size, color, shape and nature of perianth tube were studied (Mohammad et al., 2020).

b. Anatomical characterization

To study the anatomical features, leaves, stem bark and petioles were collected from the third node of mature branches of selected *Litsea* plants and preserved in FAA (10 mL of 40% formaldehyde, 5 mL of glacial acetic acid, 50 mL of 70% ethyl alcohol and 35mL of distilled water). Sections were taken using a microtome (Plant Microtome, Automatic MT3) for thick materials (Johansen, 1940). The sections were stained with specific reagents, mounted in glycerol, observed through the microscope attached with a camera (Leica DM 1000 LED- Trinocular microscope attached with Leica DFC 295 digital camera connected to the computer and Leica application software (Leica version 3.6.1) and transferred the images to the computer.

Stains and reagents for anatomical studies

1. Safranin solution: Dissolve 1 g safranin in 100 mL of distilled water.
2. Fast green solution: Dissolve 1 g fast green in 100 mL of clove oil.

c. Histochemical study

Stains used:

1. **Ferric chloride solution:** Dissolve 5 g of ferric chloride in 100 mL of distilled water to examine the presence of tannin.

2. **Iodine solution:** Dissolve 2.6 g of Iodine and 3 g of potassium iodide in 100 mL of distilled water to detect the presence of starch.
3. **Phloroglucinol:** Dissolve 1 mg of phloroglucinol in 100 mL of ethanol, and it is used to detect lignin deposition in cells.
4. **Hydrochloric acid (HCl):** To moisten the phloroglucinol
5. **Sudan red:** Dissolve 0.5 g of Sudan red in 100 mL of glacial acetic acid, which is used to indicate oil globules present in the cells.
6. **Glycerol:** Mix equal amounts of glycerol and distilled water, and it is used as a mounting medium for stained sections.

Procedure

To carry out histochemical studies, thin sections were treated with specific stains and coloring reagents. The detection of starch was done by iodine, which stains blue in the presence of starch and tannin with ferric chloride solution, which yields bluish black or greyish black. Lignified cells were located by phloroglucinol and concentrated HCl, and the cells were stained pink and fixed oil by Sudan red, which gives an orange-pink color to the cells (Johansen, 1940). The stained sections were observed through a similar microscope used for anatomical studies.

d. Physicochemical study

The dried powder of the stem bark of *L. bourdillonii*, *L. coriacea* and *L. wightiana* was subjected to physicochemical studies. Different physicochemical parameters, like water soluble extractive, alcohol soluble extractive values, total ash, crude fiber, acid-insoluble ash, and sulphated ash values, were determined (Salwan et al., 2012; Kadam et al., 2013; Anonymous, 1966).

• Determination of water-soluble extractive

The water-soluble extractive value was determined by mixing 2 g of powdered medication with 70 mL of water in a conical flask, boiling for 15 minutes, cooling, and then filtering into a weighed beaker. This process was repeated thrice,

evaporated in a water bath. The residue was dried at 110°C, cooled and weighed. The percentage of water-soluble extractives was calculated with reference to the drug.

$$\text{Water soluble extractive value (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of the plant material}} \times 100$$

- **Determination of alcohol-soluble extractive**

2 g of powdered drug was added to 30 mL of methanol and filtered into a weighed dish. The process was triplicated and then dried in a water bath. After 30 minutes of drying at 110°C, alcohol-soluble extractive values with reference to the drug were determined.

$$\begin{aligned} \text{Alcohol soluble extractive value (\%)} \\ = \frac{\text{Weight of residue from alcohol extraction}}{\text{Weight of the sample}} \times 100 \end{aligned}$$

- **Determination of total ash value**

2g of dried powdered drug was weighed accurately and incinerated in a Gooch crucible at a temperature not exceeding 450°C until free from carbon, then cooled and weighed. Calculated the percentage of ash with reference to the air-dried drug.

$$\text{Total ash value (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

- **Determination of acid-insoluble ash**

The acid-insoluble ash value of the sample drug was determined by drying the sample with 25 mL of weak hydrochloric and the sample was heated for 5 minutes. Insoluble materials were collected and rinsed with hot water in a crucible, then burned, cooled and weighed. The percentage of acid-insoluble ash was calculated with reference to the air-dried drug.

$$\text{Acid insoluble ash} = \frac{\text{Weight of acid insoluble ash}}{\text{Weight of the sample}} \times 100$$

- **Determination of sulphated ash**

The sample was filled in a crucible and wet with 1 mL of sulphuric acid and then heated gradually at a low temperature until it was completely charred. After cooling, it was heated slowly until white vapors stopped escaping, then fired at 550°C to 650°C until the residue was incinerated. The crucible was cooled in a desiccator, weighed, and the residue percentage was computed.

$$\text{Sulphated ash (\%)} = \frac{\text{Weight of sulphated ash}}{\text{Weight of the sample}} \times 100$$

- **Determination of crude fiber**

2-3 g of dried powder sample was subjected to Soxhlet extraction with petroleum ether to remove the fat content. The defatted sample was digested with a solution containing 1.25 percent H₂SO₄ and 1.2 percent NaOH. After digestion, the sample was dried for 2 hours at 130°C before being ignited for 30 minutes at 600°C. The percentage of crude fiber content was calculated using the weight loss on igniting and the weight of the ground sample before defatting.

$$\% \text{ Crude fiber in crude extract} = \frac{\text{Loss in weight on ignition}}{\text{Weight of ground sample}} \times 100$$

e. Powder microscopic study

For examining the cell structure in powder form of stem bark three samples were powdered, sieved, stained with safranin, mounted in glycerin, and observed under a microscope. The images of powder characters were transferred to the computer-controlled microscopic system and camera (Leica DM 1000 LED-Trinocular Leica microscope attached with Leica DFC 295 digital camera connected to the computer and Leica application software Leica version 3.6.1) was used for transferring and viewing images (Anonymous, 1996).

PHASE II. PHYTOCHEMICAL PROFILING

a. Extraction of plant material

The collected leaf and bark samples of the *Litsea* species under study were shade-dried and powdered. 20g of powder was subjected to Soxhlet extraction using methanol as solvent for 72 hours at 60°C. After the extraction, the solvent was evaporated by a rotary evaporator (LabTech EV11 Plus) and the extracts were stored at -20°C in the refrigerator (Tiwari et al., 2011).



Fig 3 Extraction by Soxhlet apparatus

The extractive values of each extract were calculated by the following formula:

$$\% \text{ Extractive value} = \frac{\text{Weight of dry extract}}{\text{Weight of plant sample taken for extraction}} \times 100$$

b. Qualitative phytochemical screening

The leaf and bark extracts of three *Litsea* species were subjected to preliminary Phytochemical screening following the method of Harborne (1998); Trease & Evans, (1983). Various tests were used for the qualitative analysis of phytochemicals.

Test for phenolic compounds

- **Lead acetate test:** To 2 mL of the plant extract, 2 mL of 1% lead acetate solution was added. The white precipitate formed was an indication of phenolic compounds.
- **Ferric chloride test:** The presence of phenolic compounds was confirmed when a few drops of FeCl_3 were added to the solution, and a green, brown precipitate formed.

Test for alkaloids

- **Dragendroff's test:** A small amount of the extract was dissolved in distilled water, and a few drops of Dragendroff's reagent was added to it. The presence of alkaloids was indicated by a persistent orange-red color.
- **Wagner's test:** To confirm the presence of alkaloids, Wagner's test was conducted. A few drops of Wagner's reagent was added to a small volume of the extract dissolved in distilled water, and it was observed that a dense brown precipitate was formed due to the presence of alkaloids.

Test for flavonoids

- **NaOH test:** To examine the presence of flavonoids, 1 mL of the test solution was taken, and 3 mL of dilute NaOH was added. It was observed that the sample turned yellow. Later, when a few drops of dilute HCl was added, the color disappeared. This test indicated the presence of flavonoids.
- **H₂SO₄ test:** To confirm the presence of flavonoids, the H₂SO₄ test was performed. To a 1 mL extract in a test tube, 1 mL of concentrated H₂SO₄

was added. The mixture turned into an intense yellow color due to flavonoids.

Test for tannins

- **Ferric chloride test:** To 2 mL of the sample solution, a few drops of 1% ferric chloride solution was added. A blue-black colored precipitate was formed, which indicated the presence of tannins.
- **Wagner's test for tannins:** 2 mL of the extract was added to 2 mL of Wagner's reagent. A reddish-brown precipitate was observed due to the presence of tannins.

Test for glycosides

To 100 µl of the plant extract, 2 mL of concentrated H₂SO₄ was added. The formation of reddish-brown color confirmed the presence of Glycosides.

- **Keller Killani test:** 2 mL of the methanolic extract was combined with 0.5 mL of a solution containing glacial acetic acid and 2-3 drops of ferric chloride. 1 mL of concentrated sulphuric acid was then added along the sides of the test tube. The presence of cardiac glycosides was shown by the intense blue color that formed at the intersection of two liquids.

Test for steroids

- **Liebermann-Burchard test:** The presence of steroids in the extracts was detected by the Liebermann-Burchard test. To the aqueous solution of the extract, a freshly prepared Liebermann Burchard reagent was added, which formed a green color, indicated the presence of steroids.

Test for terpenoids

- **Salkowski's test:** To 2 mL of the extract, 2 mL of chloroform was added, and the mixture was filtered. A few drops of concentrated H₂SO₄ were added to the filters, which were then shaken and left to stand. The presence

of a steroid ring is indicated by the emergence of a golden-yellow color at the interface.

Test for saponins

- **Foam Test:** 2 mL of the extract was mixed with 2 mL of water and thoroughly shaken. The formation of persistent foam indicated a positive saponin test.

Test for fatty acids

0.5 mL of the extract was added to 5 mL of ether and allowed to evaporate on filter paper. Then the filter paper was dried, and the appearance of transparency on the filter paper was an indication of fatty acids.

Test for quinones

2 mL of the plant extract was mixed with 3 or 4 drops of concentrated HCl. The solution turned yellow, indicating the presence of quinones.

c. Quantitative phytochemical analysis

- **Estimation of phenols**

The total phenol content of leaves and bark methanolic extracts was estimated using the Folin-Ciocalteu method. Different aliquots (20-100 μ l) of the sample were pipetted out and the volume of each tube was made up to 3 mL with distilled water. Folin-Ciocalteu reagent (0.5 mL) was added and mixed with 2 mL of 20% Sodium bicarbonate solution. The tubes were placed in a boiling water bath for exactly one minute. The tubes were cooled, and the absorbance was read at 650 nm in a spectrophotometer against a reagent blank. Gallic acid was used as a standard, which was also taken at the same concentration as the sample solution. The total phenol content was estimated in terms of μ g/mg of extract (Alhakmani et al., 2013).

- **Estimation of flavonoids**

The flavonoid content in the samples was analyzed by Aluminium chloride colorimetric assay with Quercetin as the standard. The reaction mixture consisted of 1 mg/mL of the extract and 4 mL of distilled water taken in a 10 mL volumetric flask. To the flask, 0.3 mL of 5% Sodium nitrite was mixed, and after 5 minutes, 2 mL of 1 M NaOH solution was added and diluted to 10 mL with distilled water. Made a series of samples ranging from 20-100 μ g/mL. A set of reference standard Quercetin(20-100 μ g/mL) was prepared in the same manner as the sample. The absorbance for test and standard solutions was determined against a reagent blank at 510 nm with a UV/Visible spectrophotometer. The total flavonoid content was expressed as μ g/mg of the extract (Jose & Anilkumar, 2018).

- **Estimation of tannins**

The amount of tannin was estimated using the Folin-Ciocalteu method. Colorimetric estimation of tannin is based on the measurement of blue color formed by the reduction of phosphotungstomolybdic acid by tannin-like compounds in an alkaline medium. 1 mL of the extract and standard solution of tannic acid (20-100 μ g/mL) was made up to 7.5 mL by adding distilled water. This solution was added with 0.5 mL of Folin-Ciocalteu reagent and 35% of 1 mL sodium carbonate solution. The volume was made up to 10 mL with distilled water, and the absorbance was measured at 700 nm (CI & Indira., 2016).

d. Liquid chromatography and mass spectrometric analysis

To identify the non-volatile compounds, present in *L. bourdillonii*, *L. coriacea* and *L. wightiana* leaf samples, (LC/MS) Q-TOF (Quadrupole Time of Flight) technique was carried out by a Shimadzu LC/MS Triple Quadrupole system coupled with UHPLC. For the LC/MS analysis of polar high molecular weight compounds, electron ionization (ESI) was the technique of choice, and the spectra were recorded in positive and negative ionization modes. The MS spectra of all three extracts were compared to the spectra of NIST (National Institute of Standards and

Technology) library, which was used for the identification of compounds based on their spectral match (Perumal et al., 2021).

e. Gas chromatography and mass spectrometric analysis

The three species of *Litsea* leaves were subjected to GC/MS analysis to find out the volatile compounds. Analysis was performed by injecting 1 µL of the sample in split less mode through a filter (Nylon 13mm, 0.2µm) into GC/MS (Model-7890 A with 5975C with triple axis detector) and column (DB 5MS 30x0.250 mm diameter x 0.25mm thickness). Helium gas (99.9995%) was used as carrier gas at a flow rate of 1 mL /min. The analysis was performed in the EI (Electron impact) mode with 70 EV of ionization energy. The injector temperature was maintained at 280°C constantly (Rashid et al., 2016).

f. Essential oil extraction and GC/MS coupled with GC-FID analysis

Based on histochemical studies, the deposition of fixed oils is noticed in *L. coriacea* leaves. So, it was decided to isolate the essential oils from *L. coriacea* leaves. The essential oil from *L. coriacea* was extracted by the hydro distillation method by the Clevenger apparatus (Ko et al., 2010). 500 g of previously weighed leaves were dissolved in 800 mL of water. The temperature of the mantle was set up to 90°C since the water is used as a solvent in the apparatus. The extraction was carried out in 12 hours, and the crude oil was separated and collected in the separating tube of the apparatus. The oil was filtered into a beaker, removed the water content by treating with anhydrous sodium sulphate and kept at 4°C in the refrigerator. The oil content in the sample was determined by the formula,

$$\% \text{ of oil content} = \frac{(\text{Volume of oil extracted (mL)})}{(\text{Weight of the sample taken (g)})} \times 100$$

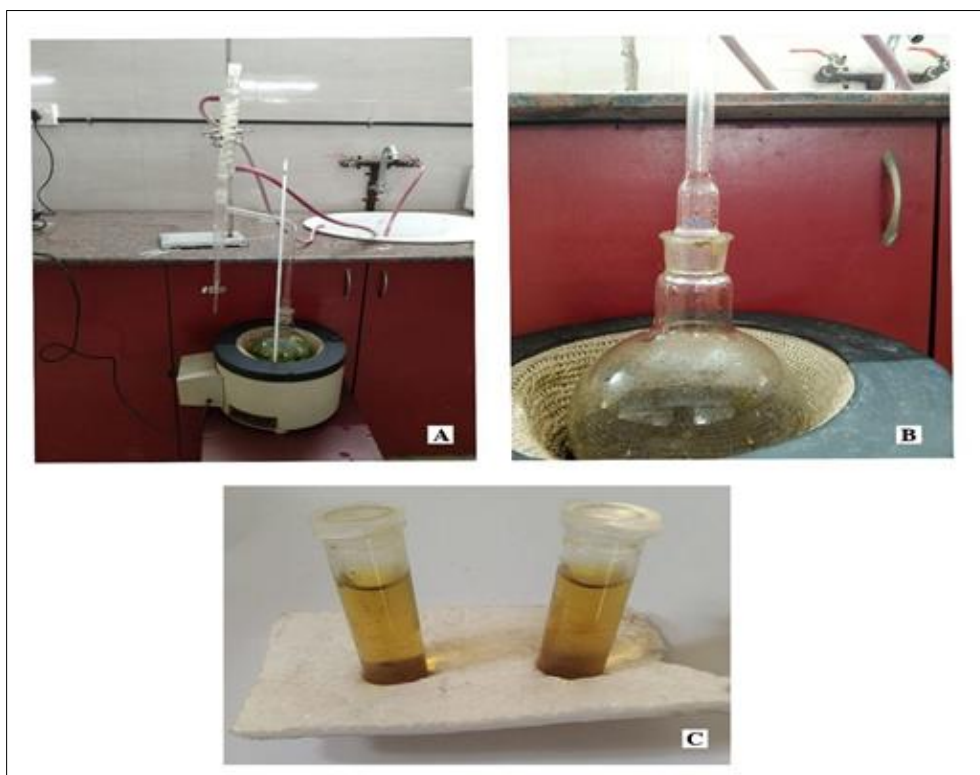


Fig 4 Essential oil extraction by Clevenger Apparatus

To analyze the oil components qualitatively and quantitatively, the essential oil extracted from *L. coriacea* leaves was subjected to GC/MS with GC-FID (Gas chromatography-Flame Ionization detection) analysis. Both analyses were performed using identical column and analysis conditions. 100 μ L sample was diluted with 1 mL of acetone, filtered through a syringe filter (Nylon 13 mm and 0.2 μ m) into vials and injected into GC/MS (Model-7890 Model-A GC with 5975C with triple axis detector and column DB 5MS 30 m x 0.250 mm diameter x 0.25 micrometer thickness). Analysis was performed by injecting 2 μ L of the sample with a split ratio of 15:1. Helium gas (99.9995%) was used as the carrier at a flow rate of 1 mL /min. The analysis was performed in EI (Electron Impact) mode with 70 EV of ionization energy. The injector temperature was maintained constantly at 220°C. The interpretation of mass spectrum by GC/MS and GC-FID was conducted by comparing the spectra in the database of National Institute Standard and Technology (NIST-08 Spectral data). The name, molecular weight, retention time, structure, and

percentage of each component of the test material were recorded (Zheljazkov et al., 2014).

PHASE III. PHARMACOLOGICAL STUDY

A. Antimicrobial activity

a. Antibacterial study by agar well diffusion method

The antibacterial activity of leaf and bark phytoextracts against microorganisms was assessed using the agar well diffusion method. Mueller Hinton Agar Medium (HiMedia-M173) was used for the assay. Petri plates containing 20 mL of Mueller Hinton Agar Medium were seeded with the bacterial culture of *E. coli*, *P. aeruginosa* and *S. aureus*. Growth of cultures was adjusted according to the McFarland Standard (0.5%). Using a well cutter, wells of approximately 10 mm were bored and different concentrations of the sample such as 250, 500 and 1000 µg/mL were added. Streptomycin (100 µg/mL) concentration was taken as the standard. The plates were then incubated at 37°C for 24 hours. Antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993; Parekh & Chanda, 2007).

Table 3 Inoculum used in antibacterial assay

Name of organism	ATTC No	Incubation condition
<i>E. coli</i>	ATCC25922	37 °C
<i>P. aeruginosa</i>	ATCC27853	37 °C
<i>S. aureus</i>	ATCC25923	37 °C

- **Determination of minimum inhibitory concentration (MIC)**

A two-fold serial dilution approach was used to obtain the minimal inhibitory concentration (MIC). The McFarland Standard was used to adjust the growth of the stock inoculum. The broth dilution was performed in 96 mm well plates. The sample was dissolved in DMSO to a final concentration of 10 mg/mL and twofold diluted. Bacterial inocula, like *E. coli*, *P. aeruginosa*, and *S. aureus*

used, were approximately 10^6 - 10^8 CFU/mL. To the medium containing inoculum, methanol extracts with 10 mg/mL were mixed and two-fold diluted (Miller et al., 2005).

b. Antifungal assay by agar well diffusion method

- **Culture media**

Rose Bengal Agar (HiMedia-M842) was used for the determination of susceptible strains to antifungal agents. 31.55 g of agar was suspended in 1000 mL distilled water. It was heated until it boiled to dissolve the medium completely. Then, it was sterilized by autoclaving at 15 psi pressure at 121°C for 15 minutes and cooled to 45-50°C. It was then mixed well and poured into sterile petri plates. Inocula were procured from the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh.

Table 4 Inoculum used in antifungal assay

Fungi	MTCC No	Incubation period
<i>Aspergillus niger</i>	281	27°C for 48 hours
<i>Candida albicans</i>	227	27°C for 48 hours

- **Preparation of fungal inoculum**

To prepare the inoculum the fungal strains *A. niger* and *C. albicans* were first inoculated in Potato dextrose Agar (PDA) medium and incubated at 25-28°C for seven days to allow sporulation. The spores were harvested by adding sterile distilled water, scraped the surface gently and filtered the suspension and removed the hyphal fragments and used for the assay.

- **Procedure**

For the analysis of antifungal activity of the sample extracts, the agar well diffusion method was adopted. 15-20 g of Rose Bengal agar was spread over sterilized glass petri plates of the same size and allowed to solidify. After solidification, the wells (4 wells/plate) were made with a sterile cork borer of

diameter 8 mm (20 mm apart from one another) and were punched aseptically in each plate. The standardized inoculum of the test organisms *viz.*, *A. niger* and *C. albicans* were uniformly spread on the surface of solidified media using a sterile cotton swab. The test volumes of the sample at desired concentrations (40µL & 80µL) were added to the first two wells, third well with 20 mcg of Clotrimazole as a positive control and fourth one filled with DMSO as a negative control. Then the agar plates were incubated at 27°C for 48 hours. After incubation, the zone of inhibition of fungal growth was observed and measured in mm (George & Dhivya, 2019).

B. ANTIOXIDANT ASSAY

i. DPPH radical scavenging assay

To determine antioxidant activity, the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method was used. DPPH reacts with antioxidant compounds which can donate hydrogen and reduce DPPH. The change in color (from deep violet to light yellow) was determined at 515 nm on a UV-visible spectrophotometer. A 60 µM DPPH solution in methanol was prepared, and 3.9 mL of it was combined with 100 µl of the test sample at various concentrations (6.25,12,25,50,100 µg/mL). The tubes were incubated in the dark for 15 minutes at room temperature, and the absorbance was measured at 515 nm. The control was prepared by DPPH only, without any extract. 95% methanol was used as the blank. For the experiment, ascorbic acid was used as the standard (Brand-Williams et al., 1995).

Radical scavenging activity was measured by the following formula

$$\% \text{ of inhibition} = \frac{(\text{Control} - \text{Test})}{\text{Control}} \times 100$$

All the determinants were conducted in triplicate. IC₅₀ values, that is, the concentration of the plant extracts (µg/mL) required for the radical scavenging by 50%, were derived by plotting the DPPH radical scavenging activity against the concentration of the extracts.

ii. Hydroxyl radical scavenging assay

This assay is based on the quantification of the degradation product of 2-deoxy ribose by condensation with tertiary butyl alcohol (TBA) (Kunchandy & Rao,1990). Hydroxyl radical was generated by the Fe^{3+} -ascorbate-EDTA- H_2O_2 system. Gallic acid was used as the reference standard. Different concentrations of sample such as 125-2000 $\mu\text{g/mL}$ from a stock concentration of 10 mg/mL were mixed with 500 μl reaction mixture (2 deoxy 2 ribose 2.8 mM), FeCl_3 (100 μM), EDTA (100 μM), Ascorbic acid (100 μM) in KH_2PO_4 -KOH buffer (20 mM , pH 7.4) was made up to final volume of 1 mL . A control without a test compound but with an equivalent amount of distilled water was taken. After incubation for 1 hour at 37°C, 1 mL of 1% TCA and 1 mL of 1% aqueous TBA were added, and the mixture was incubated at 90°C for 15 minutes to develop the color. After cooling, the absorbance was measured at 532 nm against the blank solution.

Percentage of inhibition was measured by the following formula,

$$\% \text{ of inhibition} = \frac{(\text{Control}-\text{Test})}{\text{Control}} \times 100$$

iii. Superoxide radical scavenging Assay

Superoxide is a biologically important molecule as it can form singlet oxygen and hydroxyl radical. Overproduction of superoxide anion radical contributes to redox imbalance and associated harmful physiological consequences. Superoxide anions are generated in the riboflavin- NADH system by the oxidation of NADH and assayed by the reduction of Nitro blue tetrazolium (NBT) resulting in the formation of blue formazan product. Ascorbic acid was used as standard.

Different concentrations of sample such as 125-2000 $\mu\text{g/mL}$, 0.05 mL of Riboflavin solution (0.12 M), 0.2 mL of EDTA solution (0.1 M) and 0.1 mL NBT (Nitro-blue tetrazolium) solution (1.5 M) were mixed in a test tube and reaction mixture was diluted up to 2.64 mL with phosphate buffer (0.067 M). A control without the test compound but an equivalent amount of distilled water were taken.

The absorbance of solution was measured at 560 nm after illumination for 30 minutes on UV visible spectrophotometer (Valentao et al., 2002).

The percentage of inhibition was calculated as

$$\% \text{ of inhibition} = \frac{(\text{Control} - \text{Test})}{\text{Control}} \times 100$$

iv. Ferrous reducing antioxidant power assay (FRAP)

The reducing power of extract was determined by the method of Yen and Duh (1993). Different concentrations of sample such as 125µg-2000µg/mL from a stock concentration of 10mg/mL were mixed with 2.5 mL of Phosphate buffer (200mM) (pH 6.6) and 2.5 mL of 1% potassium ferricyanide and boiled for 20 minutes at 50°C. A control without a test compound but an equivalent amount of distilled water was taken. After incubation 2.5 mL of 10% TCA were added to the mixture followed by centrifugation at 650g for 10 minutes. The supernatant 5mL was mixed with 5 mL of distilled water and 1 mL of 0.1% Ferric chloride and absorbance read at 700 nm. Quercetin was used as reference standard.

$$\% \text{ of inhibition} = \frac{(\text{Control} - \text{Test})}{\text{Control}} \times 100$$

C. ANTIDIABETIC ASSAY

i. α -amylase inhibitory assay

The antidiabetic potential of leaf and bark extracts of *Litsea* was determined by the method proposed by Keerthana et al. (2013). A total of 500 µL of 0.02M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added with 0.5 mg/mL of α -amylase and varying concentrations of the extract (in µg/mL) as inhibitors were pre-incubated at 37°C for 10 minutes. After pre-incubation 500 µL of 1% starch solution in 0.02 M sodium phosphate buffer (PH 6.9) was added to each tube and incubated at room temperature for 5 minutes. The reaction was stopped using 1.0 mL of dinitro salicylic acid (DNSA) color reagent. The test tubes were incubated in a boiling water bath for 5 min and cooled to room temperature. The volume of the reaction mixture was made up to 10 mL by adding distilled water, and the

absorbance was measured at 540 nm using a UV-visible spectrophotometer. Acarbose was used as the standard.

$$\% \text{ of inhibition} = \frac{(B - A)}{(B - C)} \times 100$$

B- Absorbance of control with starch and alpha amylase

A- Absorbance of the test

C- Absorbance of the control with starch and without alpha amylase

ii. α -Glucosidase inhibition assay

The anti-diabetic effect of the sample on α -glucosidase enzyme activity was determined according to the method described by Shai et al. (2011). 200 μ L of α -glucosidase (0.067 μ g/mL) was preincubated with different concentrations of the sample for 10 minutes. The substrate solution p-nitrophenyl glucopyranoside (pNPG) was prepared in 0.1 M Sodium phosphate buffer (pH 6.9). Then 200 μ L of 3.0 mM pNPG was added to start the reaction. The reaction mixture was incubated at 37°C for 20 minutes and stopped by adding 2 mL of 0.1M Na₂CO₃. The α -glucosidase activity was determined by measuring the yellow-colored para-nitrophenol released from pNPG at 400 nm. The results were expressed as the percentage of inhibition. The same procedure was done with Acarbose (1 mg/mL stock), which was used as the standard.

$$\% \text{ of inhibition} = \frac{(B - A)}{(B - C)} \times 100$$

B- Absorbance of control with starch and alpha glucosidase

A- Absorbance of the test

C- Absorbance of the control with starch and without alpha glucosidase

D. *IN VITRO* CYTOTOXICITY ASSAY

- **Cell line maintenance**

SK-MEL cell line, causing human skin cancer, was procured from the National Centre for Cell Sciences (NCCS), Pune, India. The cells were cultured in Dulbecco's Modified Eagle Medium DMEM (HiMedia) supplemented with 10% heat-inactivated Fetal bovine serum (FBS), 1% antibiotic penicillin (100 µg/mL), streptomycin (100 µg/mL) and amphotericin B (2.5 µg/mL). The cells containing TC flasks (25 cm²) were incubated at 37°C at 5% CO₂ in a cell culture incubator (Galaxy^R 170 Eppendorf, Germany).

- **MTT assay**

The MTT assay was used to measure cellular metabolic activity as an indicator of cell viability, proliferation and cytotoxicity. This colorimetric assay is based on the reduction of yellow tetrazolium salt (3, -(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT) to purple formazan crystals by metabolically active cells. The viable cells contain NAD(P)H-dependent oxidoreductase enzymes which reduce the MTT to formazan (Mosmann et al., 1983). The insoluble formazan crystals were dissolved using a solubilization solution (100% DMSO) and the resulting purple colored solution was quantified by measuring the absorbance at 570 nm using an ELISA plate reader (Luparello et al., 2020).

- **Cell seeding in 96-well plates**

80-90% confluent cells maintained in TC flasks were trypsinized. The trypsinization process is the detaching of adherent cells from the TC flask for subculturing or seeding in 96-well plates for experiments. For this purpose, the monolayer of cells growing in TC flasks was exposed to Trypsin/EDTA solution (0.025% EDTA in phosphate-buffered saline). The trypsinized cells were diluted in the cell culture media at a concentration of 5x10³ cells/well (in 100 µl). The 96-well plates were seeded and incubated for 3-4 days in a cell culture incubator.

- **Sample preparation and treatment**

The test samples, the leaf and bark extracts of *L. bourdillonii*, *L. coriacea* and *L. wightiana*, were prepared in DMEM media (100 mg/mL), and the filter was sterilized using a 0.2 µm millipore syringe filter. The samples were further diluted in DMEM media and seeded to cultured cells at a final concentration of 6.25µg/mL, 12.5µg/mL, 25µg/mL, 50µg/mL, and 100µg/mL, respectively. The untreated wells were kept as the control. All the experiments were done in triplicate, and the IC₅₀ values were calculated.

- **Direct microscopic observation**

After sample addition, the treated as well as control wells were observed at regular intervals up to 24 hours in an inverted phase contrast tissue culture microscope (Labomed TCM-400 with MICAPS™ HD camera). Any detectable changes in the morphology of cells, such as rounding, shrinking of cells, granulation and vacuolation in cytoplasm, were considered as indicators of cytotoxicity.

After sample treatment and incubation for 24 hrs, the media from the wells were aspirated and discarded. 100 µl of 0.5 mg/mL MTT solution in DMEM media was added to the wells. The wells were further incubated for 2-4 hrs for the development of formazan crystals. The supernatant was removed, and 100 µL DMSO (100%) were added per well. The absorbance at 570 nm was measured with a microplate reader. Two wells per plate without cells served as the blank. All experiments were done in triplicate. The cell viability was calculated using the following formula.

$$\% \text{ of viability} = \frac{\text{Average absorbance of treated}}{\text{Average absorbance of test}} \times 100$$

E. ANTI-INFLAMMATORY STUDY

L. wightiana leaves methanolic extract was subjected to *in vitro* anti-inflammatory studies using RAW 264.7 macrophage cell lines. *L. wightiana* leaf extract was used for the study on the ground of significant results obtained in

qualitative and quantitative phytochemical screening, antioxidant and anti-diabetic assays. The anti-inflammatory assays were conducted by testing the inhibition of enzymes which are normally active during inflammation, such as cyclooxygenase -2 (COX-2), lipoxygenase (LOX), myeloperoxidase (MPO), and inducible nitric oxide synthase (iNOS). The total cellular nitrite level was also determined.

i. *In vitro* anti-inflammatory activity

- **Cell culture and extract preparation**

For the anti-inflammatory studies, RAW 264.7 cells were procured from the National Centre for Cell Sciences (NCCS), Pune, India and were maintained in Dulbecco's Modified Eagle Medium, DMEM (Sigma Aldrich, USA). The cell line was grown in a 25 cm² tissue culture flask with DMEM supplemented with 10% FBS (Fetal Bovine Serum), L-glutamine, sodium bicarbonate (Merck company) and antibiotic solution comprising Penicillin (100U/mL) and Amphotericin B (2.5g/mL) in a humidified 5% CO₂ incubator (NBS Eppendorf, Germany). Cultured cell lines were incubated at 37°C. The cells grew up to 60% confluency before being activated with 1 liter of lipopolysaccharide (1g/mL). The LPS-stimulated RAW cells were subjected to various concentrations of the sample solution (25,50,100 µg/mL), and Diclofenac sodium, a standard anti-inflammatory medication, was added in diverse amounts proportional to the sample and incubated for 24 hours. Anti-inflammatory experiments were performed using cell lysate after incubation. Enzyme inhibition assays were adopted for *in vitro* experiments.

- **Cyclooxygenase (COX) activity**

The Cyclooxygenase inhibition assay, one of the common methods to determine anti-inflammatory activity, was carried out by the method of Walker and Gierse (2010). 100 µl cell lysate was incubated with Tris-HCl buffer (pH 8), glutathione 5 mM/L and hemoglobin 5 mM/L and terminated after 20 minutes incubation at 37°C by the addition of 200 µL of 1% trichloroacetic acid and 1N HCl. After the centrifugal separation and the addition of 200 µl of 1% thiobarbiturate, the tubes

were boiled for 20 minutes. After cooling, the tubes were centrifuged for 3 minutes. The COX activity was determined by reading the absorbance at 632 nm.

The percentage of inhibition of the enzyme was calculated as,

$$\% \text{ inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

- **Lipoxygenase (LOX) activity**

The determination of LOX activity was done as per the method proposed by Axelrod et al. (1981). The reaction mixture (2 mL final volume) contained Tris-HCl buffer (pH 7.4), 50 μ L of cell lysate, and sodium linoleate (200 μ L). The LOX activity was monitored as an increase of absorbance at 234 nm (Shimadzu), which reflected the formation of 5-hydroxyeicosatetraenoic acid.

The percentage of inhibition of the enzyme was calculated as

$$\% \text{ inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

- **Myeloperoxidase (MPO) activity**

Cell lysate was homogenized in a solution containing 50 mM Potassium Phosphate buffer and 0.5% Hexadecyltrimethylammonium bromide (HTAB), the samples were centrifuged at 2000 g for 30 minutes at 4°C, and the supernatant was assayed for MPO activity. MPO in the sample was activated by the addition of 50 mM phosphate buffer (pH 6) containing 1.67 mg/mL guaiacol and 0.0005% H₂O. The change in absorbance at 460 nm was measured. MPO activity was presented as units per mL of cell lysate. 1 unit of MPO activity was defined as the amount degrading 1 μ M of peroxide per minute at 25°C (Bensalem et al., 2014).

Myeloperoxidase activity was calculated by using the formula

$$U = \frac{(\Delta OD \times 4 \times V_t)}{L \times \epsilon_{470} \times \Delta t \times V_s}$$

Here, ΔOD = Density change, V_t -Total volume (mL) -0.2mL, V_s -0.01mL, L =Light path (1cm), ϵ_{470} =Extinction coefficient for tetraguaiacol ($26.6\text{mM}^{-1}\text{cm}^{-1}$).

- **Inducible Nitric oxide synthase activity**

The Cell lysate was homogenized in 2 mL of HEPES buffer. The assay system contained substrate 0.1 mL -2 $\mu\text{mol/L}$, L-Arginine, 0.1 mL -4 $\mu\text{mol/L}$ manganese chloride, 0.1-10mmol/L 30 μg dithiothreitol (DTT), 0.1 mL-1 mmol/L NADPH, 0.1mL-4 $\mu\text{mol/L}$ tetrahydropterin, 0.1mL of oxygenated hemoglobin, and 0.1 mL enzyme (sample). An increase in absorbance was recorded at 401 nm (Kim et al., 2004).

The percentage inhibition of the enzyme was calculated as,

$$\% \text{ inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

- **Estimation of cellular nitrite level**

To estimate the cellular nitrite level, 0.5 mL of cell lysate and 0.1 mL of 3% sulphosalicylic acid were added and vortexed well for 30 minutes. The samples were then centrifuged at 5000 rpm for 15 minutes. The protein-free supernatant was used for estimating the nitrite levels. To 200 μL of supernatant, 30 μL of the 10% NaOH was added, followed by 300 μL of Tris-HCl buffer and mixed well. To this, 530 μL of Griess reagent (1% sulfanilamide, 2% phosphoric acid, and 0.1% N- 1 naphthyl ethylenediamine dihydrochloride) was added and incubated in the dark for 10-15 minutes. The absorbance was read at 540 nm against the Griess reagent blank. Sodium nitrite was used as the standard. The amount of nitrite present in the sample was estimated from the standard curves obtained.

ii. Gene expression studies

Based on the activities of enzymes in *in vitro* anti-inflammatory study, the relative gene expression studies of COX2, TNF- α IL-6 and NF κ B in RAW 264.7 cells were conducted (Sanjeeva et al., 2020). RAW 264.7 cell line was initially procured from NCCS, Pune, India and maintained at Dulbecco's Modified Eagle

Medium, DMEM (Sigma Aldrich, USA). The cell line was cultured in a 25 cm² tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate (Merk Germany) and antibiotic solution containing penicillin (100U/mL) and amphotericin B (2.5 µg/mL). Cultured cell lines were kept at 37°C in a humidified 5% CO₂ incubator (NBS, Eppendorf, Germany).

- **RNA Isolation-TRIzol method**

Table 5 Reagents used in TRIzol method

Reagent	Manufacturing Company
TRIzol reagent	Invitrogen; Catalogue No.15596026
Chloroform	VETEC; Catalogue No. MMBB4798V
2 Propanol (Isopropyl alcohol)	VETEC; Catalogue No. MMBB4064
Ethanol	Fischer Scientific
RNA Quantification	Qubit™ RNA high sensitivity (HS) assay kit- Invitrogen
cDNA synthesis	iSCRIPT cDNA synthesis kit-Bio-Rad
SYBR Green	iTaq Universal SYBR Green Supermix-Bio-Rad
Oligo Dt	Eurofins
Agarose	SeaKem®LE Agarose, Lonza, Catalogue no.50004.s

Total RNA was isolated using a total RNA isolation kit according to the manufacturer's instructions (Invitrogen-Product code 10296010). The addition of TRIzol solution caused the disruption of cells and the release of RNA. The extraction was followed by centrifugation. The supernatant was mixed with isopropanol, and the RNA was precipitated as a white pellet on the side and bottom of the tube.

The RAW 264.7 cell line was cultured following the standard procedures described earlier. 45.09246 µg/mL of sample concentration from a stock of 1 mg/mL was added to LPS-stimulated cells and incubated for 24 hours. After the incubation, the DMEM media was removed aseptically, and 1 mL of TRIzol reagent was added to the culture well plate and incubated for 5 minutes. The contents were then transferred to a fresh sterile Eppendorf tube. 200 µL of chloroform was added and

shaken vigorously for 15 seconds and incubated for 2-3 minutes at room temperature, followed by centrifugation at 14000 rpm for 15 minutes at 4°C. The aqueous layer was collected, and 500 µL of 100% isopropanol was added. It was incubated for 10 minutes at room temperature and then centrifuged at 14000 rpm for 15 minutes at 4°C. The supernatant was discarded, and the pellet thus obtained was washed with 200 µL of 75% ethanol (Merk). It was then centrifuged at 14000 rpm for 5 minutes at 4°C in a cooling centrifuge (Remi CM12). The RNA pellet was dried and suspended in the Tris-EDTA (TE) buffer.

- **cDNA synthesis**

Isolated and purified RNA was used for cDNA synthesis. Template complementary DNA was synthesized using cDNA preparation kit (G Biosciences, Product code- 786-5020s, 786-5020 master premix, for first strand cDNA master). About 5 µL of RT easy mix, 0.5 µL of oligodT and 2 µL of template (0.5µg of total RNA) were added to an RNase-free tube. Then, the total reaction volume was made up to 10 µL with the addition of sterile distilled water. The solution was mixed by pipetting gently up and down. The thermal cycler (Eppendorf Master Cycler) was programmed to undergo the cDNA synthesis. The following cycling conditions were employed:

20 minutes at 42°C and 5 minutes at 85°C.

- **Gene expression analysis by qRT-PCR (Yang et al., 2012)**

Table 6 Instruments used in qRT-PCR analysis

Instrument	Model and manufacturer
Real Time PCR	LightCycler 96® ROCHE
mRNA quantification	Invitrogen™ Qubit™ Fluorometer
Centrifuge	REMI CM 12 Plus cooling centrifuge
Electrophoresis apparatus	Bio-Rad, Mini-Sub®Cell GT Complete system
Electrophoresis power supply	Life Technologies PowerEase 300 Electrophoresis Power Supply
Gel imager	Life Technologies E- Gel Imager- The compact gel imaging system

Real Time qRT-PCR analysis was carried out using SYBR Green Master Mix (G Biosciences Product code 786-5062) using Light Cycler 96® (Roche). All

reactions were performed in triplicate, and the data were analyzed according to the $\Delta\Delta C_t$ method by LightCycler 96 SW 1.1 Software.

Table 7 Various steps involved in qRT-PCR analysis

Steps	Time required	Temperature
Initial activation step	2 minutes	95°C
Denaturation	10 minutes	95°C
Annealing	1 minute	58°C
Extension	1 min/kb	72°C
Number of cycles	40 cycles	68°C
End of PCR cycling	indefinite	4°C

Table 8 List of primers used in qRT- PCR study

Name of Oligonucleotide	Forward Sequence (5' - 3')	T _m	Reverse Sequence (3' - 5')	T _m
GAPDH	ACTCAGAAGACTGTGGA TGG(20)	57.3	GTCATCATACTTGG CAGGTT(20)	55.3
COX-2	GGAGAGACTATCAAGA TAGT(20)	53.2	ATGGTCAGTAGACT TTTACA(20)	51.2
IL-6	GGTACATCCTCGACGGC ATCT(21)	61.8	GTGCCTCTTTGCTT TCAC(21)	59.8
NFKB	CCCACACTATGGATTTC CTACTTATGG (21)	63.4	(CCAGCAGCATCTT CACGTCTC (21)	61.8
TNF ALPHA	CCCAGGCAGTCAGATCA TCTTC(22)	62.1	AGCTGCCCCCTCAGC TTGA(18)	58.2

- **Agarose gel electrophoresis**

After the qRT-PCR analysis, the products were subjected to Agarose gel electrophoretic analysis. 1.5% agarose gel was prepared in a 1xTE buffer and melted in a hot water bath at 90°C. Then the melted agarose was cooled down to 45°C. 6 μ L of 10 mg/mL of ethidium bromide was added and poured into the gel comb. After setting up, the comb was removed from the gel. The electrophoresis buffer was poured into the gel tank, and the platform with the gel was placed in it to immerse in the gel. The gel was loaded with the samples and ran at 50V for 30 minutes. The

stained gel was visualized using a gel documentation system (E gel imager, Invitrogen).

iii. Docking study

Considering the results obtained in anti-inflammatory studies, molecular docking analysis was carried out. The binding affinity of twelve phytochemicals against four target proteins was checked. The phytochemicals were selected based on their anti-inflammatory property reported earlier. The SMILES representation of twelve chemicals was obtained from the PubChem database, and the 3D structure in PDB format was obtained by the CORINA tool. For the proteins, the structure was downloaded from the RCSB PDB. The PDB id of enzymes used were as follows- human myeloperoxidase (1dnu), human inducible nitric oxide synthase (4nos), cyclooxygenase II (5ikr), 5-lipoxygenase (6n2w) (Meenambiga et al., 2015; Biswas et al., 2019).

For predicting the binding affinity, knowledge regarding the active site is necessary. So, the active regions were predicted using the Castp server and PDBsum. Molecular docking was performed with AutoDock vina using the PyRx interface. Prior to docking, all the targets were subjected to preparation steps, which included removing water molecules, adding polar hydrogen atoms, and removing the non-amino acid residues present in the protein structure which is not necessary for the normal functioning of the protein. A grid box was set for each protein with respect to the position of active site residues. After docking the compound showing the highest activity against the selected targets, each best compound was subjected to interaction analysis to identify the binding mode with the targets. The tool used was Discovery Studio Visualizer (Peng et al., 2018).

iv. *In vivo* anti-inflammatory study

● Animals

Male Wistar albino rats weighing 180-200g were obtained from the animal house, Sree Lakshmi Narayana Institute of Medical Sciences, Pondicherry. All experimental animals were housed in a polypropylene cage with paddy husks as

bedding. The animals were maintained at a humidity of 30-70% and $26 \pm 1^\circ\text{C}$ temperature in 12 hours of alternating dark/light cycles. They were allowed free access to water and fed with standard commercial pellet rat chow (M/s Hindustan Lever Ltd., Mumbai). The experimental protocols were reviewed by the Institutional Animal Ethics Committee (IAEC) of Sree Lakshmi Narayana Institute of Medical Sciences, Pondicherry, India, and all the procedures were in accordance with IAEC (932/Po/Pe/S/06/CPCSEA).

- **Carrageenan-induced rat paw edema model**

The anti-inflammatory activity of *L. wightiana* methanolic extract of leaf against carrageenan-induced acute paw edema in rats was studied (Winter et al., 1962; Vasudevan et al., 2006). Two dose levels, such as 50 and 100 mg/kg of LWME as oral feed were employed in the study and indomethacin (10 mg/kg), the conventional anti-inflammatory agent, was the reference control. Twenty-four healthy male Wistar albino rats were randomly divided into four groups, each comprising six rats, namely,

Group I: Vehicle control (0.1% CMC)

Group II: Oral feeding of Indomethacin (10 mg/kg)

Group III: Oral feeding of LWME (50 mg/kg)

Group IV: Oral feeding of LWME (100 mg/kg)

After 30 minutes, 0.1 mL of 1%(w/v) carrageenan was injected in the plantar region of the left paw of the control as well as the test compounds-treated groups. The right paw served as a reference to the non-inflamed paw for comparison. Paw thickness of the left leg of the control, indomethacin and LWME-treated rats were noted at every 1 hour using a digital caliper after the administration of carrageenan to the right hind paw. The reading was taken for a total of 5 h. The reduction in paw volume was noted.

The percentage of inhibition of paw thickness at the time interval was calculated as

$$\% inhibition = \frac{(V_c - V_t)}{V_c} \times 100$$

Where, (V_c- Control mean paw thickness, V_t- Test mean paw thickness)

F. WOUND HEALING STUDY

i. *In vitro* wound healing study by scratch wound method

L929 cell lines were procured from the National Centre for Cell Science (NCCS), Pune, India and maintained at Dulbecco's Modified Eagle Medium (Gibco, Invitrogen). The cell line was cultured in a 25 cm² tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and an antibiotic solution containing penicillin (100 µg/mL), streptomycin (100 µg/mL) and amphotericin B (2.5 µg/mL). Cultured cell lines were kept at 37°C in a humidified 5% CO₂ incubator (NBS Eppendorf, Germany). Experimentally growing cells were trypsinized and seeded at a density of 200,000 cells per well into a 12-well plate for 24 h incubation (90% confluence). The scratch wounds were made by a sterile 1 mL pipette tip through a pre-marked line. After removal of the resulting debris from five linear scratches, the cell monolayer was subsequently rinsed three times with phosphate buffered saline (PBS), followed by incubation with a sample of concentration 25 µg/mL for 0 hours, 24 hours, 48 hours, and 72 hours. The wound areas were displayed by taking images just above the interchanges between scratched wound areas and pre-marked lines, and the effect of the sample on wound closure was determined microscopically (4x magnification, Olympus CKX41) after incubation (Mazutti da Silva et al., 2018). The effect of the sample on wound closure was measured in terms of the area using the MRI-Image J analysis software.

ii. *In vivo* wound healing study

● Animals

Male Wistar albino rats weighing 180-200g were obtained from the animal house of Sree Narayana Institute of Medical Sciences, Pondicherry. All experimental animals were housed in polypropylene cages with paddy husks as bedding. The animals were maintained at a relative humidity of 30-70% and $26\pm 1^{\circ}\text{C}$ temperature for 12 hours of alternating dark/light cycles. They were allowed free access to water and fed with standard commercial pellet rat chow (M/s. Hindustan Lever Ltd., Mumbai). The experimental protocols were reviewed by the Institutional Animal Ethics Committee (IAEC) of Sree Lakshmi Narayana Institute of Medical Sciences, Pondicherry, India, and all the procedures were in accordance with IAEC 932/Po/Pe/S/06/CPCSEA.

● Ointment preparation

An ointment formulation was prepared using distilled water (5 mL), Vaseline (9g), and 20 drops of Tween 80 (Polyoxyethylene, Sorbitan monooleate) as an emulsifier. Two concentrations (5&10%) of methanolic extracts of LWME were prepared. All the ingredients were melted over a water bath to make a homogenate of all components. The sample methanolic extract ointment was prepared by mixing 0.25 g and 0.5 g of the extract to make 5 & 10% (w/w) ointment, respectively. The prepared ointment was kept in a safe and clean container for further experiments (Dahmani et al., 2020).

● Excision wound model

An excision wound was inflicted by cutting away approximately 500 mm² full thickness of shaved skin at a predetermined area on the anterior dorsal side of rats under Pentobarbitone (45mg/kg., ip) anesthesia. The entire wound was left open. Animals were closely observed for any infection; those which showed signs of infection were separated, excluded from the study, and replaced.

A total of 24 Wistar albino rats were used in this study. The rats were divided into four groups of 6 animals each. An excision wound was inflicted on all the rats of four groups. Group I, Vehicle control, animals were treated with a simple ointment base. Group II, reference control, topically applied with Povidone iodine ointment once daily. In group III and Group IV animals, the wound was applied with 5% and 10% of LWME extract in sample ointment and the standard drugs were applied topically on the wound, once daily for 21 days. In this experiment, wound contraction and epithelialization period were evaluated (Udupa et al., 1995).

- **Assessment of wound contraction**

Wound contraction was monitored by metric measurement of wound area at different time intervals (1st day, 3rd day, 7th day, 14th day and 21st day). This was studied by tracing the raw wound area on a transparent polythene paper, and the traced area was measured using graph paper. The wound area and percentage decrease of the original wound size of 500 mm² for each group were measured (Arunachalam & Parimelazhakan, 2013). The rate of wound healing was calculated as

$$\% \text{ of wound closure} = \frac{\text{Wound area on day 0} - \text{Wound area on day } n}{\text{Wound area on day 0}} \times 100$$

- **Histopathological study**

For histological analysis, the cross-sectional full-thickness skin samples from control, standard and treated groups were collected from the excision model animals. Samples were treated and blocked with paraffin, sectioned into 5µm pieces, stained with hematoxylin and eosin after being fixed in 10% buffered formalin (Akkol et al., 2011) and observed under a microscope for histopathological findings, such as angiogenesis, collagen production and fibroblast proliferation.

iii. Docking Study

To validate the wound healing potential of *L. wightiana* leaf extracts, the docking experiments were conducted. Ten ligands, viz., dicentrinone, lauroilsine,

corytuberine, reticuline, eudesmin, grandisin, dehydroisoeugenol, 9,12-octadecadienoic acid (z,z) methyl ester, n-hexanoic acid and linalool, were selected against 4 target proteins such as glycogen synthase kinase-3 β (GSK-3 β), transforming growth factor-beta (TGF- β), c-myc, and β -catenin (Saleem et al., 2022). The canonical notation of ligands was obtained from the chemical database PubChem. The three-dimensional structure of molecules in .pdb format was derived by the CORINA tool. The 3-D structure of the 4 target proteins was obtained from the protein structural database PDB. The PDB ID of selected proteins were TGF beta (5e8w), GSK-3 beta (5oy4), c-myc (1nkp), and beta catenin (1jdh). The preparation steps before docking were carried out in AutoDock 4.2. Water molecules were removed, and polar hydrogen was added. The non-amino acid residues were also removed from protein structures and saved in PDBQT format. The docking process was done in AutoDock Vina open-source program. The visualization of docked conformations (target-ligand complex) and their interaction was done using the Discovery Studio Visualizer tool (Shady et al., 2022).

G. STUDY OF INSECTICIDAL ACTIVITY

Insecticidal activity study of *L. coriacea* leaves essential oil against selected insects was carried out. Contact toxicity, fumigant toxicity and repellency assays were performed.

- **Insects**

The insects selected for the present study were *Sitophilous oryzae* L. and *Tribolium castaneum* Herbst, which infest wheat and rice grains, causing a lot of damage during the storage of grains. Because of this reason, these insects were selected for the study. The insects were collected from Food Corporation of India (FCI), Puthupariyaram, Palakkad, Kerala, India.

***Sitophilous oryzae* L.**

Common name	:	Rice Weevil
Kingdom	:	Animalia
Phylum	:	Arthropoda
Class	:	Insecta
Order	:	Coleoptera
Family	:	Curculionidae
Genus	:	<i>Sitophilus</i>
Species	:	<i>Oryzae</i> (Linnaeus,1763)

The insect is primarily a pest in warm places and is found throughout India. The larvae and pupa of this insect complete their development inside the kernel of the grain. The adults of *S.oryzae* are 2.5 to 3.5 mm long with reddish brown or yellowish spots.

***Tribolium castaneum* Herbst.**

Common name	:	Red Flour Beetle
Kingdom	:	Animalia
Phylum	:	Arthropoda
Class	:	Insecta
Order	:	Coleoptera
Family	:	Tenebrionidae
Genus	:	<i>Tribolium</i>
Species	:	<i>castaneum</i> (Linnaeus, 1763)

T.castaneum is a common pest distributed in warm places worldwide. The insects are rusty red and 3-4 mm long. They have with elongated body with tiny punctures on the head and thorax. It is especially common in granaries, flour mills and warehouses.

i. Contact toxicity assay

Contact toxicity by multiple doses was conducted to evaluate the insecticidal activity of *L. coriacea* essential oil (Bisrat &Jung, 2020). The adults insects of 7-14 days of age after the first emergence were used in the study. The essential oils were diluted with acetone and applied to the ventral side of the insect's abdomen with the help of a micro applicator. Five different concentrations, like 5, 10, 20, 40 and 80 μ L of oil and 10 insects were used in each replication of all treatments. Acetone was taken as the control. The treated insects were transferred to 90 mm Petri dishes containing 10 g of wheat and incubated at $25\pm 2^{\circ}\text{C}$. The Petri dishes were monitored at 24 h intervals for 3 days, and the number of dead individuals was recorded. The immobile insects, upon being touched with a fine sable brush, were considered dead. The experiment was done with three replicates.

ii. Fumigant toxicity assay

Different doses of *L. coriacea* leaf oil were tested against *S.oryzae* and *T. castaneum*. The fumigant toxicity of essential oil against insect species was determined according to the procedure of Cam et al. (2012). Glass tubes (10 mL) were filled with 5 g of wheat and 10 adult individuals were placed in each tube. Discs of 10 mm diameter were cut from Whatman's No.1 filter paper and attached to the caps of glass tubes with a needle. Stock solutions of essential oils were diluted with acetone (v/v) and applied to filter paper with the help of a micropipette in a single-dose fumigant toxicity assay. The tubes were opened for 5 min to allow the acetone to evaporate and then closed. The tubes were incubated in a climate cabinet at $25\pm 2^{\circ}\text{C}$ and 65% relative humidity, and the number of dead insects was recorded at 8,12 and 24 hours. Five different concentrations, such as 5, 10, 20, and 40 $\mu\text{L/mL}$ were used in the experiment. The experiments were repeated three times. The mortality data from different periods were analyzed separately.

iii. Repellent activity assay

The method of McDonald et al. (1970) was followed to determine the repellent activities of essential oils. For this purpose, 9 cm discs were cut from Whatman No.1 filter paper and used in the experiments. Four different concentrations, like 0.16, 0.31, 0.47, and 0.63 $\mu\text{L}/\text{cm}^2$ were used. Half of the filter paper was treated with the solvent acetone (control) and the other half with the essential oil (treatment). The solvent was allowed to evaporate from the filter paper for 5 minutes, and 20 insects were released. *S.oryzae* and *T.castaneum* adults were placed in the middle of the filter paper. The Petri dish was sealed and incubated in dark conditions at $25\pm 1^\circ\text{C}$ for 2, 4, 8, 12, and 24 h. At the end of these periods, Petri dishes were opened, and the areas preferred by insects were determined. The repellent activity was determined according to the following formula.

Percent of repellent activity calculated as

$$PR = \frac{Nc - Nt}{Nc + Nt} \times 100$$

Here,

(PR= Percent repellent activity, Nc= Number of insects in control, Nt= Number of insects in treatment)

H. Statistical analysis

All the experimental data were replicated thrice and expressed as $\pm$ standard error mean (SEM)/standard deviation (SD). In antioxidant, antidiabetic and anti-inflammatory studies, IC_{50} values of standard and sample were calculated by ED50 PLUS V1.0 software. In *in vivo* experiments data were analyzed using one-way Analysis of Variance (ANOVA) to compare the means between groups followed by Dunnett's '*t*' test for multiple comparison using GraphPad Prism 5.01 software for windows and $P < 0.05$, 0.01, 0.001 were considered as significant. In insecticidal activity studies, ANOVA was used to compare the means of different treatments and Post ANOVA, Least Significant Difference (LSD) test was employed to identify

which treatments differ significantly from each other. Person Goodness Fit Test (Probit) was used to calculate lethal concentration/dose (LC50 and LC90/ LD50 and LD90) which indicate the concentration at 50 or 90%. Statistical analysis was done by IBM SPSS 4.4.1. Version 21. Values with $p < 0.05$ was considered statistically significant.

CHAPTER IV

RESULTS

The current study examined the pharmacognostic, phytochemical and pharmacological characteristics of three *Litsea* species, namely *L. bourdillonii*, *L. coriacea*, and *L. wightiana*. Pharmacognostic features like morphology, anatomy, histochemistry, physicochemical, and powder characters were analyzed. Preliminary qualitative and quantitative phytochemical profiling and pharmacological properties, including antimicrobial, antioxidant, antidiabetic, and cytotoxicity potentials, were evaluated. The most active and efficient *Litsea* species was selected for anti-inflammatory, wound healing and docking studies. The research revealed the bioactivity potential of the studied *Litsea* spp.

PHASE I. PHARMACOGNOSTIC CHARACTERIZATION

a. Morphological study

The macromorphological features of the three selected species of *Litsea* were studied. The details are listed in Table 9 and Figures 5, 6 & 7. The three plants showed similarities in leaf, stem, inflorescence and fruit characteristics and exhibited differences in some morphological features like the length of leaves, leaf lamina, petiole, fruit size, shape, and the perianth tube. Among the three species, *L. bourdillonii* and *L. wightiana* exhibited more resemblances when compared to *L. coriacea*.

Table 9 Comparative morphological characterization of three *Litsea* spp.

Morphological characters	<i>L. bourdillonii</i>	<i>L. coriacea</i>	<i>L. wightiana</i>
Habit	Tree	Tree	Tree
Height of the plant	8-10 m	10-18 m	10-15 m
Leaf shape	Elliptical	Elliptical lanceolate	Elliptical
Leaf length (cm)	20.16 cm	12.5 cm	18 cm
Leaf tip	Acute	Acute	Acute
Leaf margin	Entire	Entire	Entire
Leaf lamina	Hairy	Fleshy	Hairy
Leaf Petiole	Hairy	Smooth	Hairy
Phyllotaxy	Alternate	Alternate	Alternate
Stem	Woody	Woody	Woody
Bark color	Pale Brown	Pale Brown	Brown
Inflorescence	Umbel	Umbel	Umbel
Number of flowers	4-6	4-5	5
Male flowers	12 stamens	12 stamens	12 stamens
Female flowers staminodes	12	12	12
Flower colour	Brown	White	White
Fruit type	Berry	Berry	Berry
Fruit size	10-12 mm	8 mm	1.5 mm
Fruit colour	Reddish to dark purple	Reddish to dark purple	Reddish to dark purple
Fruit shape	Oblong or obovoid	Oblong or obovoid	Ellipsoid
Perianth tube	Enlarged obconic	Enlarged cup-shaped	Cup-shaped

b. Anatomical study. The anatomical characteristics of the leaf, petiole and stem parts of three *Litsea* plants were studied and compared the results with each other.

- ***L. bourdillonii* stem**

The outline of the stem was circular. The outermost layer was the epidermis, which was uniseriate, irregularly arranged, squarish cells. Epidermal cells give rise to



Fig 5 Macromorphological characters of *L.bourdillonii*. a.Habit; b. Twig with leaf and bud; c. Twig with flower; d. Young and mature fruits; d & e. Bark; g. Seeds

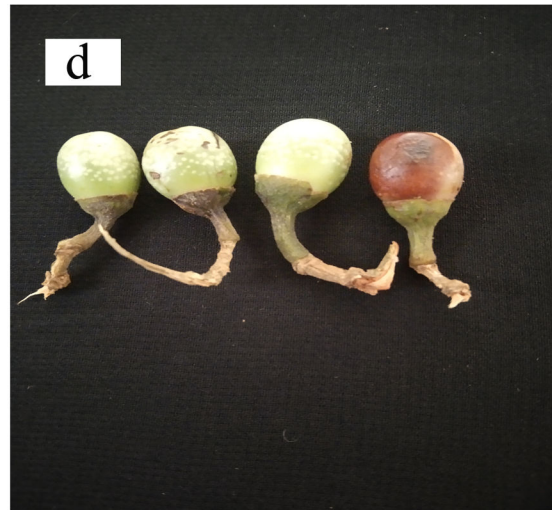
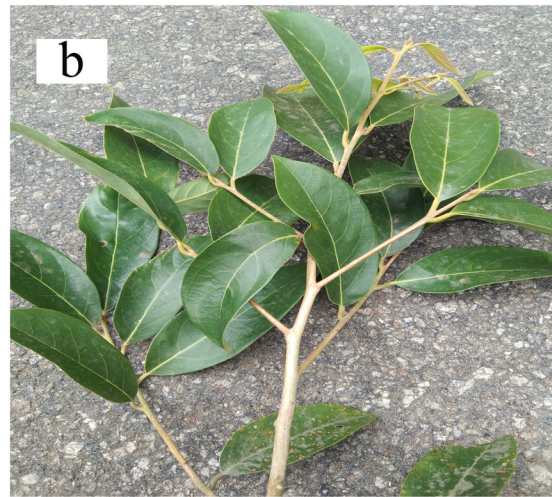


Fig 6 Macromorphological characters of *L. coriacea*. a. Habit; b. Twig with leaf; c. Twig with flower; d. Young and mature fruits; e & f. Bark;g. Seeds

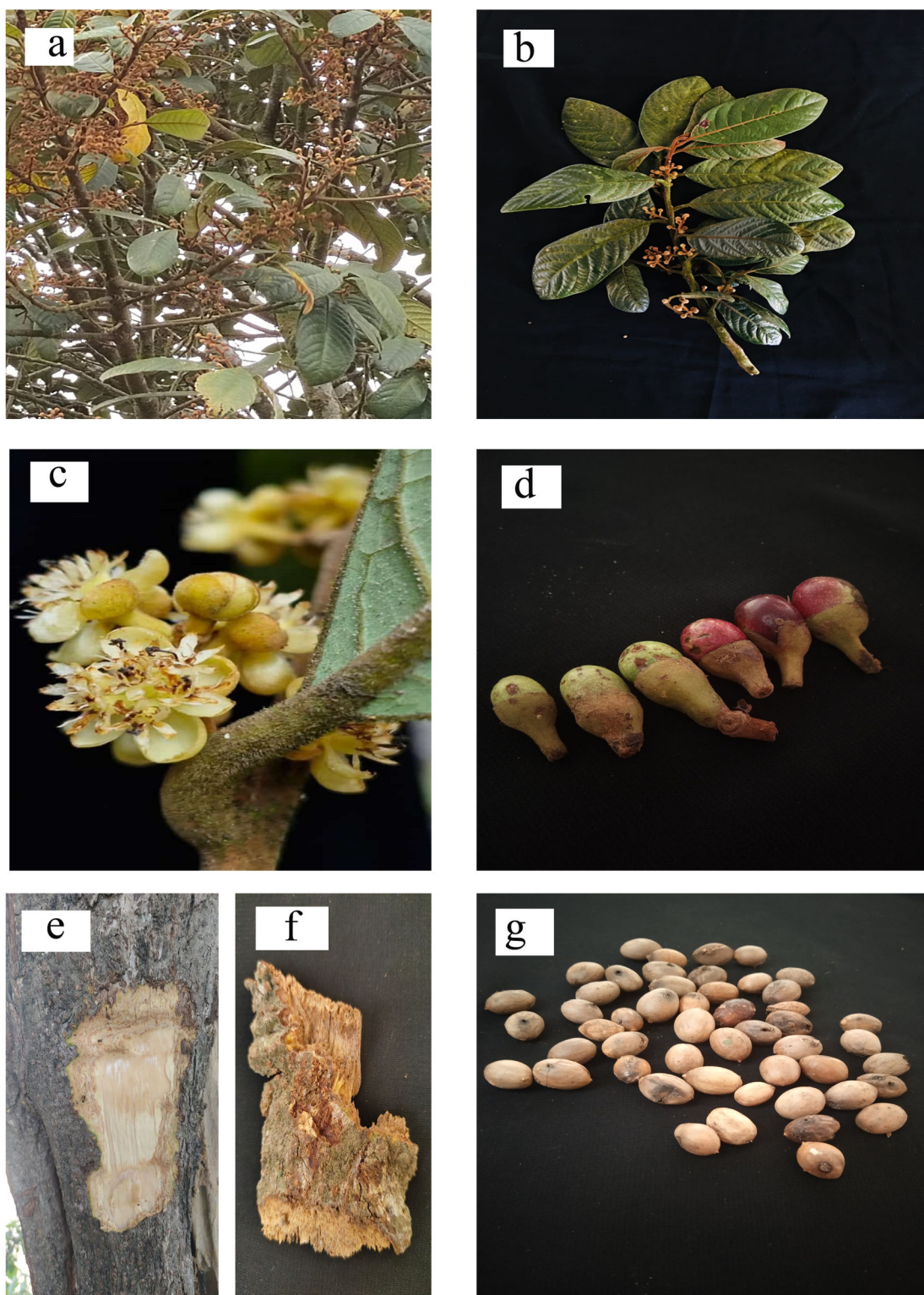


Fig 7 Macromorphological characters of *L.wightiana*. a. Habit;b. Twig with leaves and bud; c. Twig with flower;d. Young & mature fruits. e & f. Bark; g. Seeds

elongated unicellular trichomes. The cortex was multilayered and composed of parenchyma cells intermingled with a few sclerenchyma cells. A few cells in the inner cortex contained starch grains. Sclerenchymatous pericyclic fibers were present. The vascular bundles showed xylem strands with numerous tracheids mixed with large vessels. The phloem faced towards the outside with phloem parenchyma and fibers. The central part of the stem was occupied by a large pith with loosely arranged polygonal parenchyma cells (Table 10 & Fig 8).

- ***L. coriacea* stem**

The structure was circular in outline. Epidermis was single layered with squarish cells. The outer cortex was multilayered with simple polygonal parenchymatous cells with angular thickening. The inner cortex was multilayered with parenchyma cells, which were loosely arranged. One layer of sclerenchyma cells was present between the outer and inner cortex. Continuous patches of pericyclic fibers were also present. The secondary phloem contained fibers and parenchyma. The xylem has parallel rows of tracheids mixed with vessel elements. The pith consisted of polygonal parenchymatous cells (Table 10 & Fig 9).

- ***L. wightiana* stem**

The internal structure of the stem showed a wavy outline. The epidermis was found to be single layered with small squarish type cells. Some cells gave rise to unicellular glandular trichomes. The outer cortex is multilayered with compactly arranged parenchyma cells intermingled with a few sclerenchyma cells. The inner cortex was multilayered with loosely arranged thin-walled parenchyma cells. Discontinuous patches of pericyclic fibers were present. In the xylem, numerous rows of tracheids were intermingled with large vessel elements. External to the xylem, the phloem consisted of phloem parenchyma and fibers. A large pith was present in the center with compactly arranged polygonal parenchyma cells (Table 10 & Fig 10).

Table 10 Comparative anatomical characterization of the stem of three *Litsea* spp.

Microscopic characters	<i>L. bourdillonii</i>	<i>L. coriacea</i>	<i>L. wightiana</i>
Epidermis	Uniseriate, squarish cells with cuticle	Uniseriate, squarish cells with cuticle	Uniseriate, small, squarish cells, with cuticle
Trichomes	Unbranched, long unicellular	Unicellular, broad, small and glandular	Small, unicellular, and glandular
Cortex	The outer and inner cortex-multilayered parenchyma cells; the inner cortex-1-2-layered sclerenchyma cells	The outer cortex-multilayered, with compactly arranged parenchyma cells; the inner cortex-loosely arranged parenchyma cells; the middle cortex single-layered with sclerenchyma cells	The outer cortex-multilayered with compactly arranged parenchyma cells intermingled with a few sclerenchyma cells; the inner cortex-multilayered with loosely arranged thin-walled parenchyma cells
Pericyclic fibres	Continuous patch of pericyclic fibers	Continuous patch of pericyclic fibres	Discontinuous patch of pericyclic fibers
Xylem	Groups of tracheids intermingled with varying sizes of vessels and parenchyma	Elongated patches of xylem tracheids with varying size of vessels	Elongated patches of xylem tracheids intermingled with large vessels
Phloem	Patches of condensed phloem with parenchyma and fibres	Continuous patch of phloem cells with phloem fibers and parenchyma	Continuous patch of phloem cells with fibers and parenchyma
Medullary rays	Less prominent	Prominent with uniseriate parenchyma cells	Narrow and less prominent
Vessels	Large, single or in groups	Varying sizes in groups	Large varying sizes
Pith	Polygonal parenchyma cells	Polygonal parenchyma cells	Polygonal, compactly arranged parenchyma cells

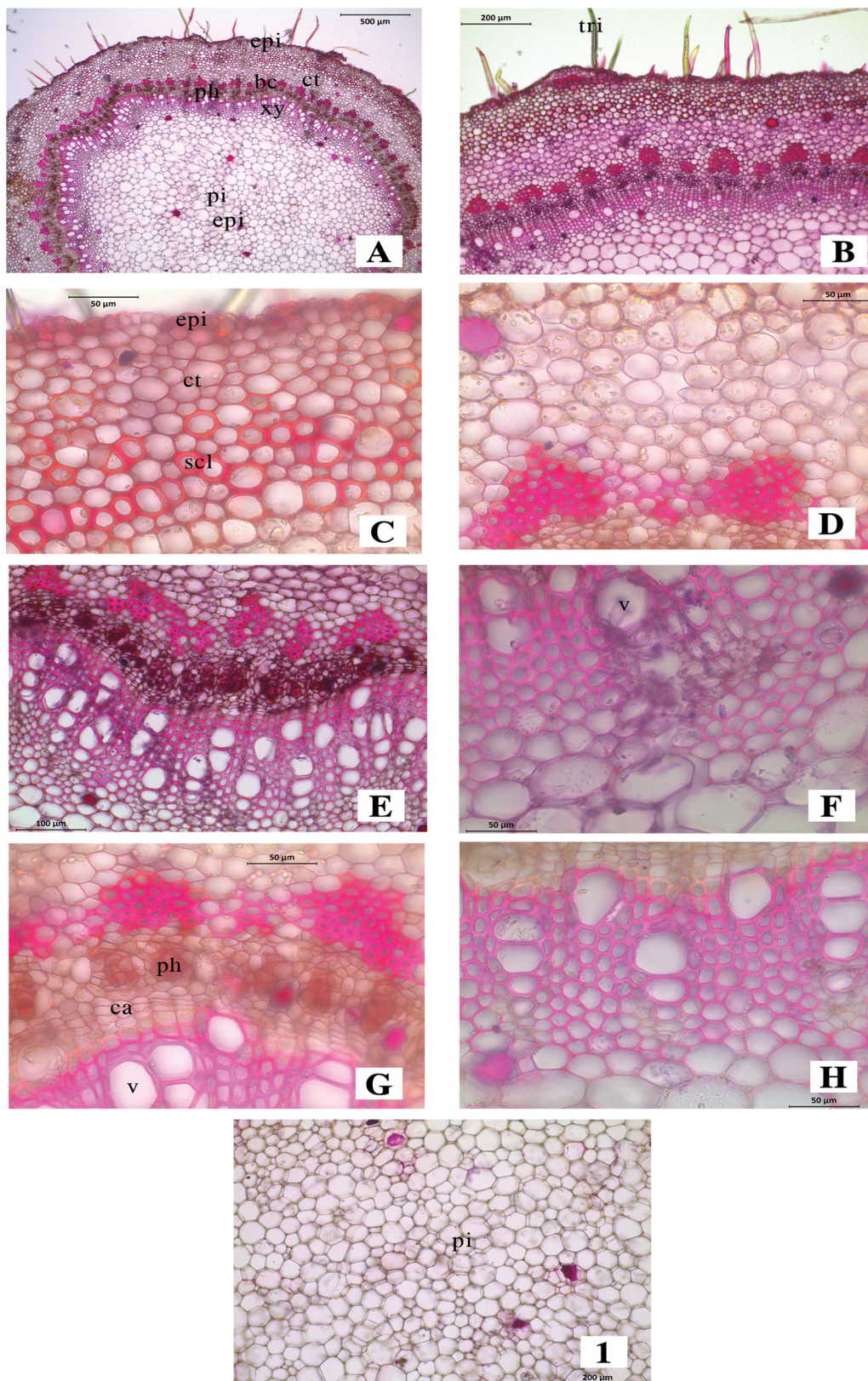


Fig 8 Microscopic characters of *L.bourdillonii* stem A. Ground plan; B. upper portion of stem; C. hypodermis parenchyma & middle cortex sclerenchyma; D. Inner cortex parenchyma and pericyclic fibres; E, F, G, & H- Xylem with large vessels, phloem and cambium; I. Pith parenchyma

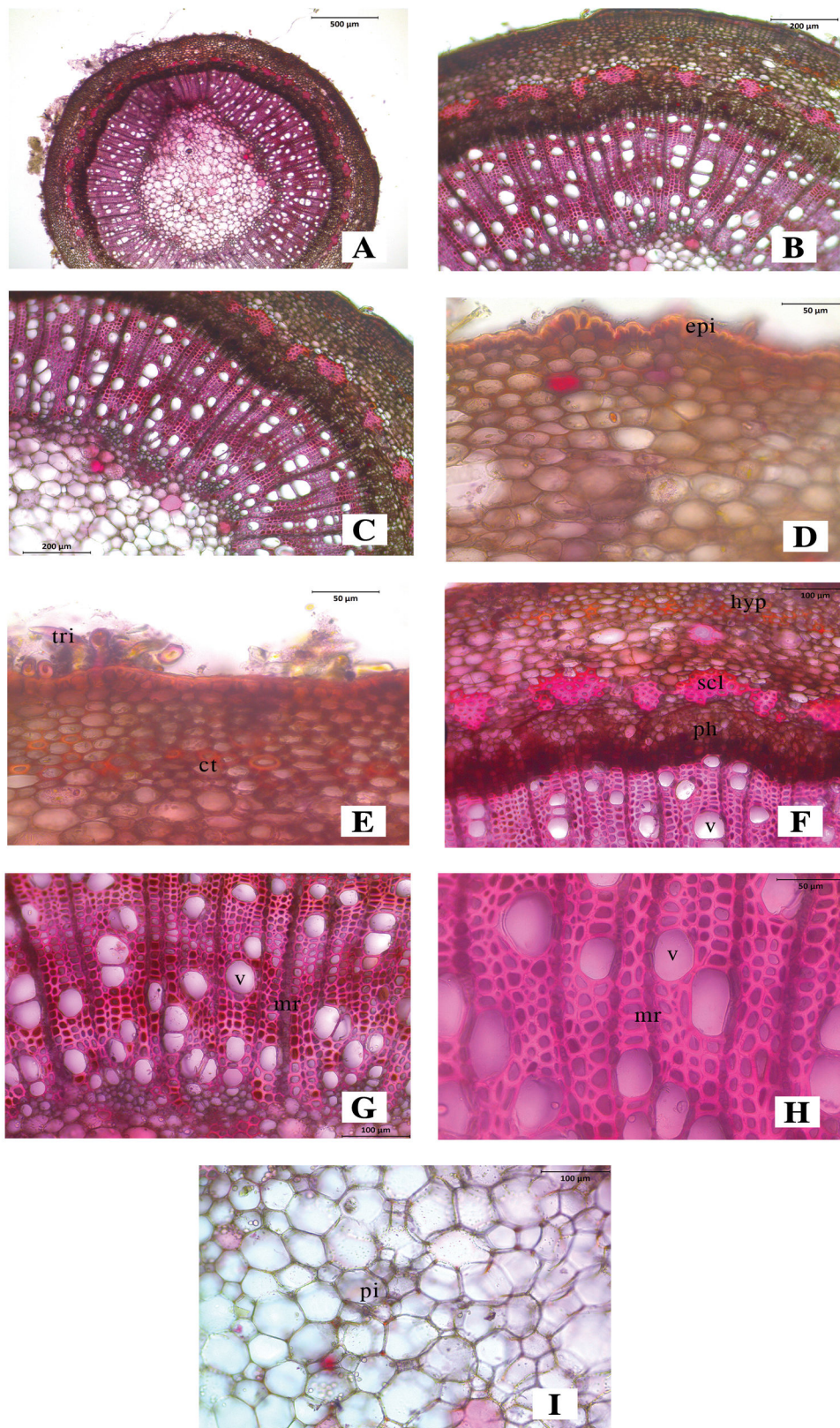


Fig 9 Microscopic characters of *L. coriacea* stem. A-C. Ground plan full view & portion enlarged; D&E-Epidermis with trichomes, outer and inner cortex parenchyma and middle sclerenchyma layer; F-H-Stelar region, xylem with large vessels and tracheids impregnated with medullary ray, phloem, cambium and pericyclic fibres; I. pith parenchyma

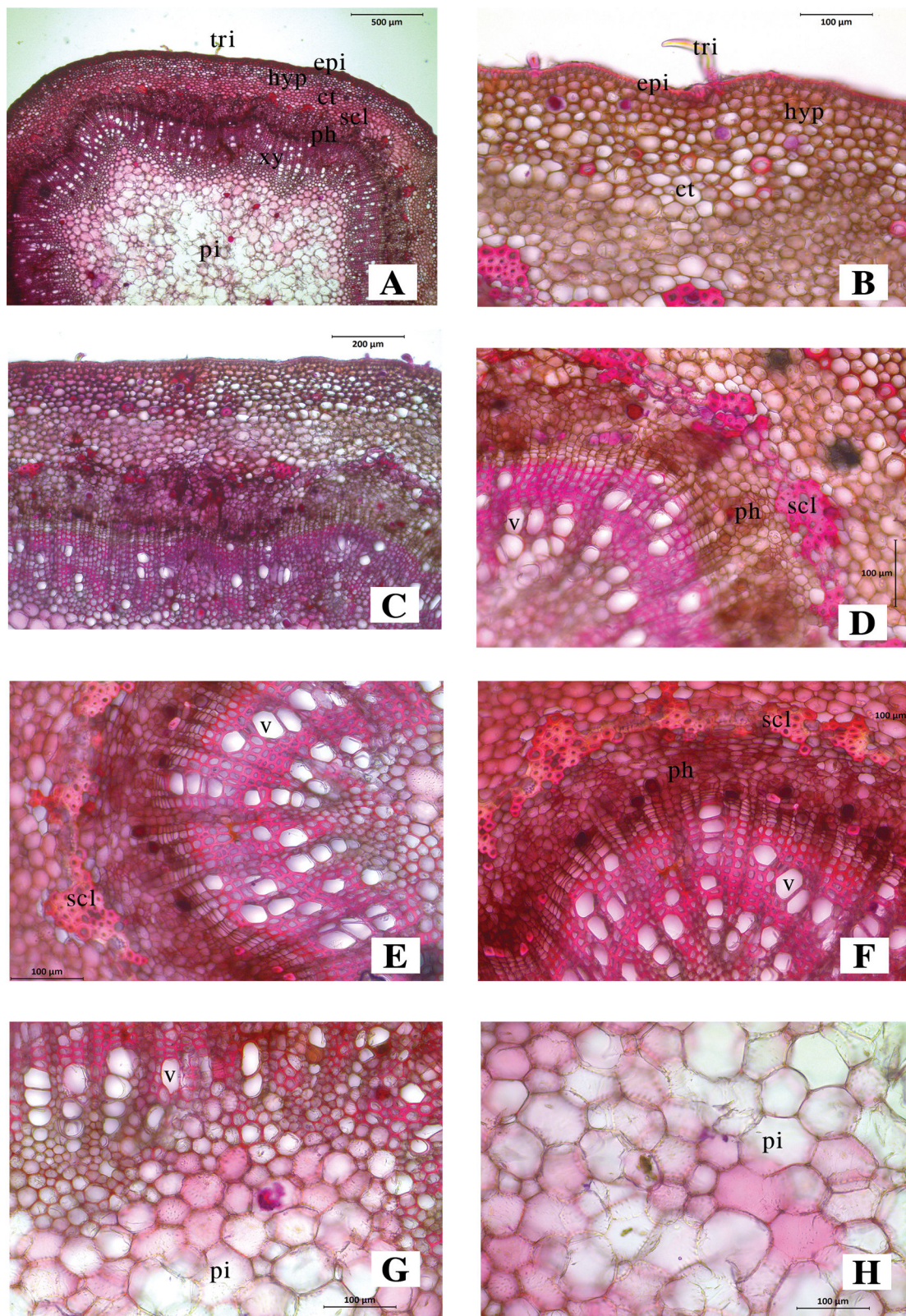


Fig 10 Microscopic characters of *L.wightiana* stem. A. Ground plan; B.Hypodermis and cortex parenchyma,C,D,E&F.Pericyclic fibres,thick patch of phloem,cambium, xylem with large vessels and tracheids intermingled with medullary rays G&H.pith parenchyma

- ***L. bourdillonii* leaf**

The internal structure showed a prominent midrib, lateral veins and uniformly thin lamina. The upper epidermis in the midrib consisted of a uniseriate layer of squarish, thin-walled parenchyma cells. The upper epidermal cells gave rise to a few unicellular trichomes in the midrib region. The lower epidermal cells produced a few narrow, unicellular elongated trichomes from the midrib and numerous from the laminar regions. The cuticle cells in the upper epidermis were uniformly distributed, and the cuticle in the lower epidermis were undulate and irregularly arranged.

In the midrib region below the epidermis, multilayered ground tissue with thin-walled round parenchyma cells were present. A major portion of the midrib was occupied by wide vasculature with collateral bundles consisting of continuous parallel rows of xylem with two large, distinct bundles, somewhat fused, in the upper part. The phloem was seen as a continuous horizontal band below the xylem. A regularly arranged sclerenchymatous bundle cap was present below the phloem patch. Lateral veins with distinct collateral bundles surrounded by the sclerenchyma bands.

In the laminar region, the adaxial epidermis with squarish cells was evenly distributed with a thin cuticle. Abaxial surface cells with short, irregularly arranged, somewhat columnar and undulate cells, covered by a thick cuticle. There were no stomata in the adaxial epidermis, but a few were in the abaxial epidermis. Below the epidermis, mesophyll consisted of two layers of thick, columnar, pillar-like palisade tissue interspersed with a few secretory idioblasts. Lower spongy mesophyll was composed of loosely and irregularly arranged, thin-walled round, and some elongated parenchyma cells (Table 11 & Fig 11).

- ***L. coriacea* leaf**

The section showed a prominent midrib with lateral veins and uniformly thin lamina. The upper epidermal cells were squarish, covered with a thin cuticle and the lower layer with columnar cells with an undulated cuticle. The upper and lower epidermis did not bear trichomes. In the midrib region, the ground tissue comprised 1-2 irregular layers of sclerenchyma, followed by a broad zone of round, thin-walled

parenchyma cells. Below, a broad vascular strand covering the central part of the midrib appeared as two fused half rings, with conjoint collateral vascular bundles with parallel rows of xylem. Two distinct xylem bundles were visible in the upper part, while the phloem forms a continuous horizontal band below the xylem strands. Phloem was surrounded by a thin band of regularly arranged sclerenchyma. The lateral veins had collateral bundles surrounding sclerenchyma bands.

The adaxial epidermal cells were squarish in the laminar region, evenly distributed and covered with thin cuticle. The abaxial epidermal cells were columnar, unevenly distributed, with an undulate cuticular layer. A few stomata exist in the abaxial side and no stomata exist in the adaxial side. Below the epidermis appear mesophyll tissue with 2-3 layers of thick, elongated, pillar-like palisade cells and a lower spongy layer with loosely arranged spherical parenchyma cells. The mesophyll cells were intervened with secretory idioblasts (Table 11 & Fig 12).

- ***L. wightiana* leaf**

The section of the leaf showed a prominent midrib with lateral veins and uniformly thin lamina. The upper epidermis consisted of squarish cells, evenly distributed and covered with a thin cuticle. The lower epidermis had irregularly distributed, small columnar cells with papillose and undulate cuticle. The entire leaf epidermis had numerous elongated, unicellular, and narrow trichomes. In the midrib, the ground tissue consisted of multilayered parenchyma cells. The central part of the midrib was occupied by a wide vasculature that consisted of collateral bundles arranged as two half rings, of which the lower was broader and the upper narrower. Phloem was seen as a continuous horizontal band below the xylem strands. Surrounding the phloem, there was a thin band of regularly arranged sclerenchyma. The lateral veins had distinct collateral bundles surrounding sclerenchyma bands.

In the laminar region, the upper epidermis had thick-walled, regular, squarish-type cells. The lower epidermis was composed of irregularly arranged parenchyma cells with a thick cuticle covering. Below the upper epidermis, mesophyll tissue with 2-3 layers of elongated palisade cells, interspersed with large idioblasts, was seen (Table 11& Fig 13).

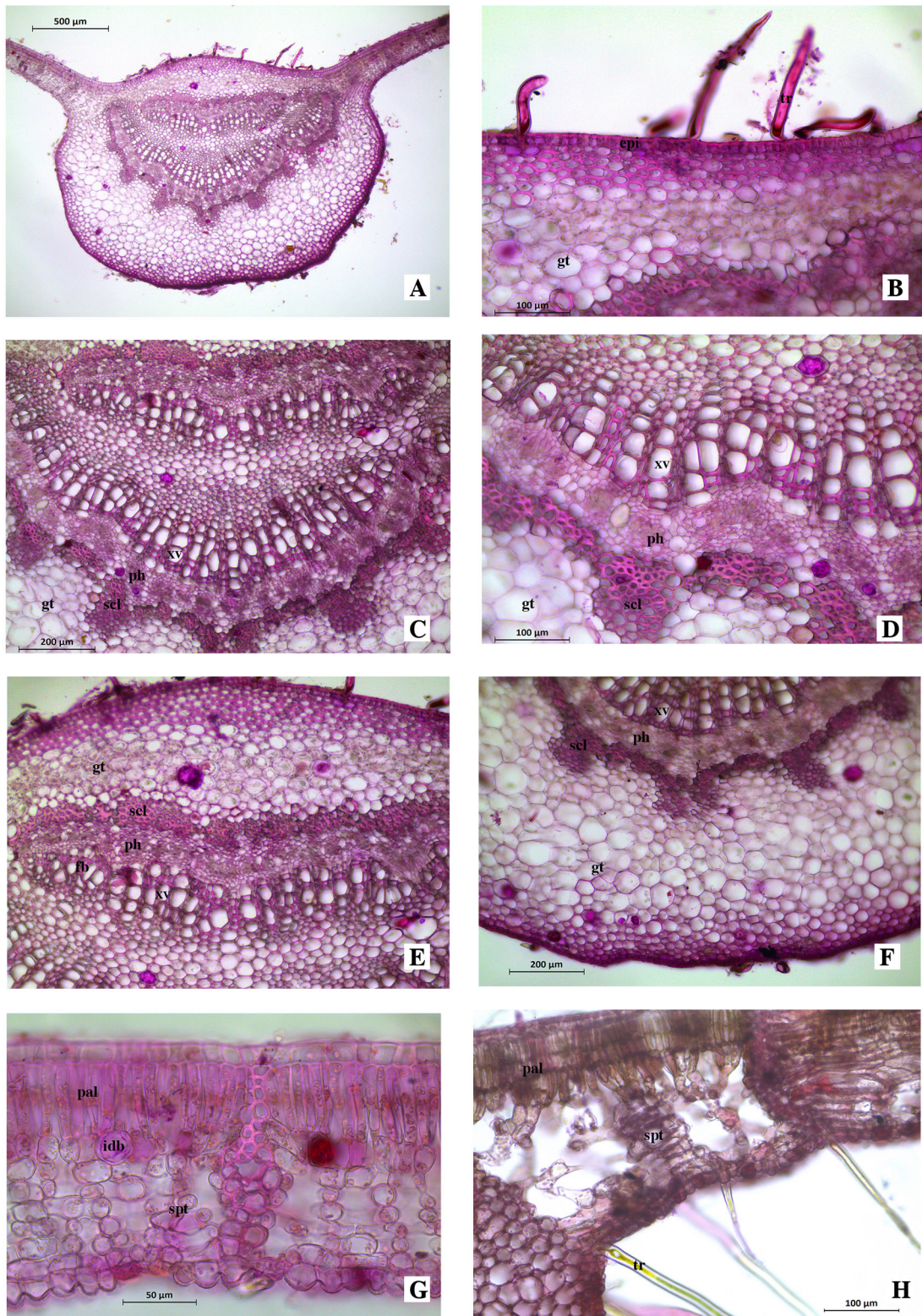


Fig 11 Microscopic characters of *L.bourdillonii* leaf A. ground plan, B. trichome, C,D & E. mid rib& vasclature, F.ground tissue, G.palisade & spongy tissue, H. lower epidermis with trichomes.

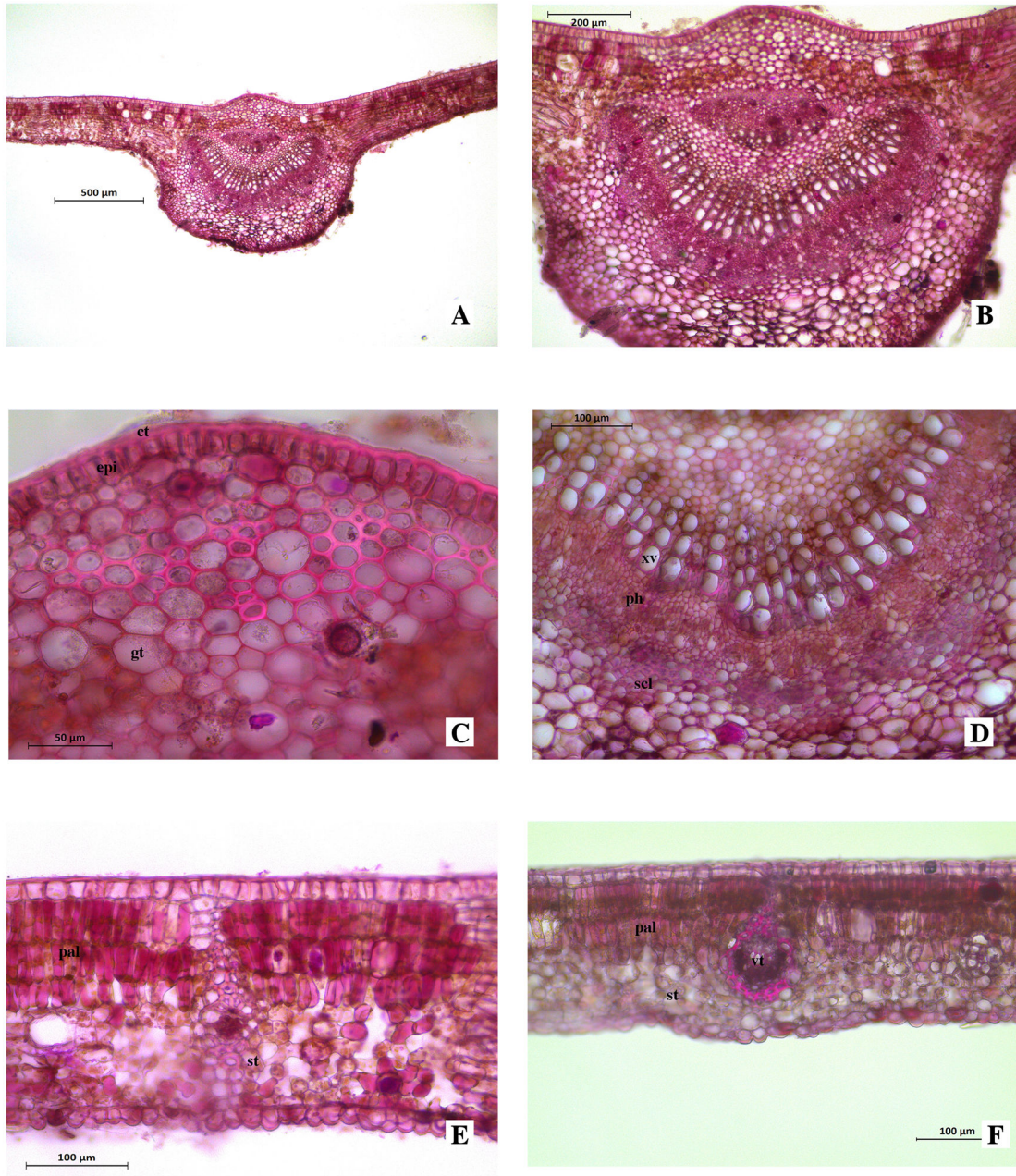


Fig 12 Microscopic characters of *L. coriacea* leaf. A. ground plan, B. mid rib, C. ground tissue, D. Vascular tissue E&F. lamina with palisade & spongy tissue

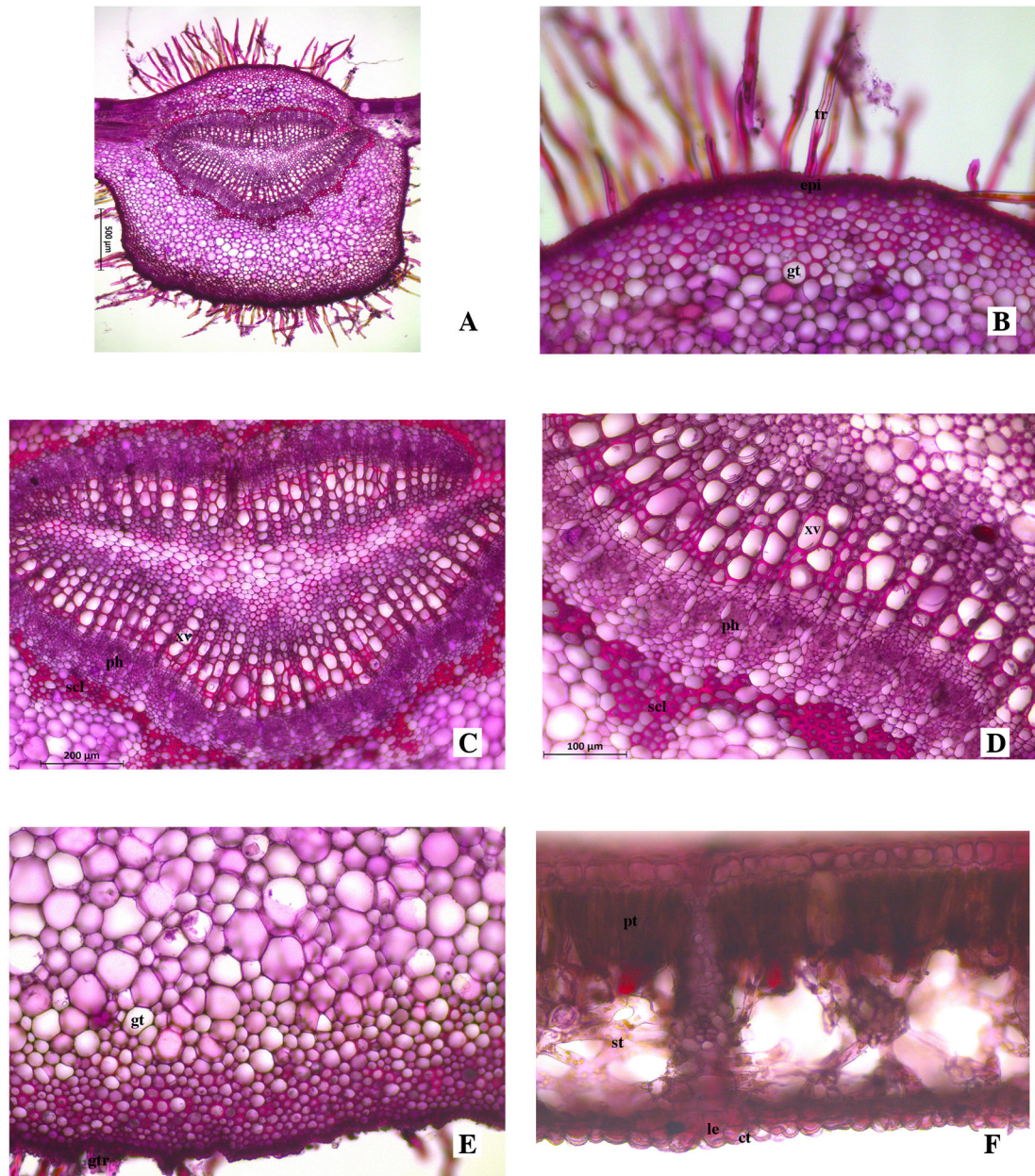


Fig 13 Microscopic characters of *L. wightiana* - A. ground plan, B. trichome, C&D. mid rib vasculature E. ground tissue, F.lamina with palisade & spongy tissue.

Table 11 Comparative anatomical characterization of the leaves of three *Litsea* spp.

Microscopic characters	<i>L. bourdillonii</i>	<i>L. coriacea</i>	<i>L. wightiana</i>
Epidermis	Upper uniseriate, small, narrow, thin-walled, and squarish parenchyma cells, lower columnar cells	Upper uniseriate, small, narrow, thin-walled, and squarish parenchyma cells, lower columnar cells	Upper uniseriate, small, squarish, and thin-walled parenchyma cells, lower columnar cells
Cuticle	Upper: thin-walled and uniformly arranged Lower: undulate and irregularly arranged	Upper: thin-walled and uniformly arranged Lower: undulate and irregularly arranged	Upper: thin-walled and uniformly arranged Lower: undulate and irregularly arranged
Trichomes	Unicellular and elongated	Absent	Numerous, elongated, and unicellular
Ground tissue	Multilayered and round parenchyma in the midrib region	Multilayered parenchyma and 1-2 irregular sclerenchyma	Multilayered parenchyma
Vasculature	Wide vasculature with collateral bundles, with two large, fused bundles on the upper side	Wide vasculature with two fused half rings of bundles, the upper small and the lower large	Wide vasculature consists of collateral bundles arranged as two half rings, the lower broader and the upper narrower ring.
Palisade tissue	Two layers of elongated, compactly arranged, pillar-like columnar cells interspersed with a few secretory idioblasts	Thick, elongated, pillar-like columnar cells interspersed with secretory idioblasts	2-3 layers of elongated palisade cells interspersed with large secretory idioblasts
Spongy mesophyll	Loosely arranged parenchyma cells	Loosely arranged parenchyma cells	Loosely arranged parenchyma cells
Bundle sheath	Continuous sclerenchymatous layer	Discontinuous sclerenchymatous layer	Continuous sclerenchymatous layer

● ***L. bourdillonii* petiole**

The section of the petiole showed a crescent outline. The epidermis was single-layered with thin-walled parenchyma cells covered by a thick cuticle. The entire epidermis gave rise to numerous short and narrow unicellular trichomes. The ground tissue was wide with thin-walled parenchyma cells. The center of the petiole consisted of crescent-shaped vasculature that contained numerous narrow bundles.

The xylem tissue had tracheids and vessels, and the phloem tissue had phloem parenchyma and a few fibers. The centre of the petiole was occupied by pith that consisted of loosely arranged polygonal parenchyma cells (Table 12 & Fig 14).

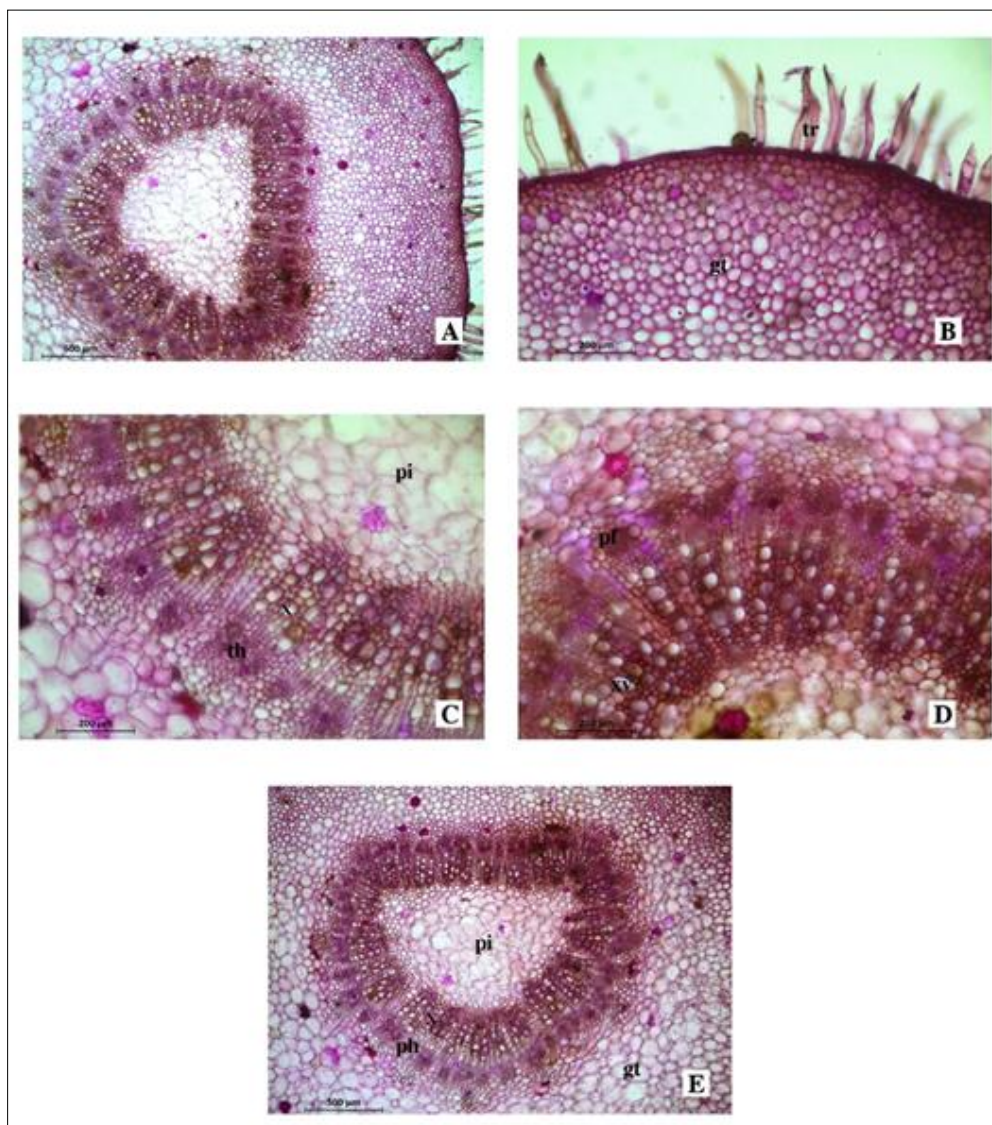


Fig. 14 Petiole anatomy of *L. bourdillonii*. A. Ground plan; B. Trichome; C&D Vasculature; E. Pith

● ***L. coriacea* petiole**

The section showed a crescent-shaped outline. The epidermis was uniseriate with small, squarish-type cells covered with a thin cuticle. The epidermis did not give rise to trichomes. The ground tissue was wide and consisted of thin-walled parenchyma cells. Numerous stone cells were scattered in the cortex. Crescent-

shaped vasculature had a hooked upper end. Extensive xylem strands had tracheids, vessels and parenchyma. Outside the xylem, the phloem cells contained parenchyma and a few fibers. A single layer of sclerenchyma was seen outside the phloem above the hook. The centre of the petiole was occupied with a narrow and condensed pith with a few polygonal parenchyma cells (Table 12 & Fig 15).

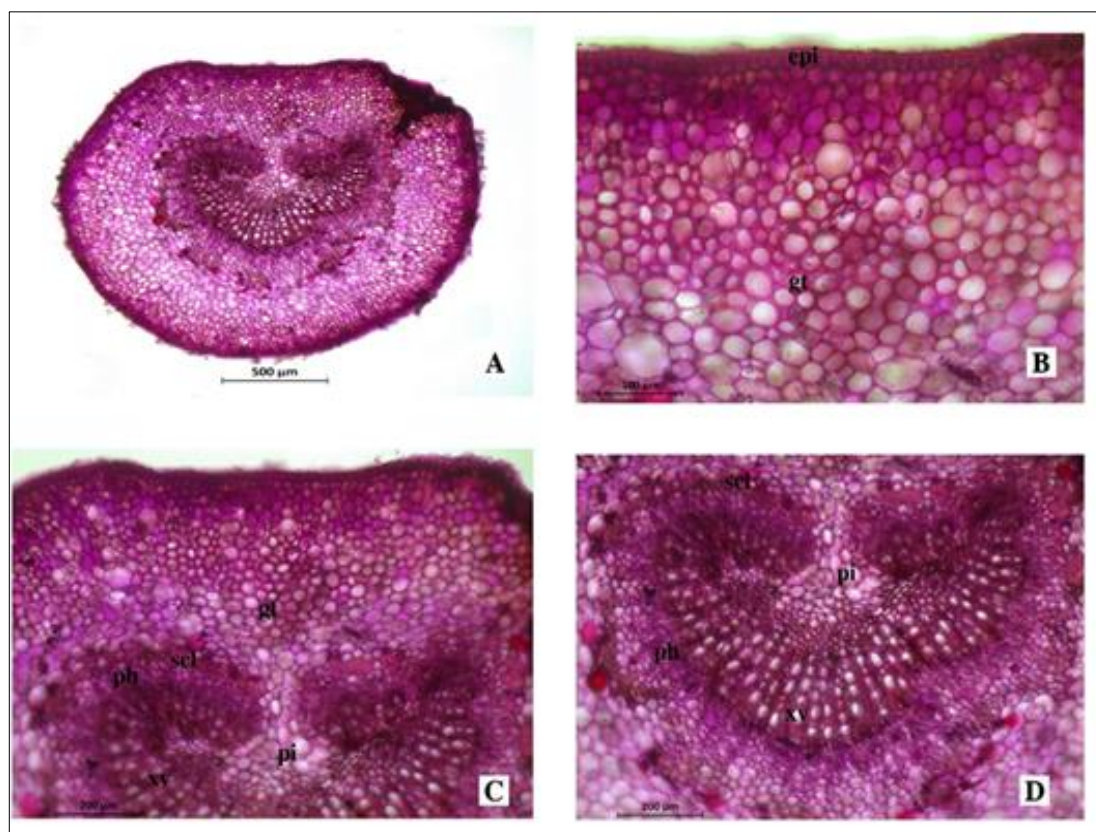


Fig 15 Petiole anatomy of *L. coriacea*. A. Ground plan; B. Ground tissue; C&D. Vasculature

- ***L. wightiana* petiole**

The section showed a horizontally flattened crescent-shaped outline. The outer epidermis contained small parenchyma cells covered by a thick cuticle. The epidermis gave rise to numerous long unicellular trichomes. The ground tissue was wide with thin-walled parenchyma cells and a few lignified cells. Numerous stone cells were seen scattered in the cortex. Vasculature was horizontal and crescent shaped. Numerous long, narrow xylem strands encompassed with uniformly distributed vessels and tracheids. Outer to the xylem, a band of uniseriate phloem

was present. The centre of the petiole was occupied by a crescent-shaped pith that consisted of loosely arranged polygonal parenchyma cells (Table 12 & Fig 16). The comparative anatomical features of petiole of three species of *Litsea* represented in Table 12.

Table 12 Comparative anatomical characterization of the petiole of three *Litsea* spp.

Microscopic characters	<i>L. bourdillonii</i>	<i>L. coriacea</i>	<i>L. wightiana</i>
Shape	Crescent-shaped	Crescent-shaped	Horizontal crescent-shaped
Epidermis	Uniseriate with thin-walled parenchyma covered by a thick cuticle	Uniseriate with thin-walled and squarish parenchyma covered by a thin cuticle	Uniseriate with thin-walled parenchyma covered by a thick cuticle
Trichome	Short, narrow, and unicellular	Absent	Numerous, long, and unicellular
Ground tissue	Wide with thin-walled parenchyma	Wide with thin-walled parenchyma	Wide with thin-walled parenchyma and numerous scattered stone cells
Shape of Vasculature	Crescent-shaped	Crescent-shaped with two hooks at the end	Horizontal and crescent-shaped
Xylem	Tracheids and vessels	Tracheids, vessels and parenchyma	Vessels and tracheids
Phloem	Parenchyma and a few fibres	Parenchyma and a few fibres	Parenchyma
Bundle cap	Absent	Sclerenchymatous above the hooks	Absent
Pith	Crescent-shaped with loosely arranged polygonal parenchyma	Narrow and condensed with few polygonal parenchyma	Crescent-shaped with loosely arranged parenchyma

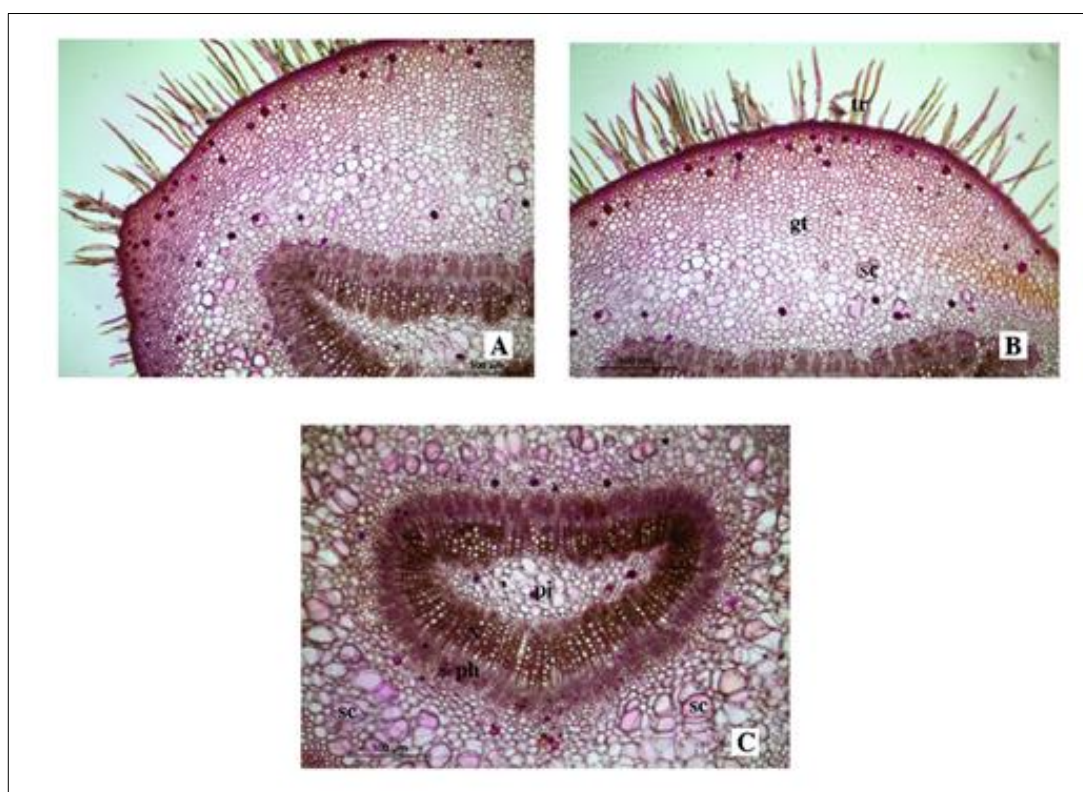


Fig 16 Petiole anatomy of *L. wightiana*. A. Ground plan; B. Ground tissue; C. Vasculature & pith

c. Histochemical study

The results of the histochemical study revealed significant differences in the localization of compounds such as starch, tannin, lignin and fixed oils in various cells of the leaf and stem of selected *Litsea* species. In stem, starch is found abundantly in the stele and cortical regions of *L. coriacea* than that of *L. wightiana*. In *L. bourdillonii*, starch deposition was observed very little. Lignin deposition was mainly located in pericyclic fibers and in the wall of xylem tracheids and tannin was present in cortical regions of all the three species. Fixed oil content was observed to be higher in the hypodermal, cortical and pith region of *L. coriacea* compared to *L. wightiana* and *L. bourdillonii* (Table 13 and Figures 17, 18 & 19).

Table 13 Comparative histochemical characterization of the stem of three *Litsea* spp.

Test for histochemicals	<i>L. bourdillonii</i>	<i>L. coriacea</i>	<i>L. wightiana</i>
Starch	Present in a few cells in the inner cortex	Abundant in stele and cortex	Present in inner cortex, phloem parenchyma than <i>L.bourdillonii</i>
Lignin	Present in bundle cap, xylem tracheids	Present in bundle cap, abundant in xylem tracheids and vessels	Abundant in the outer and the inner cortex and the secondary xylem
Tannins	Present in hypodermis, cortex, phloem and pith	Present abundantly in the cortex, pericyclic fibres and xylem	Abundant in hypodermis, cortex and less in pith
Fixed oils	Small deposition in hypodermal cells	Increased deposition in the hypodermis, cortex and pith	More in the hypodermis, and fewer in the inner cortex and pith

Histochemical studies in the leaves indicated various histochemicals in three *Litsea* plants. Starch was present in *L. coriacea* and *L. wightiana* and absent in *L. bourdillonii*. Lignin was located mainly in the bundle sheath and xylem wall of *L. coriacea* and *L. wightiana* compared to *L. bourdillonii*. Tannin was found to be present in the midrib region and was abundant in *L. wightiana* than *L. bourdillonii* and *L. coriacea*. Oil content was localized more in cortical cells of *L. coriacea* and scarcely present in *L. wightiana* and absent in *L. bourdillonii* (Table 14 & Fig 20).

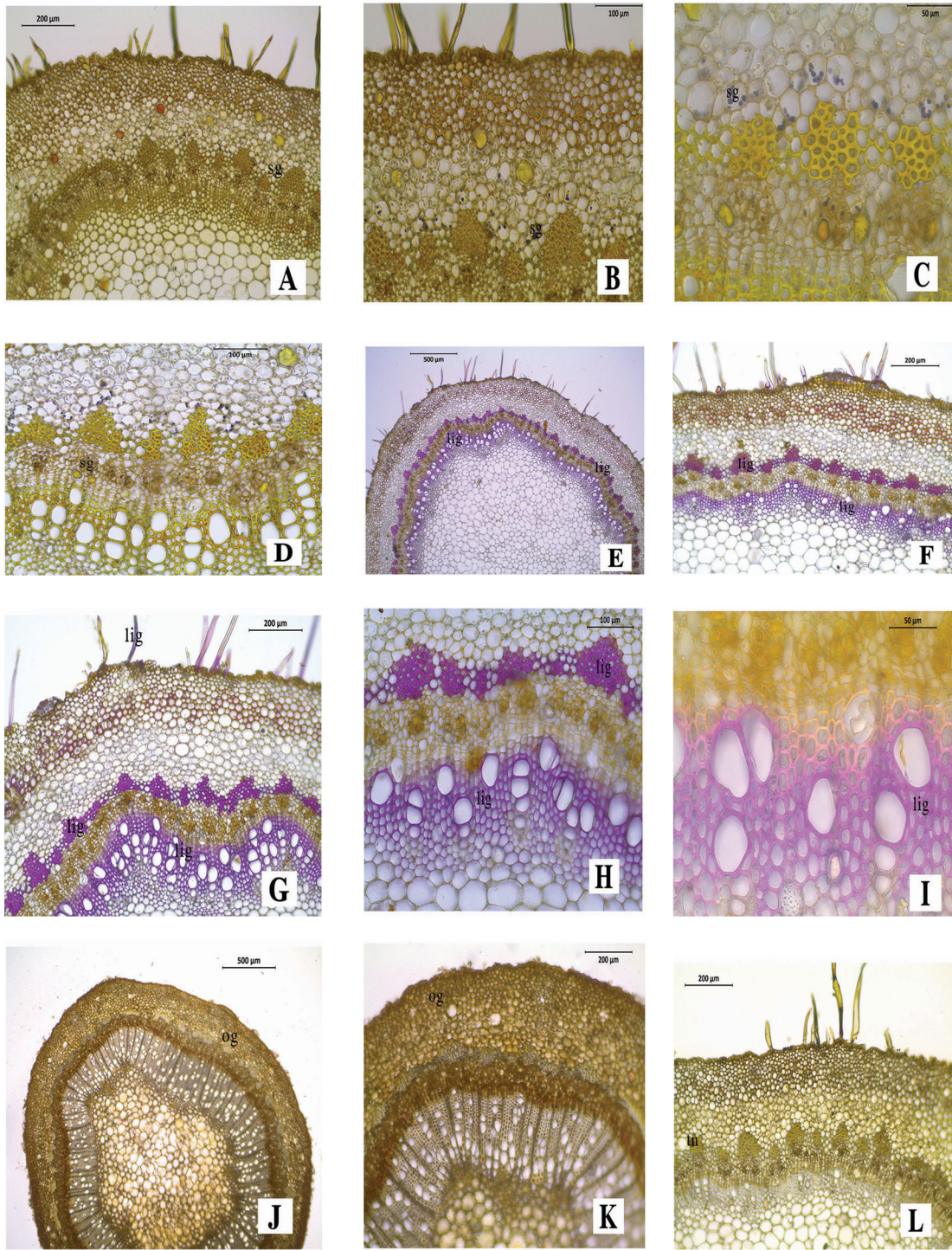


Fig 17 Histochemical localization of *L. bourdillonii* stem. A-D. Starch; E-I. Lignin; J&K. Fixed oil; L. Tannins

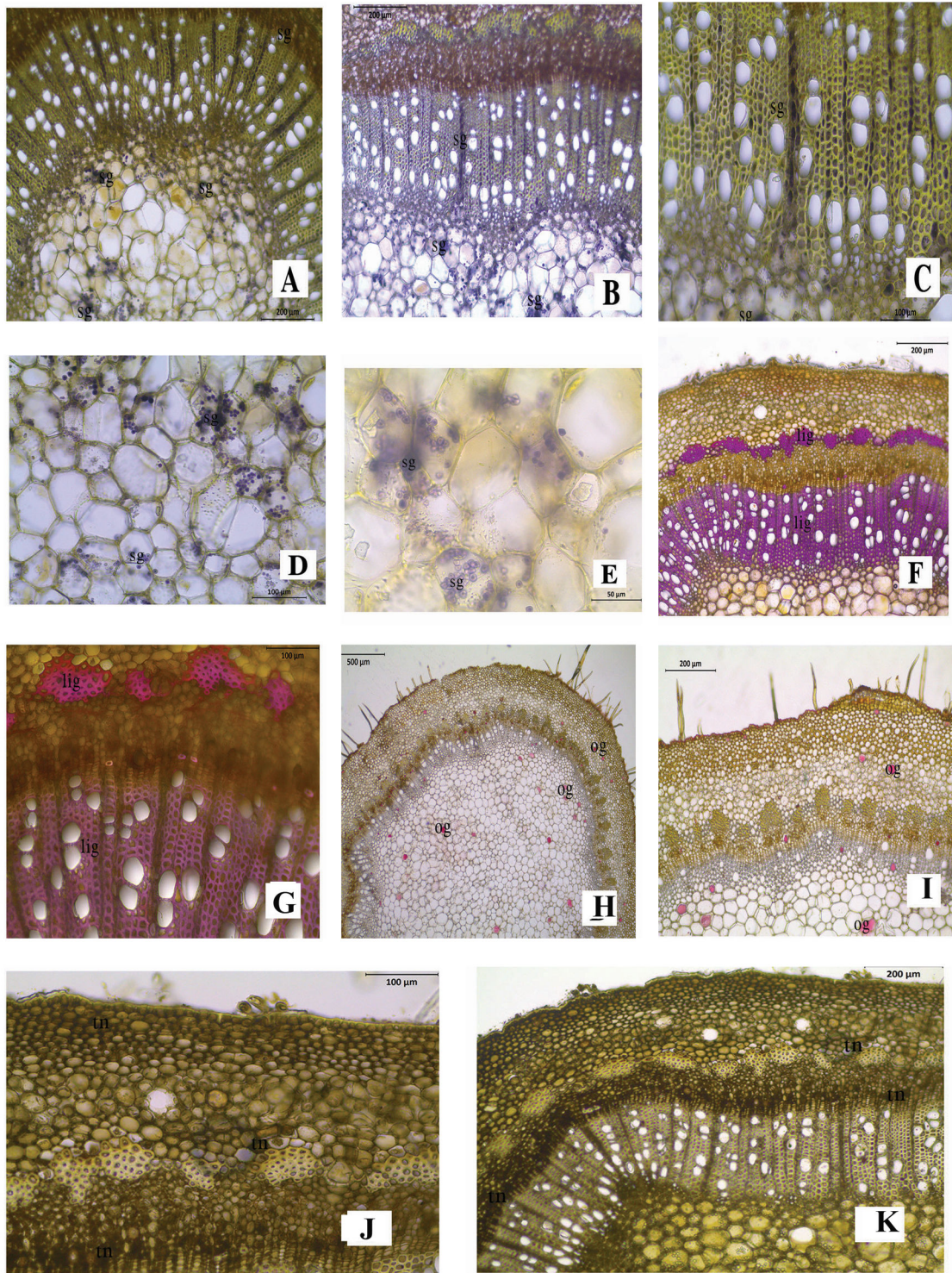


Fig 18 Histochemical localization of *L. coriacea* stem. A-E. Starch; F&G. Lignin; H&I. Fixed oil; J&K. Tannin

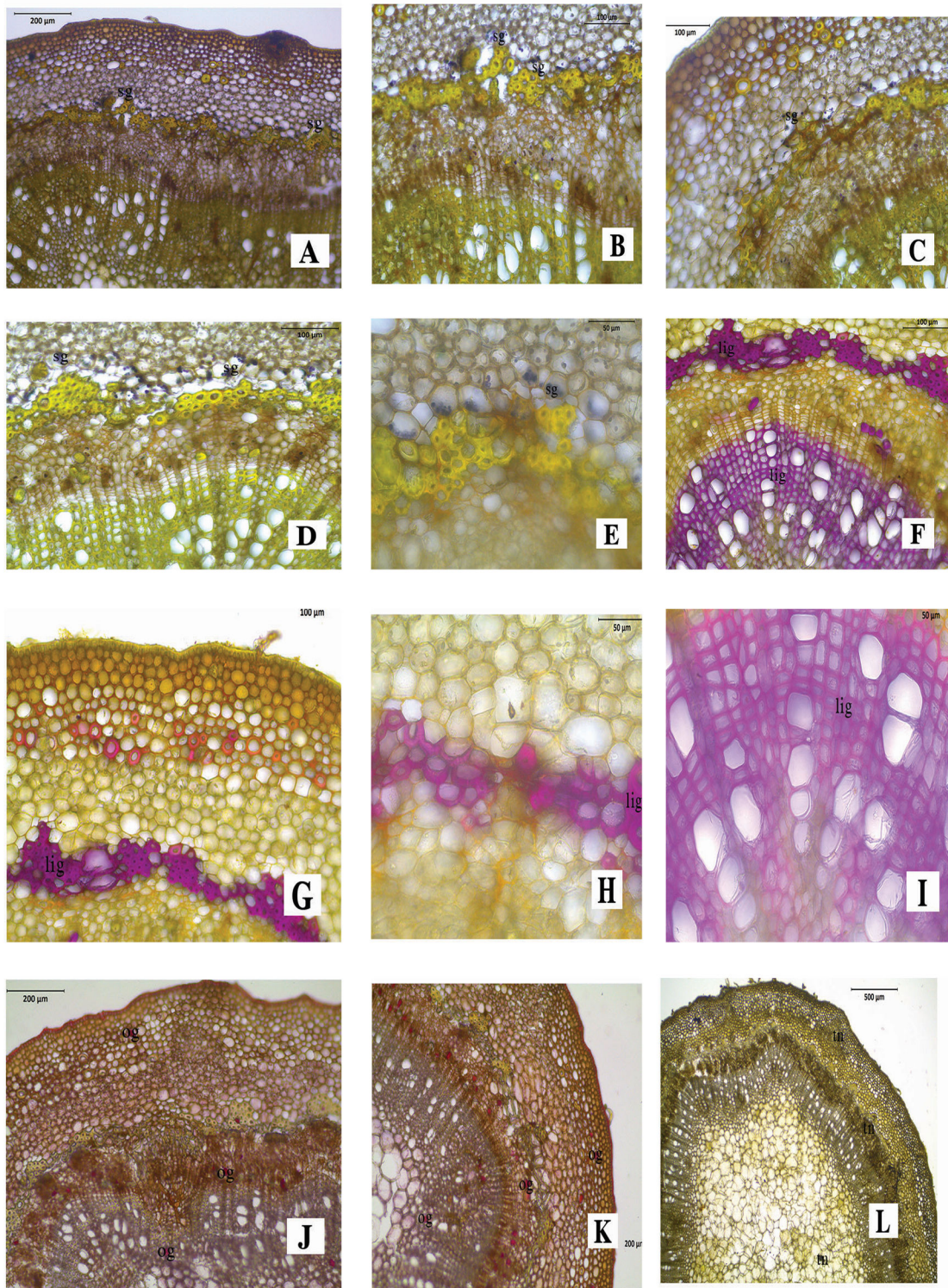


Fig 19 Histochemical localization of *L.wightiana* stem. A-E. Starch; F-I. Lignin; J&K Fixed oil; L. Tannin

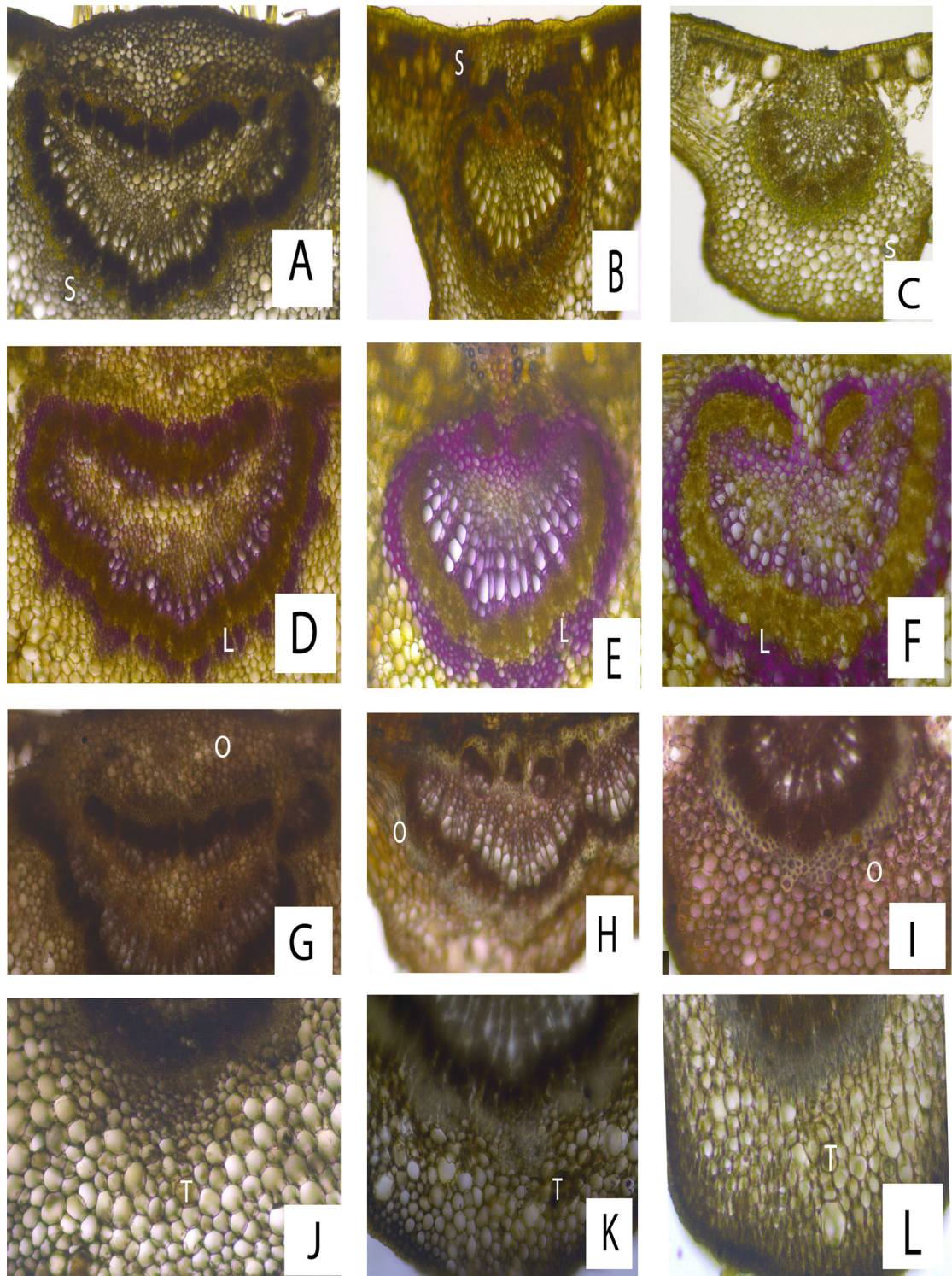


Fig 20 Histochemical localization of leaf of *L.bourdillonii*, *L.coriacea*, &*L.wightiana*. A-C. Starch; D-F. Lignin; G-I. Fixed oil; J-L. Tannin

Table 14 Comparative histochemical characterization of the leaves of three *Litsea* spp.

Test for histochemicals	<i>L. bourdillonii</i>	<i>L. coriacea</i>	<i>L. wightiana</i>
Starch	Absent	Present in cortical cells in the midrib region	A few deposits in cortical cells in the midrib region
Lignin	Present in the bundle sheath and xylem tracheids	Abundant in the bundle sheath and xylem, and a few in mesophyll cells	Present in the midrib, hypodermis, bundle sheath, and xylem wall
Tannins	Present in the midrib hypodermis and outer epidermal cells	Present in the midrib hypodermis. More than that in <i>L. bourdillonii</i>	Abundant in the midrib hypodermis. More than that in <i>L. coriacea</i> and <i>L. bourdillonii</i>
Fixed oils	Absent	High deposition of oil in mesophyll, hypodermis, and cortical cells in the midrib	Present in mesophyll and cortex but less than that in <i>L. coriacea</i>

d. Physicochemical study

Physicochemical analysis was performed on powder samples of *L. bourdillonii*, *L. coriacea* and *L. wightiana* leaves and bark samples. According to the study the percentage of water-soluble extractive, alcohol soluble extractive, crude fiber, total ash, acid-insoluble ash and sulphated ash in the leaves and bark samples varied noticeably according to the comparative physicochemical study. In leaves, the extractive values of both water and alcohol-soluble extracts were found to be higher in *L. wightiana* (10.36% and 10.76%) compared to those of *L. coriacea* (8.53% and 5.42%), and *L. bourdillonii* (6.71% and 5.97%), respectively. The total ash content was found to be high in *L. coriacea* (7.60%), followed by *L. bourdillonii* (6.60%), and *L. wightiana* (3.68%). The acid-insoluble ash value was 0.94% in *L. bourdillonii*, 0.54% in *L. coriacea* and 0.88% in *L. wightiana*. The sulphated ash and crude fiber content were observed to be 4.13% and 4.40% respectively in *L. wightiana*, 3.94% and 3.83% in *L. bourdillonii*, and comparatively lower in *L. coriacea* (1.96% and 3.17%) (Table 15).

In the bark samples, the water-soluble extractive values were found to be higher in *L. bourdillonii* (6.55%) compared to *L. wightiana* (4.63%) and *L. coriacea* (3.67%). At the same time, the alcohol-soluble extractives were higher in *L. wightiana* (6.07%) compared to *L. bourdillonii* (2.47%) and *L. coriacea* (1.59%). The total ash content was 24.64% in *L. coriacea* and 22.4% and 20.20% in *L. wightiana* and *L. bourdillonii*, respectively. The acid-insoluble ash was found higher in *L. wightiana* (0.59%) than in the other two species. In *L. bourdillonii*, the acid-insoluble ash was 0.54%, followed by 0.34% in *L. coriacea*. The total sulphated ash content was higher in *L. bourdillonii* (15.86%) followed by *L. coriacea* (9.62%) and *L. wightiana* (4.98%). Crude fibre content was also highest in *L. wightiana* (6.83%), followed by *L. coriacea* (5.2%) and *L. bourdillonii* (2.2%) (Table 16).

Table 15. Comparative physicochemical characterization of the leaves of three *Litsea* spp.

Parameters	<i>L. bourdillonii</i>	<i>L. coriacea</i>	<i>L. wightiana</i>
Water-soluble extractive	6.71%	8.53%	10.36%
Alcohol-soluble extractive	5.97%	5.42%	10.76%
Total ash	6.60%	7.60%	3.68%
Acid-insoluble ash	0.94%	0.54%	0.88%
Sulphated ash	3.94%	1.96%	4.13%
Crude fibre	3.83%	3.17%	4.40%

Table 16. Comparative physicochemical characterization of the bark of three *Litsea* spp.

Parameters tested	<i>L. bourdilonii</i>	<i>L. coriacea</i>	<i>L. wightiana</i>
Water-soluble extractive	6.55%	3.67%	4.63%
Alcohol-soluble extractive	2.47%	1.59%	6.07%
Total ash	20.20%	24.64%	22.41%
Acid-insoluble ash	0.54%	0.34%	0.59%
Sulphated ash	15.86%	9.62%	4.98%
Crude fibre	2.2%	5.2%	6.83%

e. Powder microscopic study

The stem bark of the three *Litsea* plants in powder form was subjected to powder characterization. The studies revealed the occurrence of cork cells, fibers, stone cells, starch grains and crystals in all three powdered stem barks and exhibited much similarity in structure. But the samples showed minor variations in powder characteristics like the shape of cells in sectional view, the nature of fibers, and the size of starch grains. It was observed that the fibers were long with a narrow lumen and pointed ends in *L. coriacea* and *L. wightiana*, but blunt ended in *L. bourdillonii* (Fig 21).

PHASE II. PHYTOCHEMICAL PROFILING

a. Determination of extractive value

Extracts of the leaves and bark samples of the three *Litsea* species selected for phytochemical studies were prepared by the Soxhlet extraction method. Methanol was used as the solvent. Before the phytochemical analysis, the extractive values of each sample were determined. The extractive value of *L. wightiana* bark was found to be higher (43.5%), followed by *L. bourdillonii* (35.65%) and *L. coriacea* (37%) leaf extracts. The least extractive value was found in *L. bourdillonii* bark (4.8%) (Table 17).

Table 17. Extractive values of the leaf and bark samples of three *Litsea* spp.

Sample	Weight of the sample (g)	Weight of dry extract (g)	Extractive value (%)
<i>L. bourdillonii</i> leaf	20	7.13	35.65
<i>L. coriacea</i> leaf	20	7.4	37
<i>L. wightiana</i> leaf	20	4.86	24.3
<i>L. bourdillonii</i> bark	20	0.96	4.8
<i>L. coriacea</i> brk	20	2.25	11.25
<i>L. wightiana</i> bark	20	8.7	43.5

Preliminary phytochemical screening of the methanolic leaf and bark extracts of the three *Litsea* plant samples were carried out both qualitatively and

quantitatively. The identification of non-volatile compounds was done by LC/MS-Q-TOF technique and GC/MS technique was adopted to identify the volatile compounds.

b. Qualitative analysis of phytochemicals

The results of qualitative phytochemical profiling of the leaves and bark showed the presence of phenols, flavonoids, tannins, and glycosides in *L. bourdillonii*, *L. coriacea* and *L. wightiana*. Alkaloids were absent in the bark of *L. wightiana*. Sterols and quinones were present in leaves of all the three *Litsea* spp. Terpenoids were found in leaves of all the three plants and the bark of *L. wightiana*. Fatty acid was observed in the leaves of *L. bourdillonii*, *L. coriacea* and *L. wightiana* and were absent in all other samples. The saponins were screened from the bark of *L. bourdillonii* and the leaves of *L. wightiana* (Table 18)

Table 18 Qualitative phytochemical screening of the methanolic extracts of three *Litsea* spp.

Sl. No	Class of phytochemicals	LBB	LBL	LCB	LCL	LWB	LWL
1	Phenols	+	+	+	+	+	+
2	Alkaloids	+	+	+	+	-	+
3	Flavonoids	+	+	+	+	+	+
4	Tannins	+	+	+	+	+	+
5	Glycosides	+	+	+	+	+	+
6	Phytosterols	-	+	-	+	-	+
7	Terpenoids	-	+	-	+	+	+
8	Saponins	+	-	-	-	-	+
9	Fatty acids	-	+	-	+	-	+
10	Quinones	-	+	-	+	-	+

(+) positive (Present), (-) negative (Absent)

LBB- *L. boudillonii* bark, LBL- *L. bourdillonii* leaf, LCB- *L. coriacea* bark, LCL- *L. coriacea* leaf, LWB- *L. wightiana* bark, LWL- *L. wightiana* leaf

c. Quantitative analysis of phytochemicals

The Aluminium chloride colourimetric method was used to assess the total flavonoid content and represented in terms of quercetin equivalent ($\mu\text{g QE/mg DW}$)

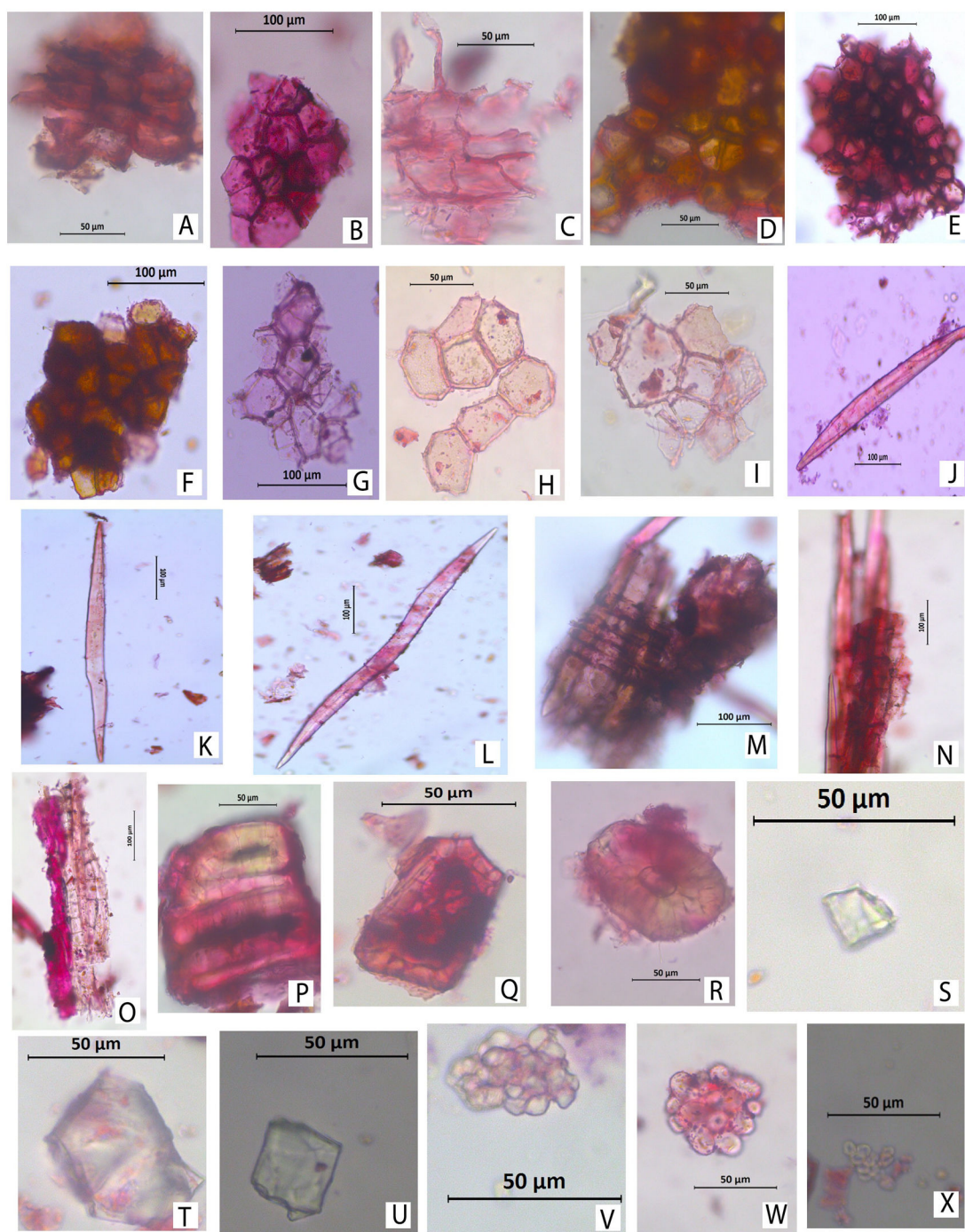


Fig 21 Powder microscopic characterization of stem bark of *L.bourdillonii*, *L.coriacea* & *L.wightiana*. A-C. Cork cell fragments in sectional view; D-F. Cork cell fragments in surface view; G-I. Cortical cells; J-L. Xylem fibres; M-O. Fibre crossing medullary rays; P-R. Stone cells; S-U. Prismatic crystals of calcium oxalate; V-X. Group of starch grains

as shown in Fig 22. The quantitative phytochemical estimation of methanolic extracts of leaves and bark extracts of selected *Litsea* samples showed that the total flavonoid content was high in *L. wightiana* leaf extracts ($37 \pm 0.04 \mu\text{g}/\text{mg}$) and lower in the bark of *L. bourdillonii* ($4 \pm 0.05 \mu\text{g}/\text{mg}$). Phenol content was quantified by the Folin-Ciocalteu method and expressed in terms of gallic acid equivalent (μg GAE/mg DW) as represented in Fig 23. The phenol content was observed to be higher in *L. wightiana* leaves ($119.14 \pm 0.26 \mu\text{g}/\text{mg}$) and lower in the bark of *L. coriacea* ($8.14 \pm 0.20 \mu\text{g}/\text{mg}$).

The tannin estimation was carried out by the Folin-Ciocalteu method and expressed in terms of tannic acid equivalent (μg TA/mg DW) as shown in Fig 24. The higher amount of tannin was noticed in the leaves of *L. wightiana* ($294.66 \pm 0.42 \mu\text{g}/\text{mg}$) and a lesser amount in the bark of *L. coriacea* ($21.5 \pm 0.04 \mu\text{g}/\text{mg}$) (Table 19 & Fig 25). The quantitative analysis revealed that the selected plant parts contained enough phytochemicals that may provide significant therapeutic value for the plants.

Table 19 Quantitative phytochemical analysis of the methanolic extracts of three *Litsea* spp.

Plant extracts	Flavonoids (μg QE/mg DW) $\pm$ SD	Phenols (μg GAE/mg DW) $\pm$ SD	Tannins (μg TAE/mg DW) $\pm$ SD
LBB	4 ± 0.05	79 ± 0.04	173 ± 0.03
LBL	25 ± 0.05	82.42 ± 0.30	163.5 ± 0.36
LCB	15 ± 0.52	8.14 ± 0.20	21.5 ± 0.04
LCL	29 ± 0.03	19.2 ± 0.15	30 ± 0.42
LWB	32 ± 0.04	21.4 ± 0.15	30.5 ± 0.30
LWL	37 ± 0.04	119.14 ± 0.26	294.66 ± 0.42

LBB- *L. boudillonii* bark, LBL- *L. bourdillonii* leaf, LCB- *L. coriacea* bark, LCL- *L. coriacea* leaf, LWB- *L. wightiana* bark, LWL- *L. wightiana* leaf. Values expressed as mean $\pm$ SD

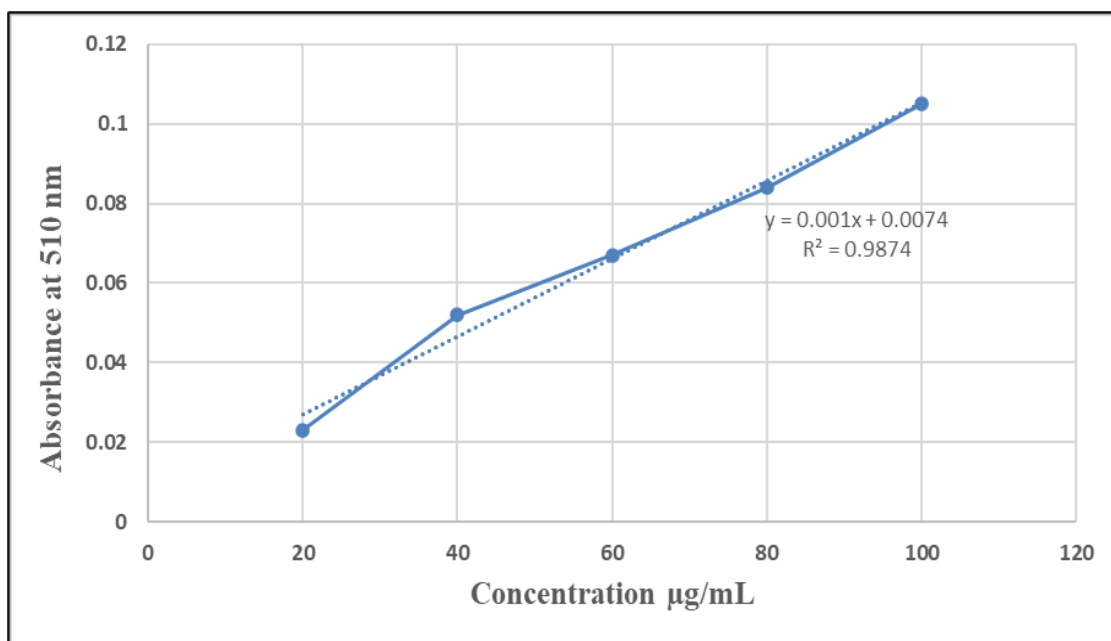


Fig 22 Standard graph of quercetin

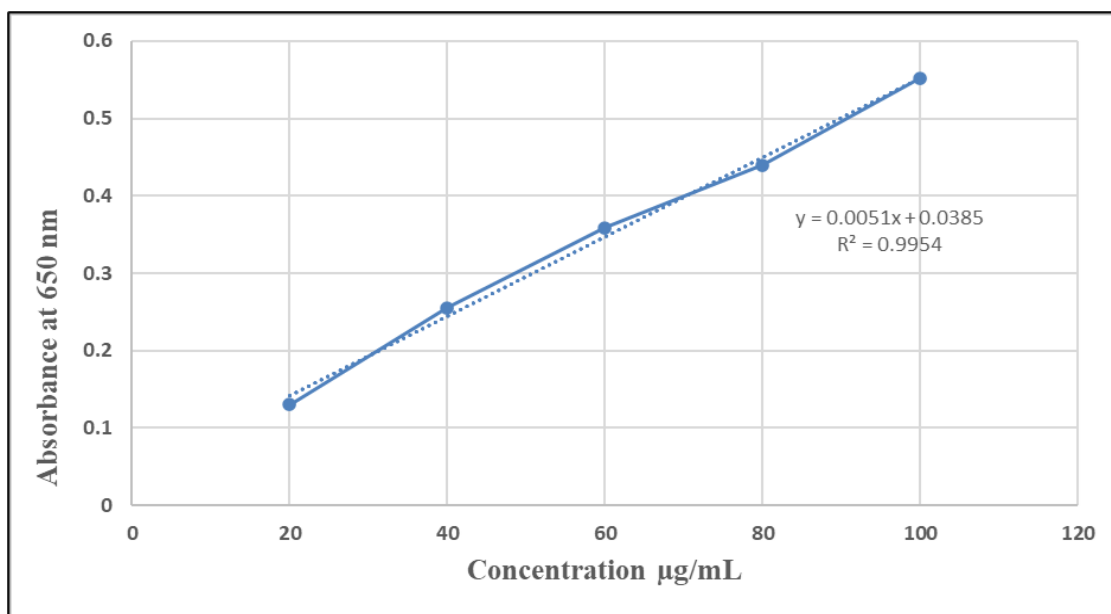


Fig 23 Standard graph of gallic acid

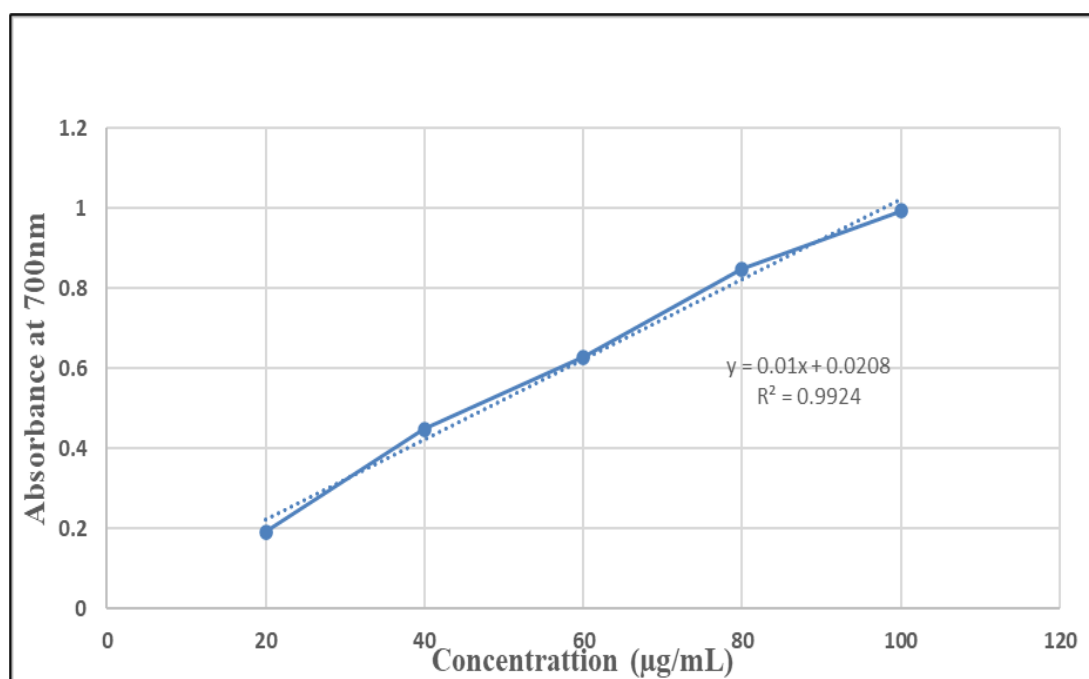


Fig 24 Standard graph of tannic acid

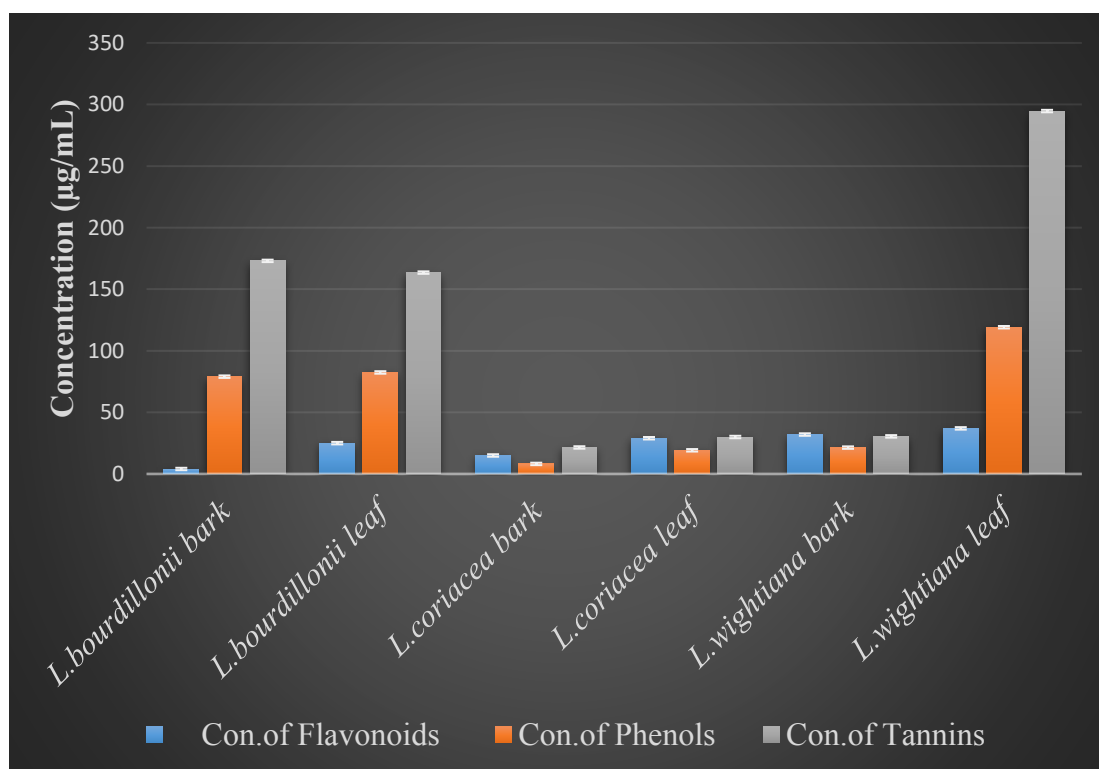
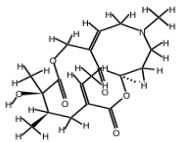
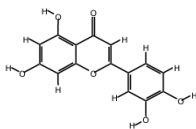
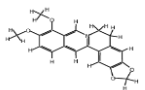


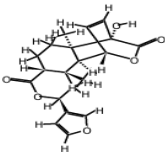
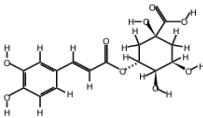
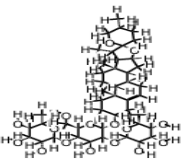
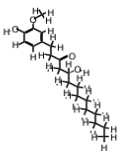
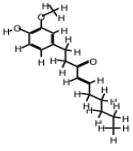
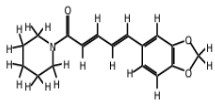
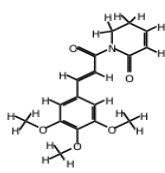
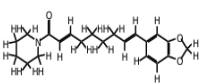
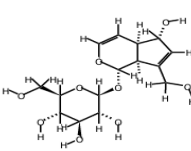
Fig 25 Quantitative analysis of phytochemicals

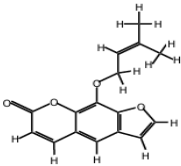
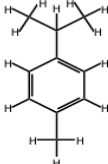
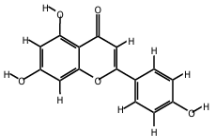
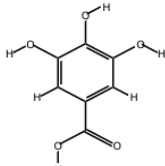
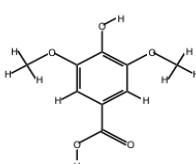
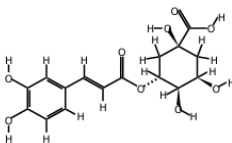
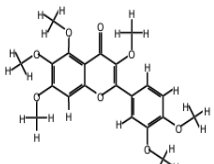
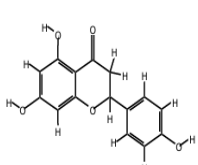
d. Liquid chromatography and mass spectrometric analysis

Liquid chromatography and mass spectrometric analysis was used for the identification of non-volatile compounds in the leaves of *L. bourdillonii*, *L. coriacea* and *L. wightiana*. LC/MS Quadrupole Time-of-Flight (Q-TOF) method was adopted. The results are given in Tables 20, 21 & 22 with the details of compounds like name, structure, molecular formula, retention time and mass. LC/MS chromatograms and mass spectra are depicted in Figures 26 & 27 (i-iv), 28 & 29 (i-v), 30 & 31 (i-iv). *L. bourdillonii* and *L. coriacea* showed the most similarity in the composition of compounds compared to *L. wightiana*. Compounds such as myristic acid, shogaol, columbin, p-cymene, chlorogenic acid, syringic acid, gingerol, luteolin, aucubin, apigenin and hexamethyl quercetagenin were found in *L. bourdillonii* and *L. coriacea* leaves and lauric acid were found in the leaves of three plants. The compounds such as senkirkine, berberine, dioscin, hesperidin are the phytochemicals present only in *L. bourdillonii* leaves. Caffeic acid, magnoflorine, diosgenin, and arbutin are the compounds present only in *L. coriacea*. Chalcone, corytuberine, dicentrinone, xanthoplanine, laurilitsine, isocorydine, reticuline, linalool are the specific compounds found in *L. wightiana* leaves.

Table 20 LC/MS analysis data of the leaves extract of *L. bourdillonii*

Sl. No	Name of compound	Structure	Molecular formula	RT (min)	Mass
1	Senkirkine		C ₁₉ H ₂₇ NO ₆	31.989	365.1845
2	Luteolin		C ₁₅ H ₁₀ O ₆	28.833	286.0453
3	Berberine		C ₂₀ H ₁₈ NO ₄	41.127	336.1229

4	Columbin		$C_{20}H_{22}O_6$	43.005	358.1367
5	Chlorogenic acid		$C_{16}H_{18}O_9$	41.775	354.0929
6	Dioscin		$C_{45}H_{72}O_{16}$	50.265	868.4798
7	(10)-Gingerol		$C_{21}H_{34}O_4$	35.861	350.2405
8	Shogaol		$C_{17}H_{24}O_3$	36.259	276.1716
9	Piperine		$C_{17}H_{19}NO_3$	20.675	285.1380
10	Piperlongumine		$C_{17}H_{19}NO_5$	3.812	317.1263
11	Pipernonaline		$C_{21}H_{27}NO_3$	41.592	341.1987
12	Aucubin		$C_{15}H_{22}O_9$	32.338	346.1267

13	Pentosalen		$C_{16}H_{14}O_4$	45.115	270.0863
14	p-Cymene		$C_{10}H_{14}$	29.514	134.1093
15	Apigenin		$C_{15}H_{10}O_5$	26.191	270.0516
16	Gallic acid		$C_7H_6O_5$	41.645	170.0224
17	Syringic acid		$C_9H_{10}O_5$	13.035	198.0501
18	Chlorogenic acid		$C_{16}H_{18}O_9$	37.691	354.0977
19	Hexamethyl quercetagenin		$C_{21}H_{22}O_8$	41.396	402.1265
20	5,7 – Dihydroxy-2-(4-hydroxyphenyl) Chroman-4-one		$C_{15}H_{12}O_5$	3.532	272.0699

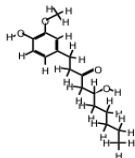
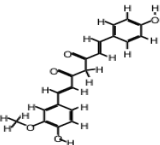
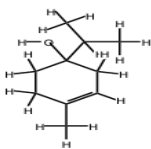
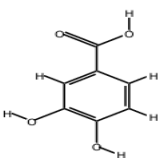
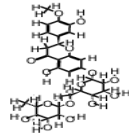
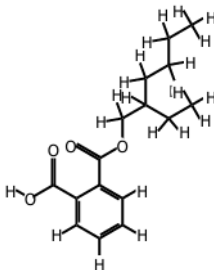
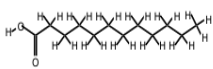
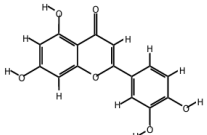
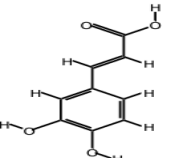
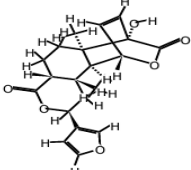
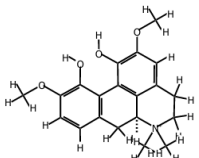
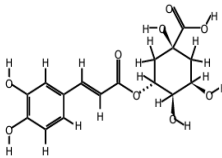
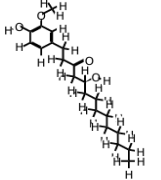
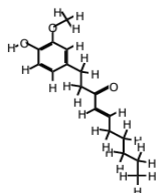
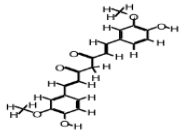
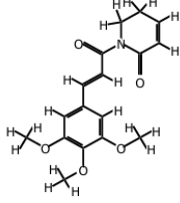
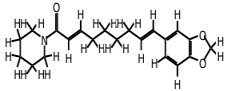
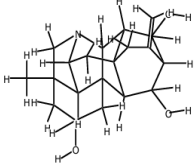
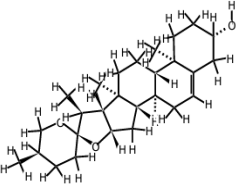
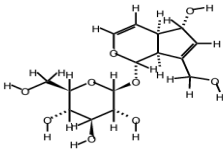
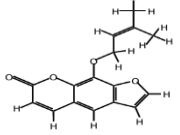
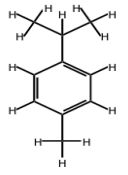
22	Gingerol		$C_{17}H_{26}O_4$	35.730	294.1786
23	Demethoxycurcumin		$C_{20}H_{18}O_5$	40.814	338.1139
24	4-Carvomenthenol		$C_{10}H_{18}O$	38.172	154.1351
25	3,4-Dihydroxybenzoic acid		$C_7H_6O_4$	9.812	154.0273
26	Hesperidin		$C_{28}H_{34}O_{15}$	24.084	610.1885
27	2-(((2-Ethylhexyl)oxy)carbonyl)benzoic acid		$C_{16}H_{22}O_4$	40.714	278.1484
28	Lauric acid		$C_{12}H_{24}O_2$	38.172	200.1770
29	Myristic acid		$C_{14}H_{28}O_2$	42.010	228.2090

Table 21 LC/MS analysis data of the leaves extract of *L. coriacea*

Sl. No	Name of compound	Structure	Molecular formula	RT (min)	Mass
1	Luteolin		C ₁₅ H ₁₀ O ₆	38.836	286.0477
2	Caffeic acid		C ₉ H ₈ O ₄	6.504	180.0466
3	Columbin		C ₂₀ H ₂₂ O ₆	42.840	358.1421
4	Magnoflorine		C ₂₀ H ₂₄ NO ₄	36.410	342.1705
5	Chlorogenic acid		C ₁₆ H ₁₈ O ₉	4.195	354.0960
6	(10)-Gingerol		C ₂₁ H ₃₄ O ₄	4.045	350.2426
7	Shogaol		C ₁₇ H ₂₄ O ₃	42.491	276.1726

8	Curcumin		$C_{21}H_{20}O_6$	42.009	368.1255
9	Piperlongumine		$C_{17}H_{19}NO_5$	3.813	317.1257
10	Pipernonaline		$C_{21}H_{27}NO_3$	40.082	341.1970
11	Hetisine		$C_{20}H_{27}NO_3$	21.241	329.1952
12	Diosgenin		$C_{27}H_{42}O_3$	21.241	329.1952
13	Aucubin		$C_{15}H_{22}O_9$	42.474	346.1264
14	Pentosalen		$C_{16}H_{14}O_4$	48.787	270.0862
15	p-Cymene		$C_{10}H_{14}$	24.614	1134.109 1

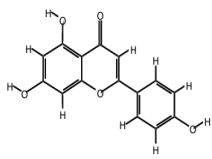
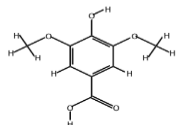
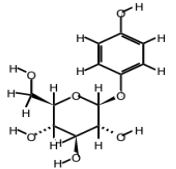
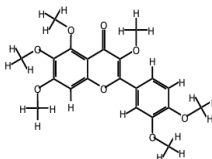
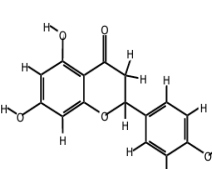
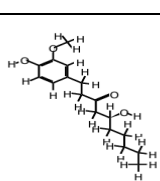
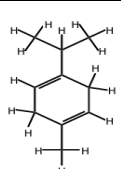
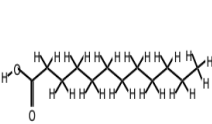
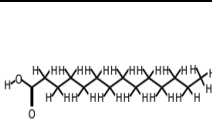
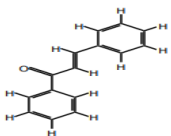
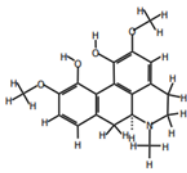
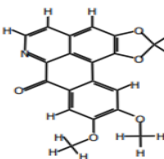
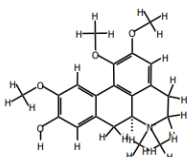
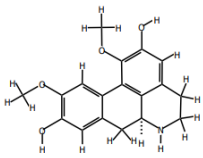
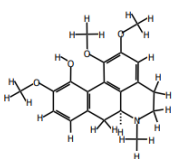
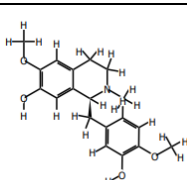
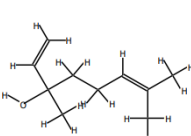
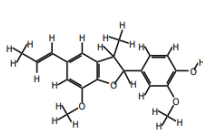
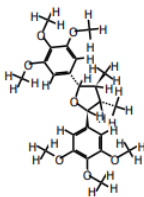
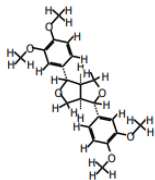
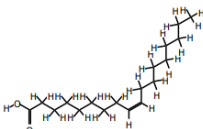
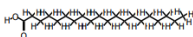
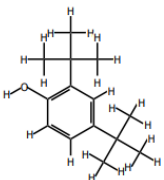
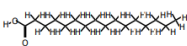
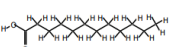
16	Apigenin		$C_{15}H_{10}O_5$	42.125	270.0528
17	Syringic acid		$C_9H_{10}O_5$	16.907	198.0528
18	Arbutin		$C_{12}H_{16}O_7$	24.251	272.0896
19	Hexamethylquercetin		$C_{21}H_{22}O_8$	30.514	402.1315
20	5,7-Dihydroxy-2(-4-hydroxyphenyl)chroman 4-one		$C_{15}H_{12}O_5$	24.001	272.0688
21	Gingerol		$C_{17}H_{26}O_4$	35.399	294.1837
22	Gamma-Terpinene		$C_{10}H_{16}$	41.064	136.1261
23	Lauric acid		$C_{12}H_{24}O_2$	37.260	200.1776
24	Myristic acid		$C_{14}H_{28}O_2$	37.077	228.2089

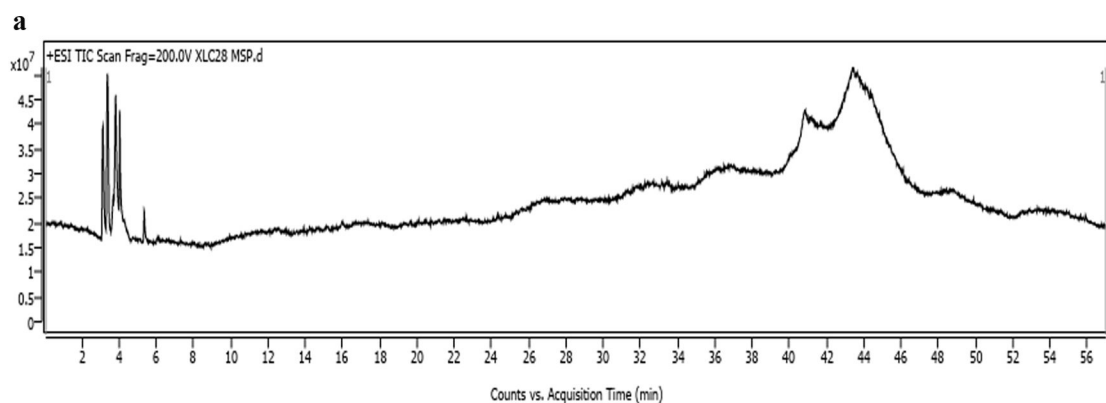
Table 22 LC/MS analysis data of the leaves extract of *L. wightiana*

Sl.No	Name of compound	Structure	Molecular formula	RT (min)	Mass
1	Chalcone		C ₁₅ H ₁₂ O	31.361	208.0875
2	Corytuberine		C ₁₉ H ₂₁ NO ₄	8.881	327.1467
3	Dicentrinone		C ₁₉ H ₁₃ NO ₅	19.215	335.0792
4	Xanthoplanine		C ₂₁ H ₂₆ NO ₄	33.936	356.1860
5	Lauro litsine		C ₁₈ H ₁₉ NO ₄	9.479	313.1320
6	Isocorydine		C ₂₀ H ₂₃ NO ₄	11.307	341.1621
7	Reticuline		C ₁₉ H ₁₉ NO ₄	9.263	329.1620
8	Linalool		C ₁₀ H ₁₈ O	1.936	154.1354
9	Dehydroisoeugenol		C ₂₀ H ₂₂ O ₄	12.437	326.1517

10	Grandisin		$C_{24}H_{32}O_7$	26.044	432.2124
11	(+)-Eudesmin		$C_{22}H_{26}O_6$	21.907	386.1731
12	Oleic acid		$C_{18}H_{34}O_2$	34.951	282.2579
13	Arachidic acid		$C_{20}H_{40}O_2$	34.835	312.303
14	2,4-Di- tert-butylphenol		$C_{14}H_{22}O$	33.838	206.1687
15	Palmitic acid		$C_{16}H_{32}O_2$	31.180	256.2416
16	Lauric acid		$C_{16}H_{32}O_2$	34.154	200.1788

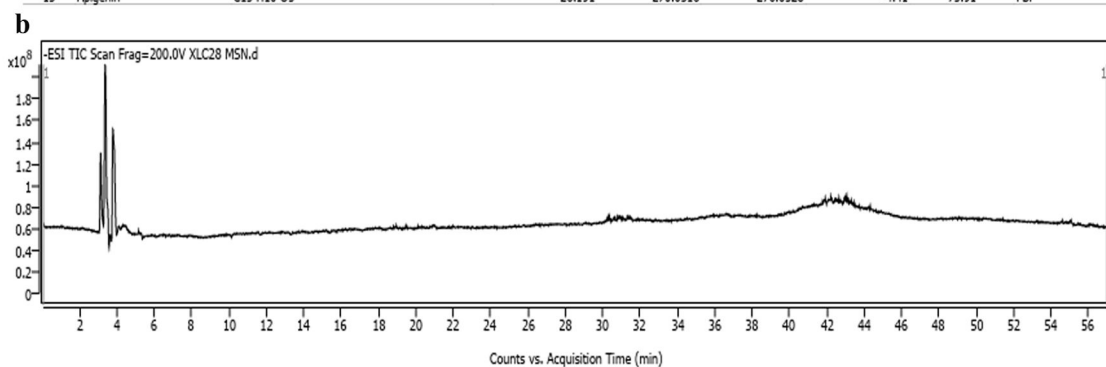
e. Gas chromatography and mass spectrometric analysis

The methanolic leaf extracts of all the three *Litsea* spp. were subjected to GC/MS analysis to find out the volatile compounds present in the samples. The GC/MS analysis of *L. wightiana* leaves revealed various compounds which include various classes of phytochemicals such as alkaloids, flavonoids and phenols (Table 23) Figures 32 & 33 (i-iv). The major compounds identified were Copaene (5.68%), 1H-cycloprop[e]azulene,decaydro-1,1,7-trimethyl-4-methylene(2.04%), naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1methylethyl)-(4.45%), naphthalene,1,2,4a,5,6,8a-hexahydro-4,7 dimethyl-1-(1-methylethyl)-



Compound Summary

Cpd	Name	Formula	CAS	RT	Mass	Mass (Tgt)	Diff (Tgt, ppm)	Score	Algorithm
1	Senkirkine	C19 H27 N O6		31.989	365.1845	365.1838	1.94	73.75	FBF
2	Luteolin	C15 H10 O6		28.833	286.0453	286.0477	-8.56	57.56	FBF
3	Berberine	C20 H18 N O4		41.127	336.1229	336.1236	-2.02	73.88	FBF
4	Columbin	C20 H22 O6		43.005	358.1367	358.1416	-13.72	57.98	FBF
5	Chlorogenic Acid	C16 H18 O9		41.775	354.0929	354.0951	-6.12	62.79	FBF
6	Dioscin	C45 H72 O16		50.265	868.4798	868.4820	-2.60	57.06	FBF
7	(10)-Gingerol	C21 H34 O4		35.861	350.2405	350.2457	-14.93	56.78	FBF
8	Shogaol	C17 H24 O3		36.259	276.1716	276.1725	-3.54	70.63	FBF
9	Piperine	C17 H19 N O3		20.675	285.1380	285.1365	5.42	65.42	FBF
10	Piperlongumine	C17 H19 N O5		3.812	317.1263	317.1263	-0.19	56.85	FBF
11	Pipernonaline	C21 H27 N O3		41.592	341.1987	341.1991	-1.25	73.75	FBF
12	Aucubin	C15 H22 O9		32.338	346.1267	346.1264	0.78	80.90	FBF
13	Pentosalen	C16 H14 O4		45.115	270.0863	270.0892	-10.76	60.50	FBF
14	p-CYMENE	C10 H14		29.514	134.1093	134.1096	-2.20	75.46	FBF
15	Apigenin	C15 H10 O5		26.191	270.0516	270.0528	-4.41	73.91	FBF



Compound Summary

Cpd	Name	Formula	CAS	RT	Mass	Mass (Tgt)	Diff (Tgt, ppm)	Score	Algorithm
1	Gallic acid	C7 H6 O5		41.645	170.0224	170.0215	5.10	58.11	FBF
2	Syringic acid	C9 H10 O5		13.035	198.0501	198.0528	-13.63	65.22	FBF
3	Chlorogenic acid	C16 H18 O9		37.691	354.0977	354.0951	7.34	61.75	FBF
4	Hexamethylquercetagenin	C21 H22 O8		41.396	402.1265	402.1315	-12.40	61.34	FBF
5	5,7-Dihydroxy-2-(4-hydroxyphenyl)chroman-4-one	C15 H12 O5		3.532	272.0699	272.0685	5.16	64.53	FBF
6	Gingerol	C17 H26 O4		35.730	294.1786	294.1831	-15.49	64.31	FBF
7	Demethoxycurcumin	C20 H18 O5		40.814	338.1139	338.1154	-4.44	80.03	FBF
8	4-Carvomenthenol	C10 H18 O		38.172	154.1351	154.1358	-4.23	74.20	FBF
9	4-Carvomenthenol	C10 H18 O		41.346	154.1367	154.1358	6.15	62.90	FBF
10	3,4-Dihydroxybenzoic acid	C7 H6 O4		9.812	154.0273	154.0266	4.28	70.16	FBF
11	Hesperidin	C28 H34 O15		24.084	610.1885	610.1898	-2.04	72.63	FBF
12	2-(((2-Ethylhexyl)oxy)carbonyl)benzoic acid	C16 H22 O4		40.714	278.1484	278.1518	-12.18	64.36	FBF
13	Lauroic acid	C12 H24 O2		38.172	200.1770	200.1776	-3.06	86.83	FBF
14	Myristic acid	C14 H28 O2		42.010	228.2090	228.2089	0.09	77.89	FBF

Fig 26 LC/MS chromatogram of *L.bourdillonii* leaf methanolic extract. a. ESI+ spectrum and compounds; b. ESI- spectrum and compounds

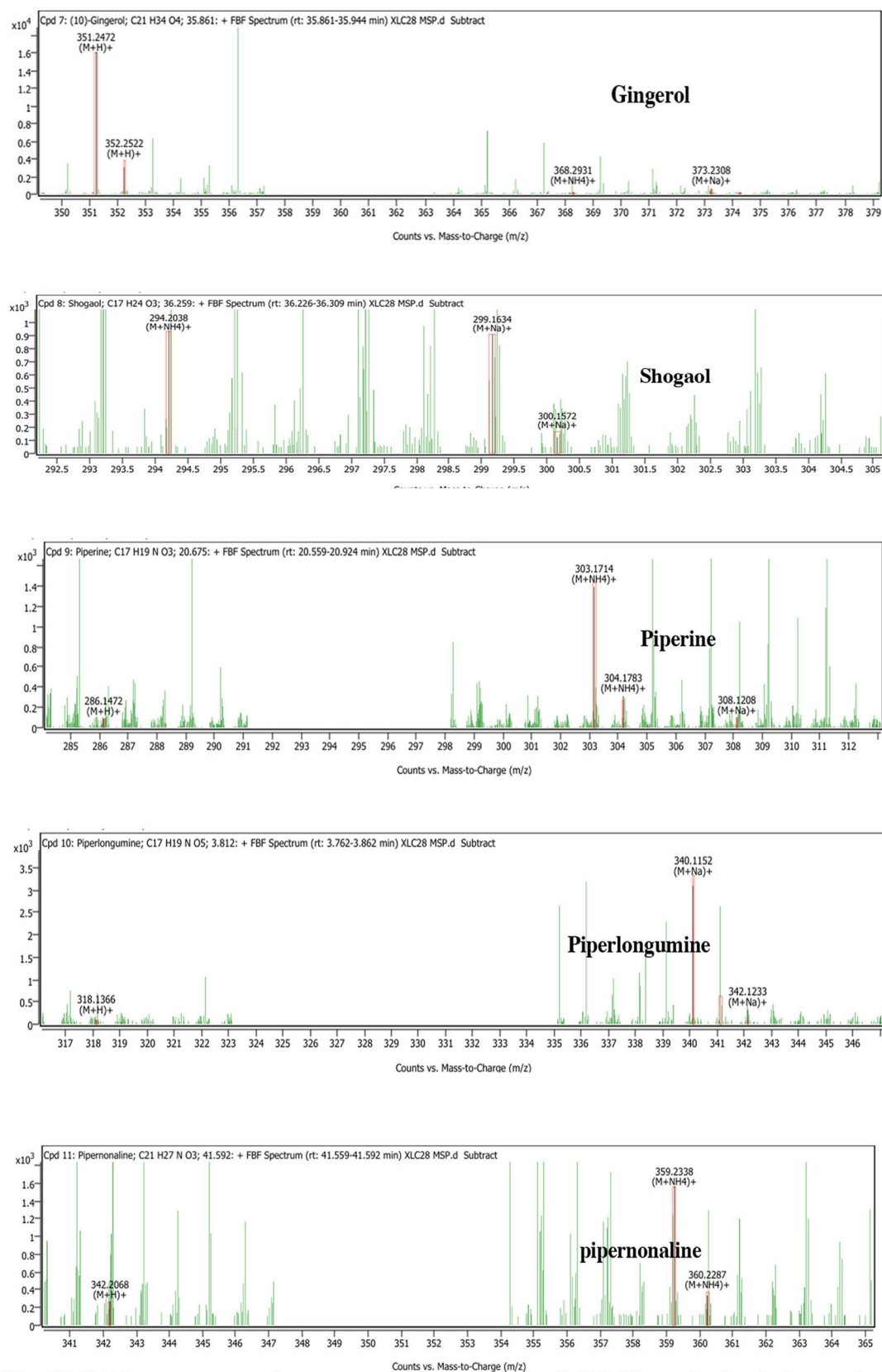


Fig 27(i) Mass spectra of compounds detected by LC/MS analysis of the methanolic extract of *L. bourdillonii* leaves

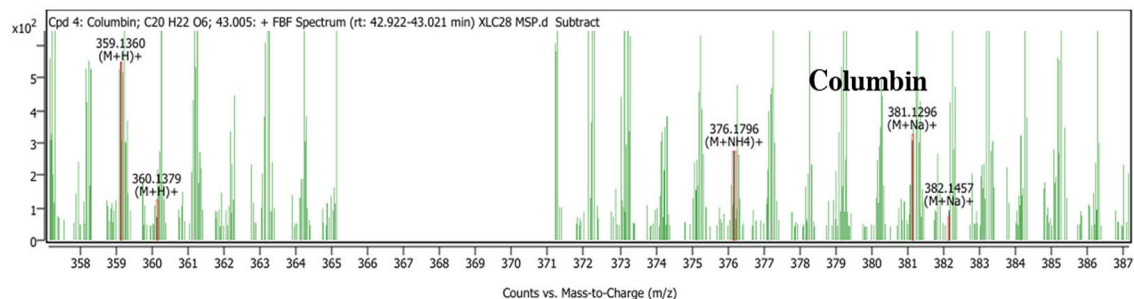
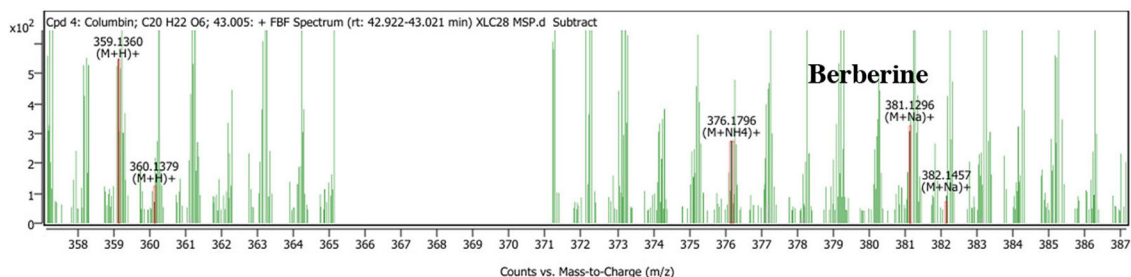
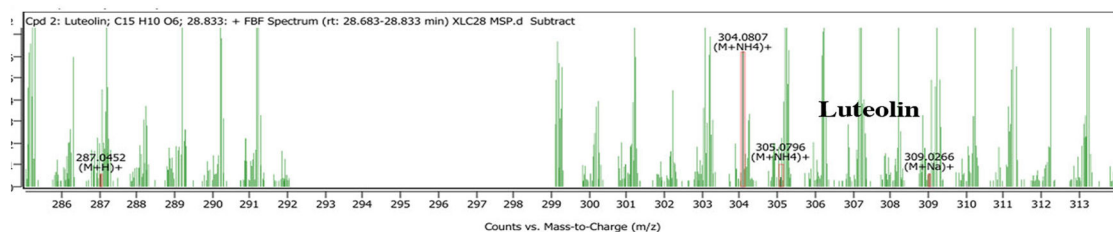
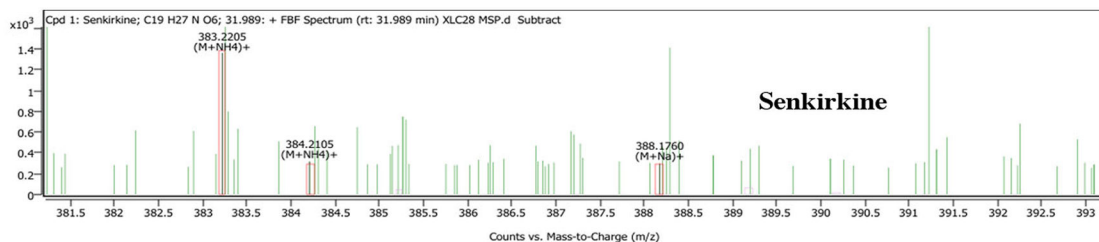
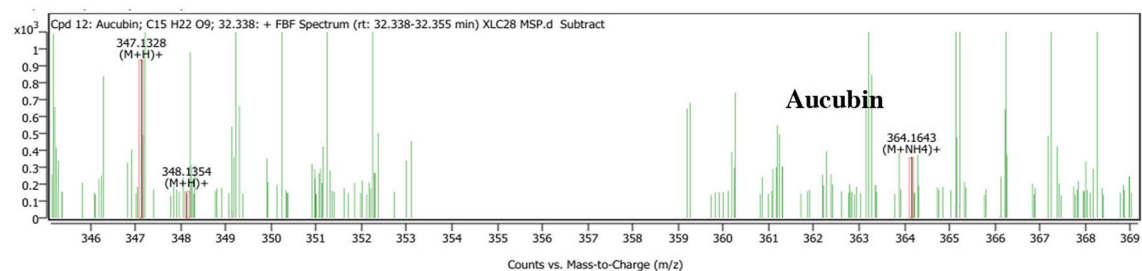


Fig 27(ii) Mass spectra of compounds detected by LC/MS analysis of the methanolic extract of *L.bourdillonii* leaves

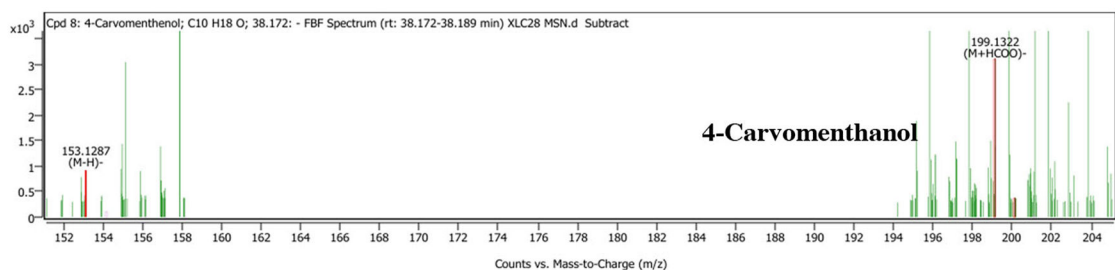
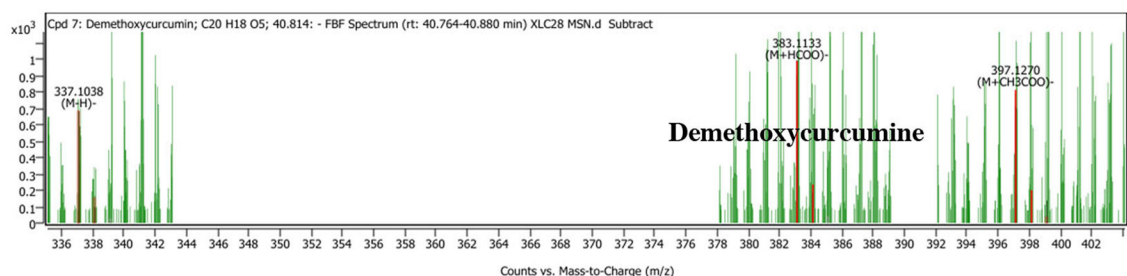
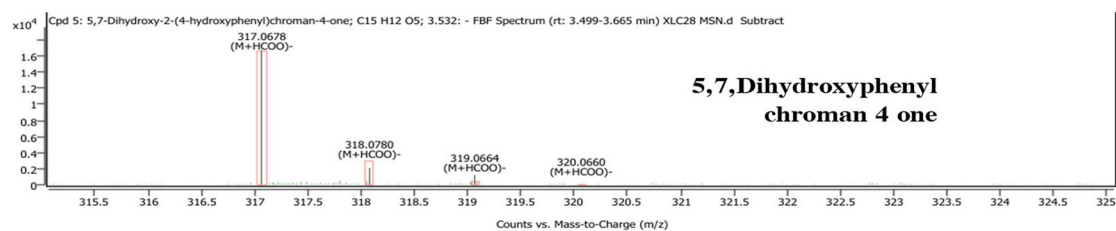
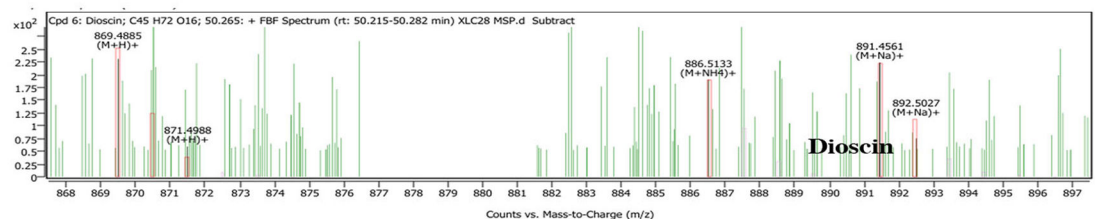
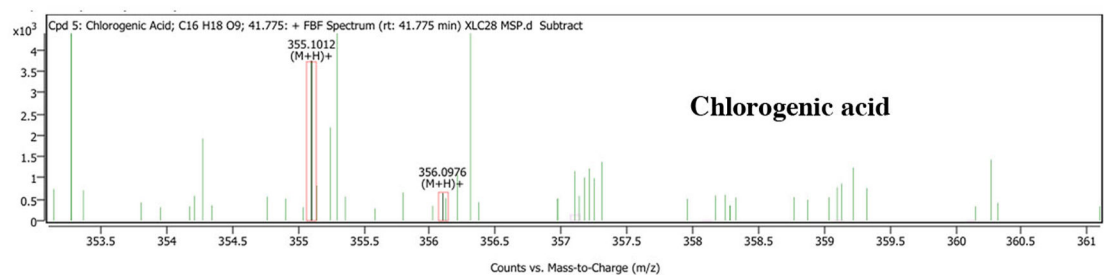


Fig 27(iii) Mass spectra of compounds detected by LC/MS analysis of the methanolic extract of *L.bourdillonii* leaves

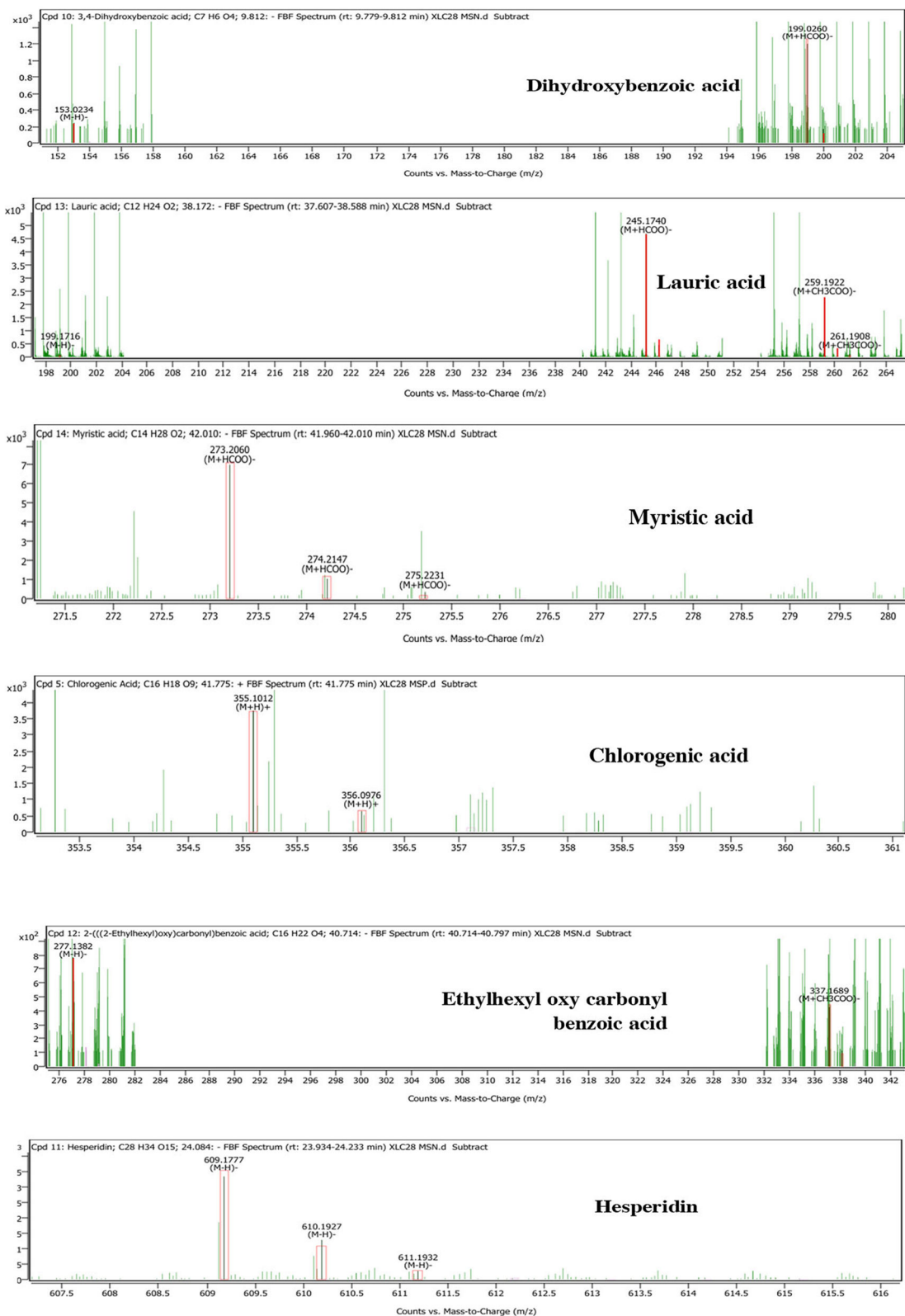
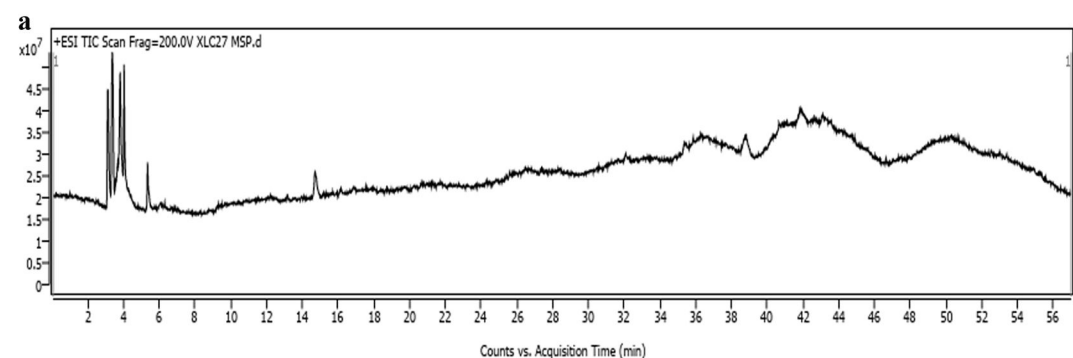
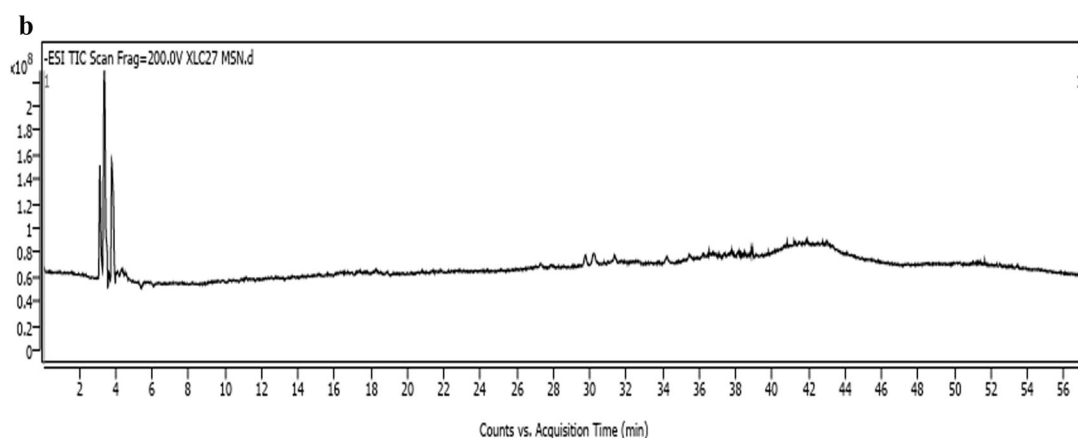


Fig 27(iv) Mass spectra of compounds detected by LC/MS analysis of the methanolic extract of *L.bourdillonii* leaves



Compound Summary

Cpd	Name	Formula	CAS	RT	Mass	Mass (Tgt)	Diff (Tgt, ppm)	Score	Algorithm
1	Luteolin	C15 H10 O6		38.836	286.0478	286.0477	0.34	98.12	FBF
2	Luteolin	C15 H10 O6		34.831	286.0466	286.0477	-3.99	88.97	FBF
3	Caffeic Acid	C9 H8 O4		6.504	180.0403	180.0423	-10.78	61.31	FBF
4	Columbin	C20 H22 O6		42.840	358.1421	358.1416	1.25	59.04	FBF
5	Magnoflorine	C20 H24 N O4		36.410	342.1722	342.1705	4.78	73.28	FBF
6	Chlorogenic Acid	C16 H18 O9		4.195	354.0960	354.0951	2.60	69.22	FBF
7	(10)-Gingerol	C21 H34 O4		4.045	350.2426	350.2457	-8.79	63.82	FBF
8	Shogaol	C17 H24 O3		42.491	276.1726	276.1725	0.24	65.53	FBF
9	(10)-Shogaol	C21 H32 O3		41.809	332.2272	332.2351	-23.77	65.39	FBF
10	Curcumin	C21 H20 O6		42.009	368.1255	368.1260	-1.35	56.29	FBF
11	Piperlongumine	C17 H19 N O5		3.813	317.1257	317.1263	-2.05	62.33	FBF
12	Piperonaline	C21 H27 N O3		40.082	341.1970	341.1991	-6.20	58.82	FBF
13	Hetisine	C20 H27 N O3		21.241	329.1952	329.1991	-11.68	55.69	FBF
14	Diosgenin	C27 H42 O3		35.911	414.3149	414.3134	3.57	55.13	FBF
15	Aucubin	C15 H22 O9		42.474	346.1292	346.1264	8.11	56.65	FBF
16	Pentosalen	C16 H14 O4		48.787	270.0862	270.0892	-11.31	83.93	FBF
17	p-CYMENE	C10 H14		24.614	134.1091	134.1096	-3.51	74.21	FBF
18	Apigenin	C15 H10 O5		42.125	270.0523	270.0528	-1.90	84.10	FBF



Compound Summary

Cpd	Name	Formula	CAS	RT	Mass	Mass (Tgt)	Diff (Tgt, ppm)	Score	Algorithm
1	Syringic acid	C9 H10 O5		16.907	198.0510	198.0528	-9.37	67.85	FBF
2	Arbutin	C12 H16 O7		24.251	272.0916	272.0896	7.17	71.72	FBF
3	Hexamethylquercetagenin	C21 H22 O8		30.514	402.1303	402.1315	-2.95	67.50	FBF
4	5,7-Dihydroxy-2-(4-hydroxyphenyl)chroman-4-one	C15 H12 O5		24.001	272.0688	272.0685	1.05	61.94	FBF
5	Gingerol	C17 H26 O4		35.399	294.1837	294.1831	1.84	70.45	FBF
6	gamma-Terpinene	C10 H16		41.064	136.1261	136.1252	6.95	78.70	FBF
7	Lauric acid	C12 H24 O2		37.260	200.1757	200.1776	-9.84	80.94	FBF
8	Myristic acid	C14 H28 O2		37.077	228.2077	228.2089	-5.56	66.74	FBF

Fig 28 LC/MS chromatogram of *L. coriacea* leaf methanolic extract. a. ESI+ spectrum and compounds; b. ESI- spectrum and compounds

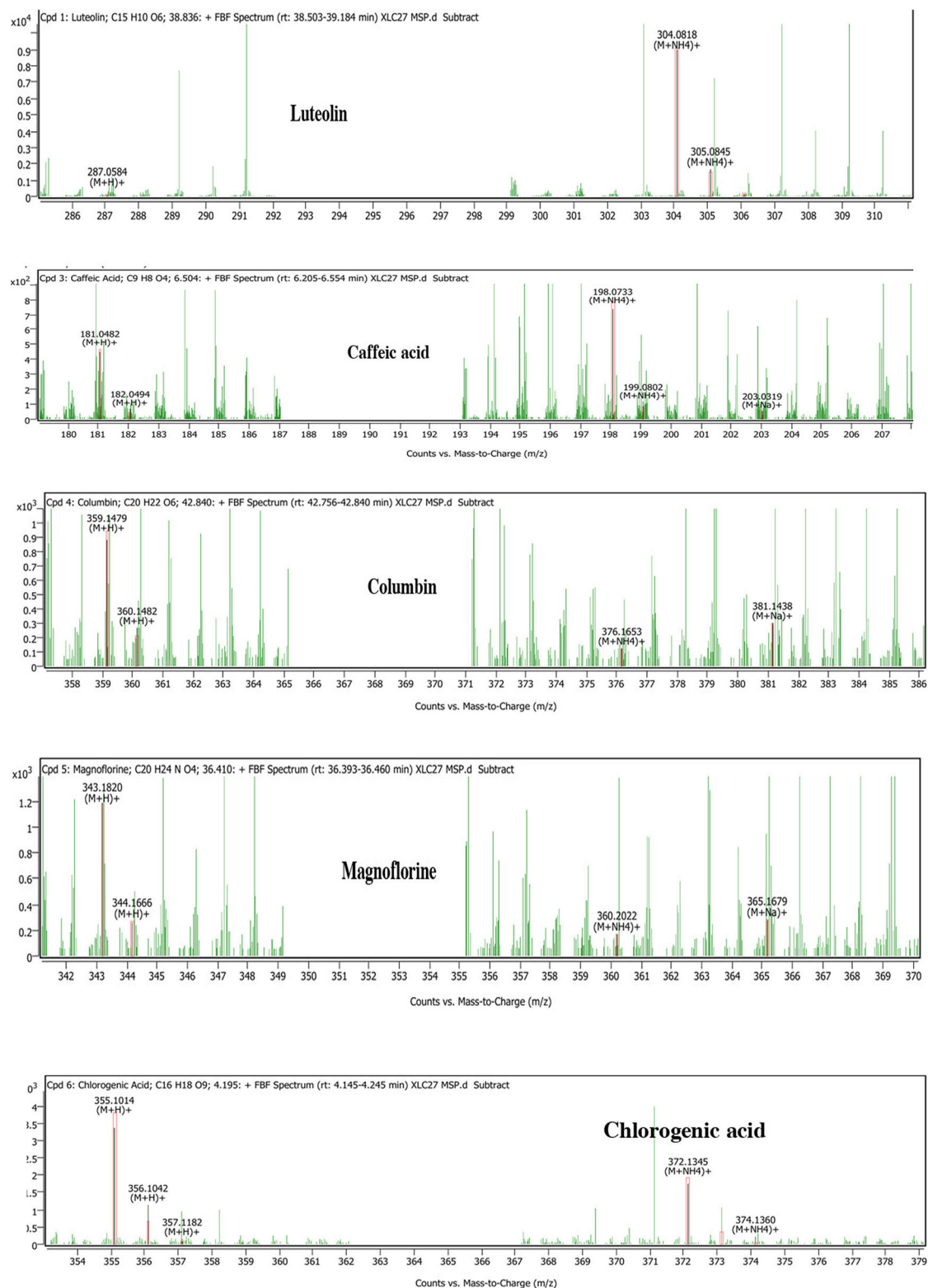


Fig 29(i) Mass spectra of compounds detected by LC/MS analysis of the methanolic extract of *L.coriaceae* leaves

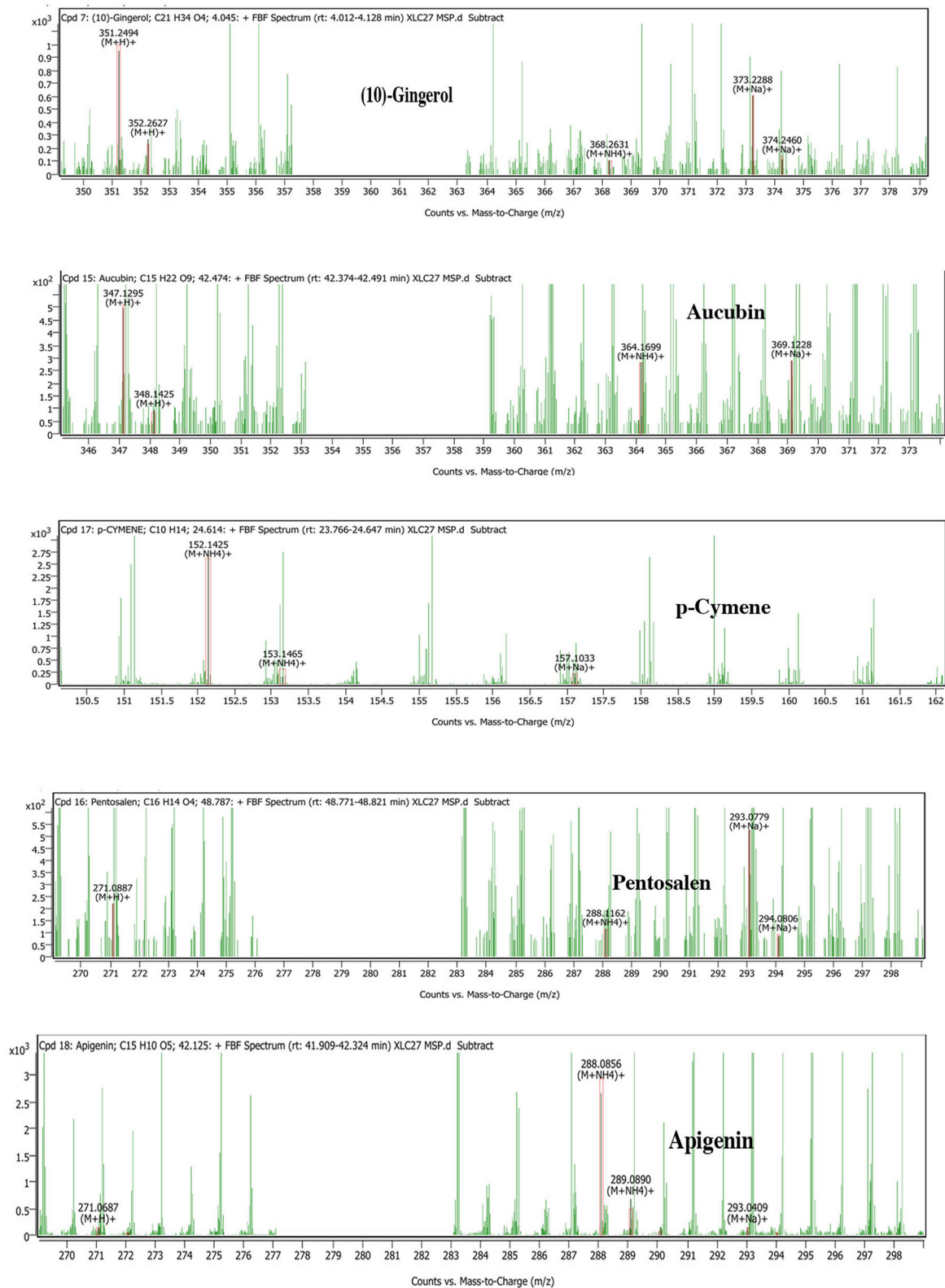


Fig 29(ii) Mass spectra of compounds detected by LC/MS analysis of the methanolic extract of *L.coriacea* leaves

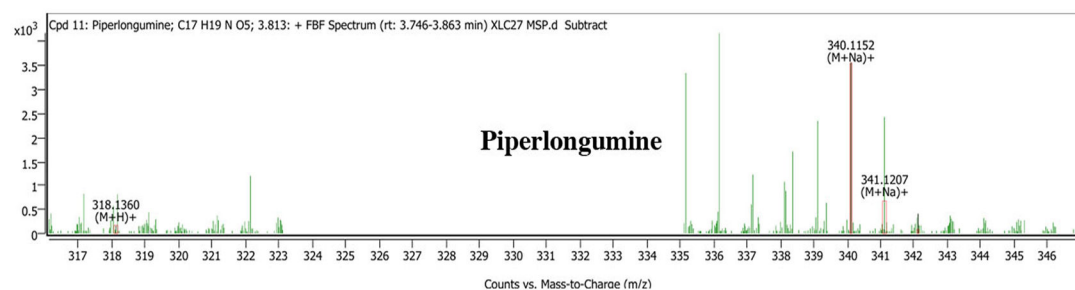
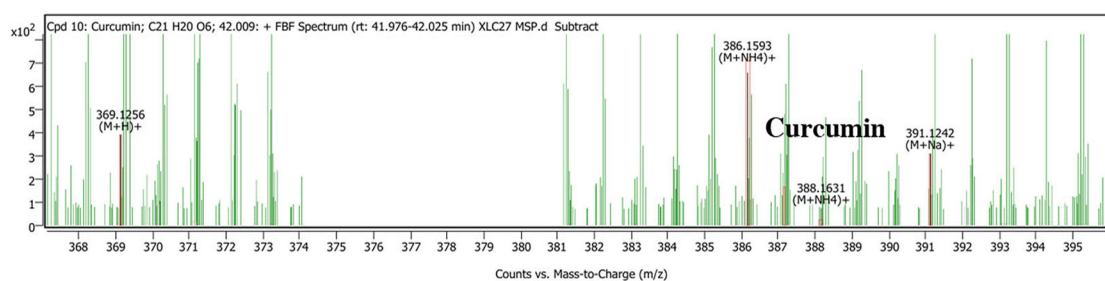
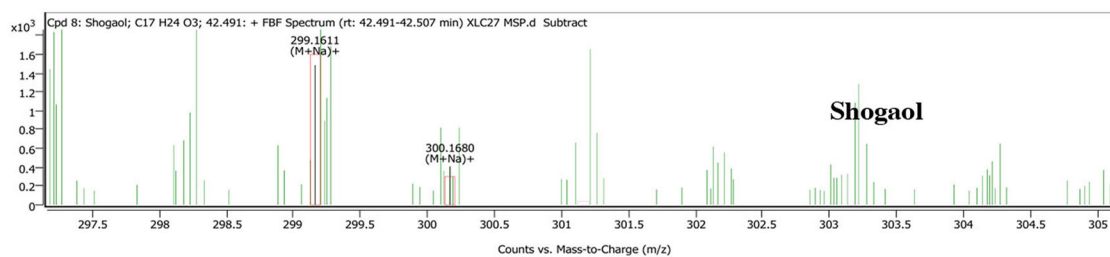
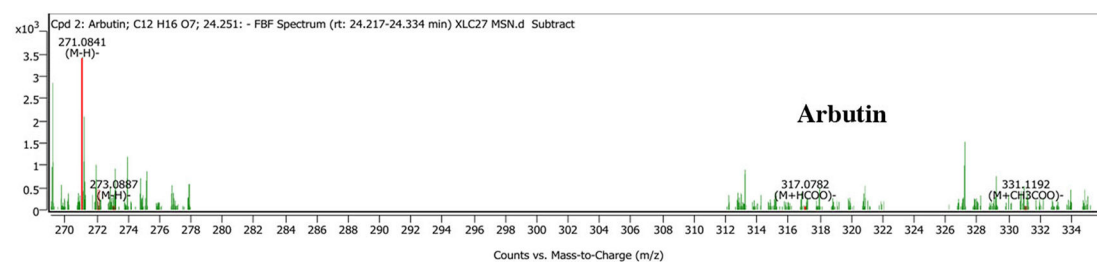
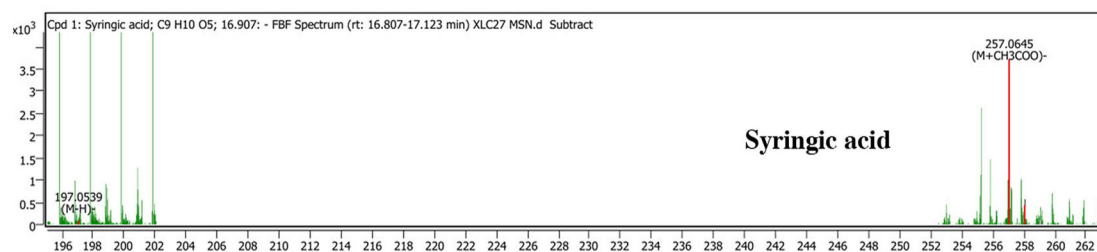


Fig 29(iii) Mass spectra of compounds detected by LC/MS analysis of the methanolic extract of *L.coriaacea* leaves

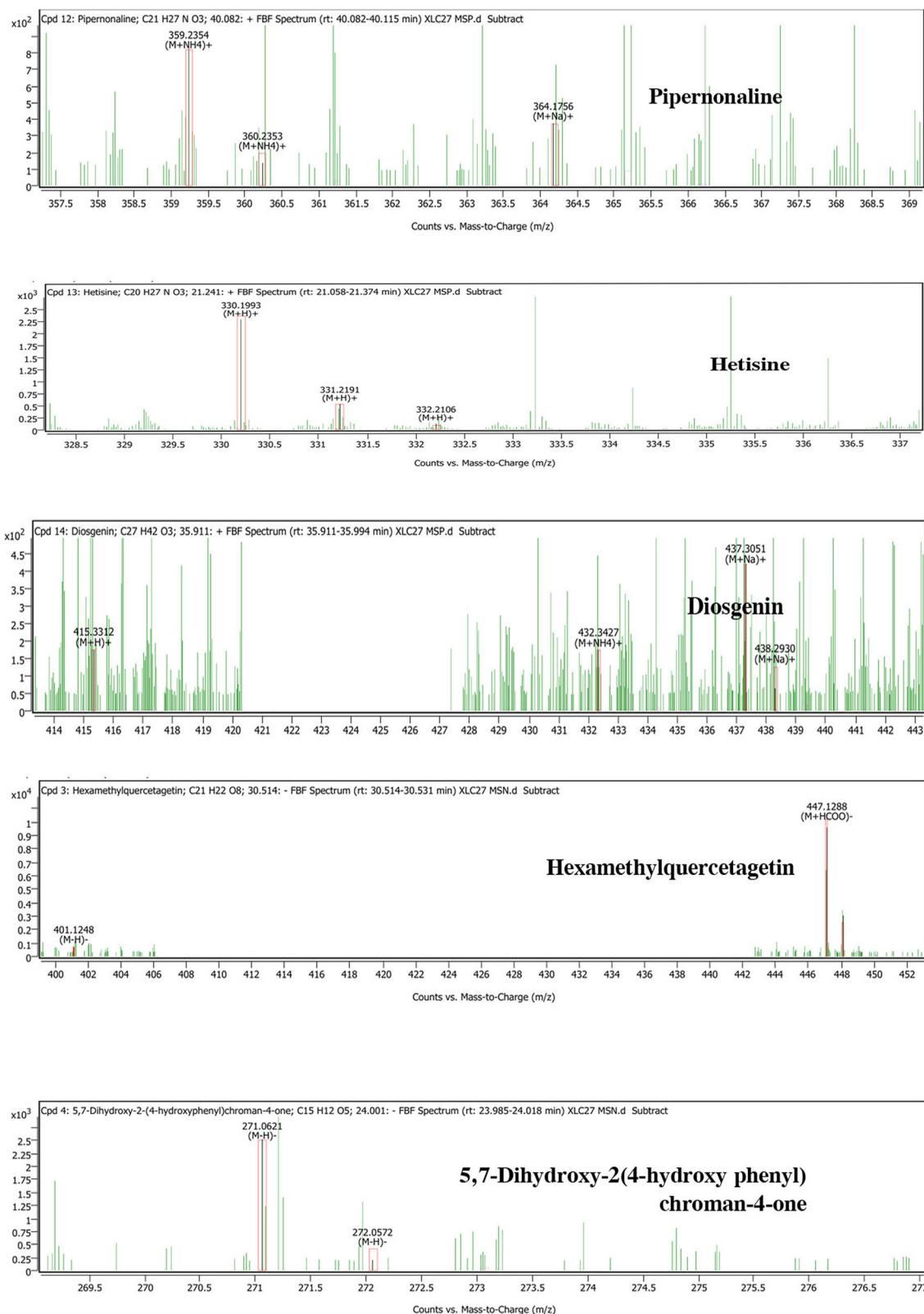


Fig 29(iv) Mass spectra of compounds detected by LC/MS analysis of the methanolic extract of *L. coriacea* leaves

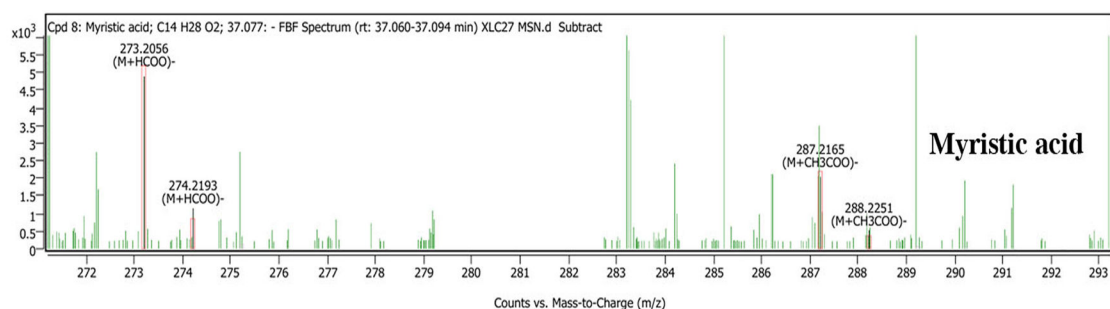
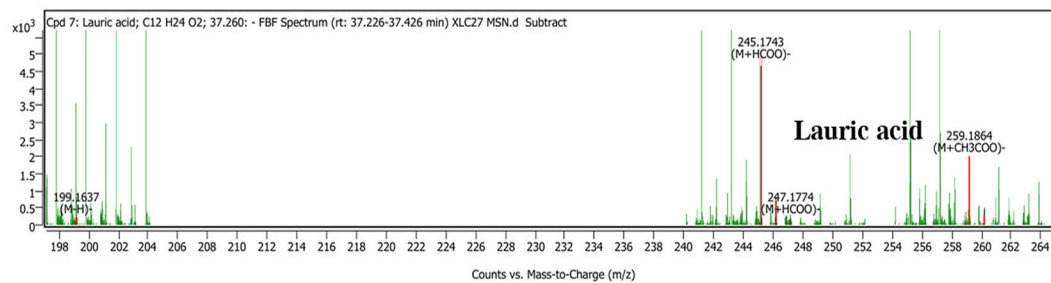
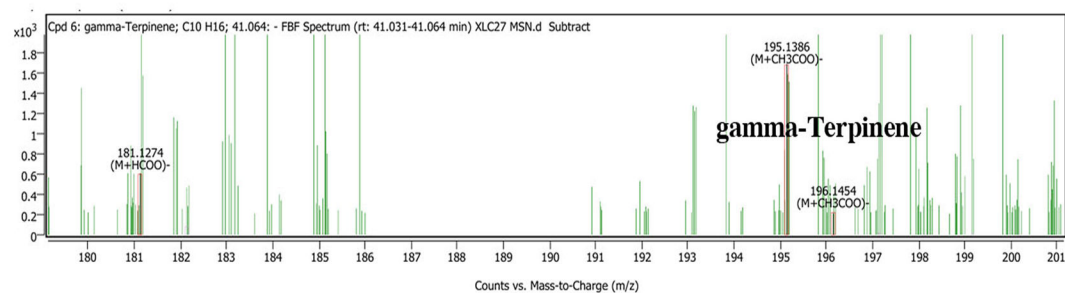
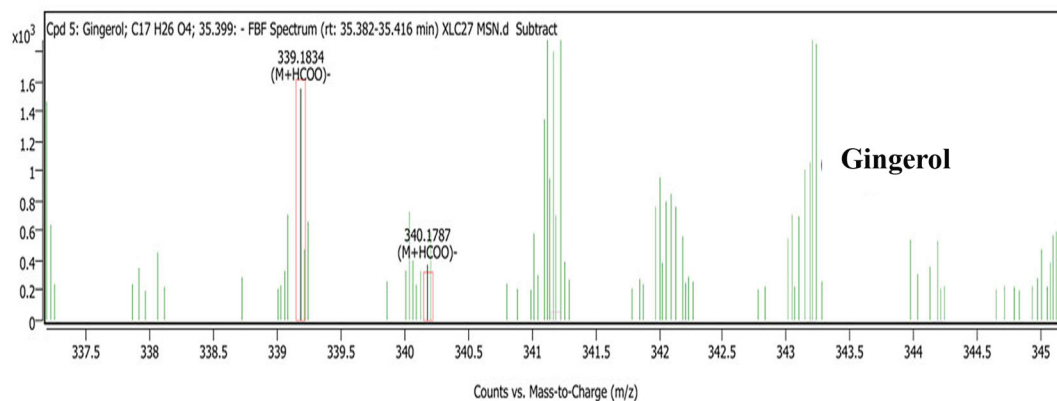
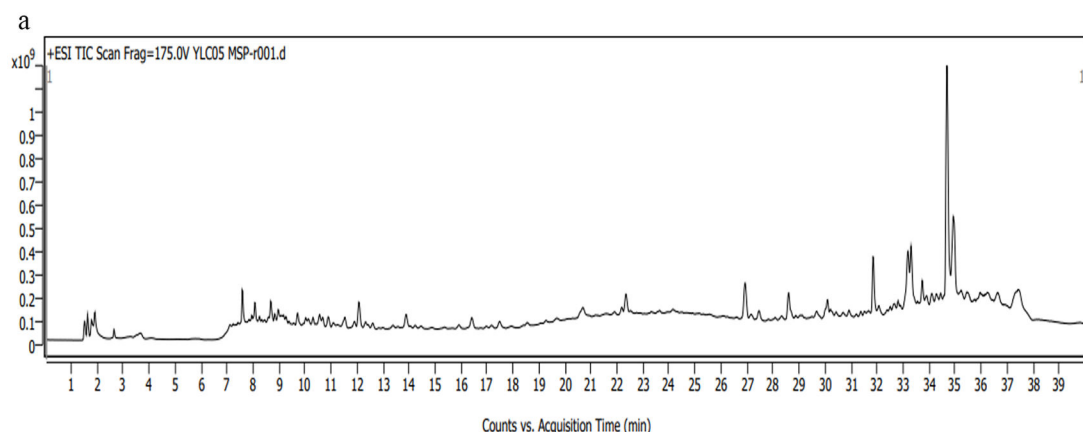
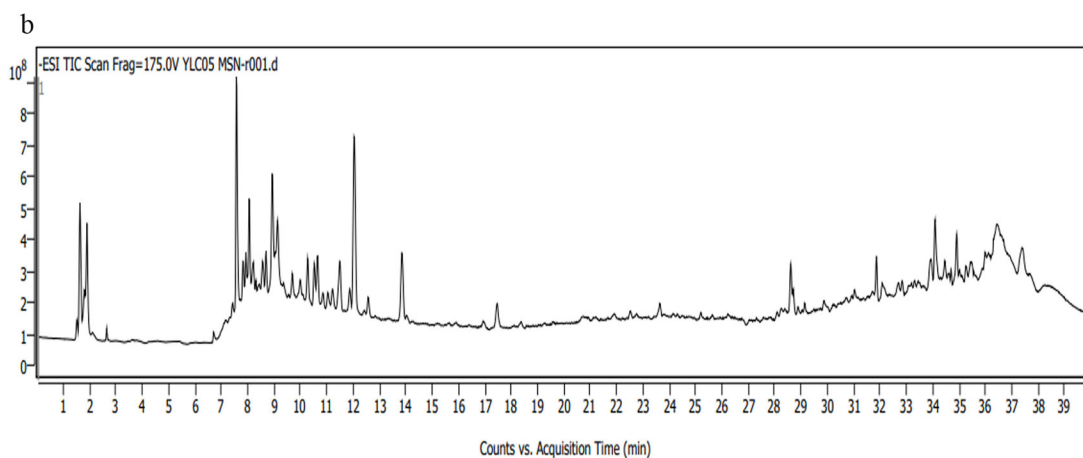


Fig 29(v) Mass spectra of compounds detected by LC/MS analysis of the methanolic extract of *L.coriaacea* leaves



Compound Summary

Cpd	Name	Formula	CAS	RT	Mass	Mass (Tgt)	Diff (Tgt, ppm)	Score	Algorithm
1	Chalcone	C15 H12 O		31.361	208.0875	208.0888	-6.35	90.02	FBF
4	Corytuberine	C19 H21 N O4		8.881	327.1467	327.1471	-0.96	98.85	FBF
5	Dicentrinone	C19 H13 N O5		19.215	335.0792	335.0794	-0.48	95.90	FBF
6	Xanthoplanine	C21 H26 N O4		33.936	356.1860	356.1862	-0.51	80.05	FBF
7	Lauroitsine	C18 H19 N O4		9.479	313.1320	313.1314	1.99	98.55	FBF
8	Isocorydine	C20 H23 N O4		11.307	341.1621	341.1627	-1.73	97.63	FBF
10	Reticuline	C19 H23 N O4		9.263	329.1620	329.1627	-2.18	94.55	FBF
11	Linalool	C10 H18 O		1.936	154.1364	154.1358	4.14	77.35	FBF
12	Dehydrolisoeugenol	C20 H22 O4		12.437	326.1517	326.1518	-0.19	95.82	FBF
13	Grandisin	C24 H32 O7		26.044	432.2124	432.2148	-5.49	84.99	FBF
14	(+)-Eudesmin	C22 H26 O6		21.907	386.1731	386.1729	0.43	83.10	FBF



Cpd	Name	Formula	RT	Mass	Mass (Tgt)	Diff (Tgt, ppm)	Score	Algorithm
1	Oleic acid	C18 H34 O2	34.951	282.2579	282.2559	7.08	76.07	FBF
2	Arachidic acid	C20 H40 O2	34.835	312.3031	312.3028	0.92	93.53	FBF
3	2,4-Di-tert-butylphenol	C14 H22 O	33.838	206.1687	206.1671	7.94	87.67	FBF
4	Palmitic acid	C16 H32 O2	31.180	256.2416	256.2402	5.19	92.19	FBF
5	Lauric acid	C12 H24 O2	34.154	200.1788	200.1776	5.66	75.25	FBF

Fig 30 LC/MS chromatogram of *L.wightiana* leaf methanolic extract. a. ESI+ spectrum and compounds; b. ESI- spectrum and compounds

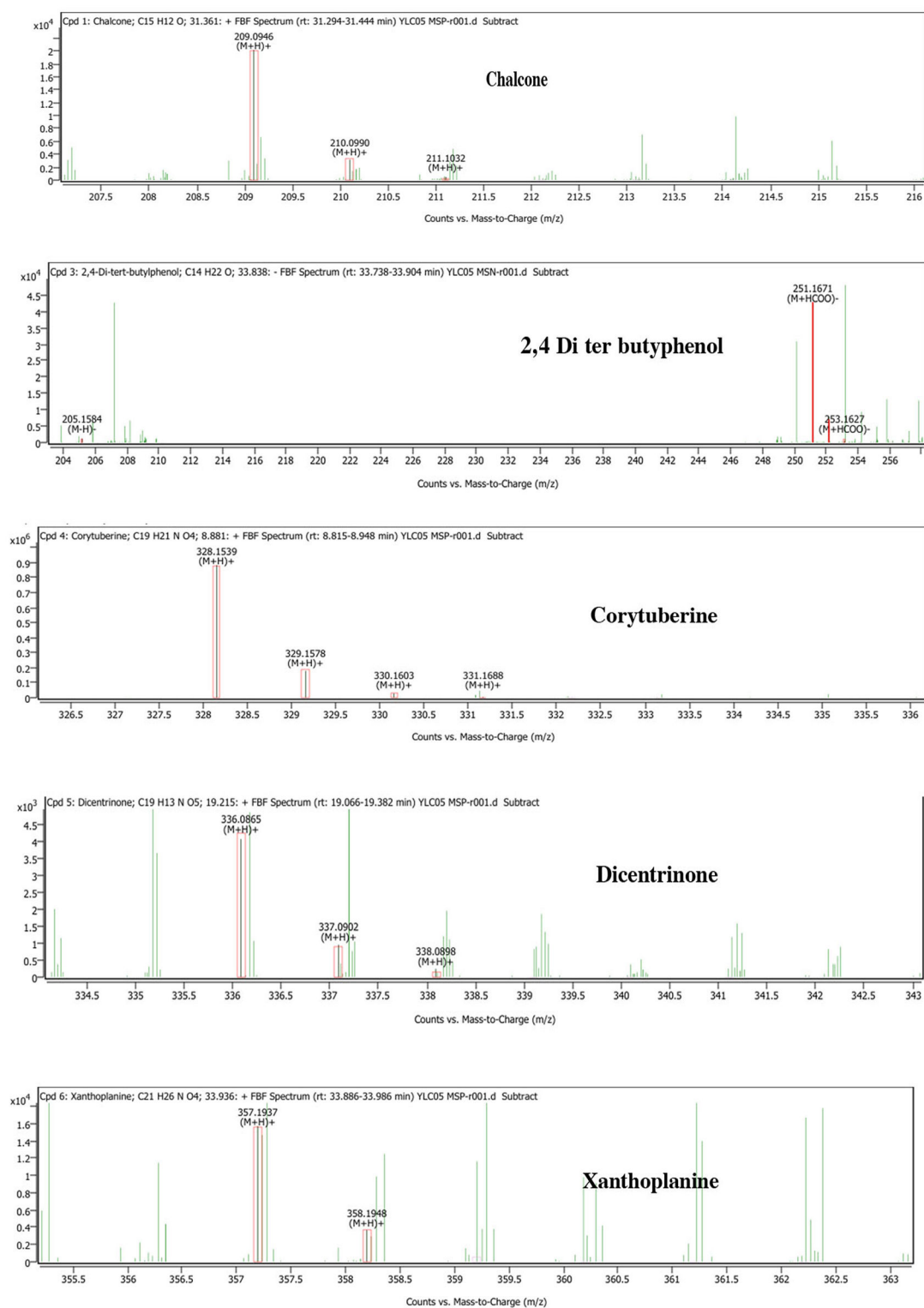


Fig 31(i) Mass spectra of compounds detected by LC/MS analysis of the methanolic extract of *L.wightiana* leaves

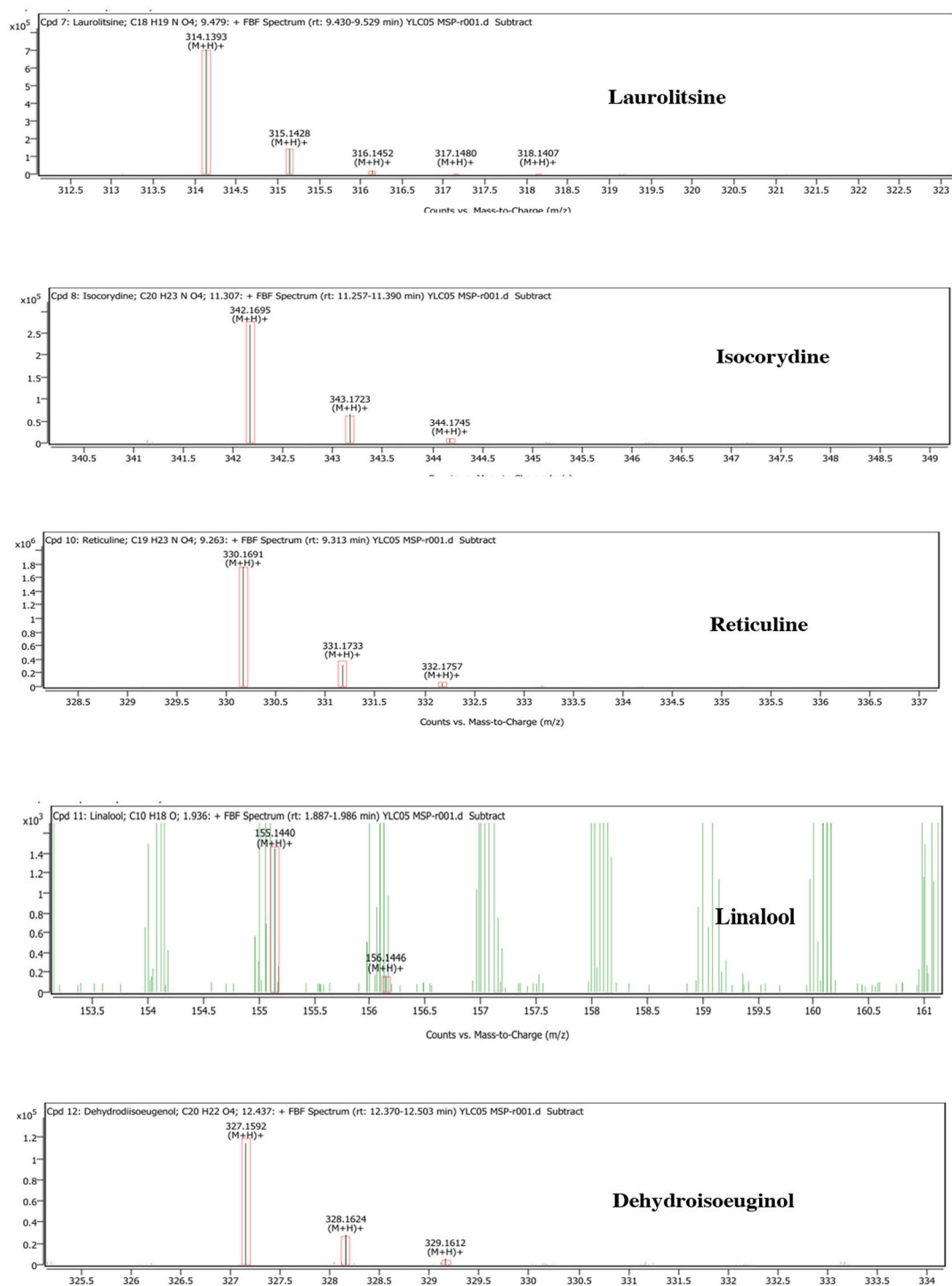


Fig 31(ii) Mass spectra of compounds detected by LC/MS analysis of the methanolic extract of *L.wightiana* leaves

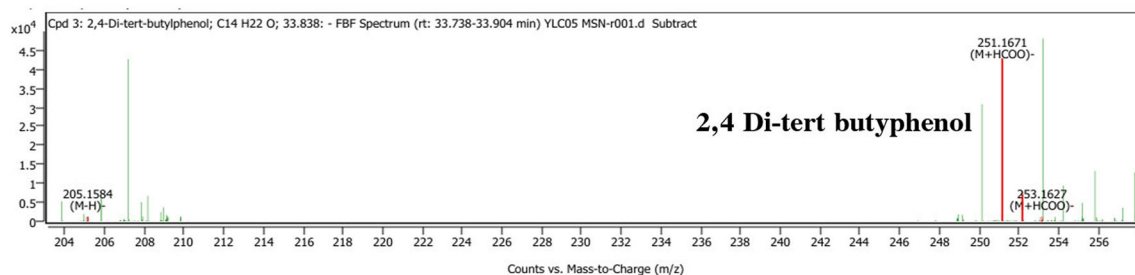
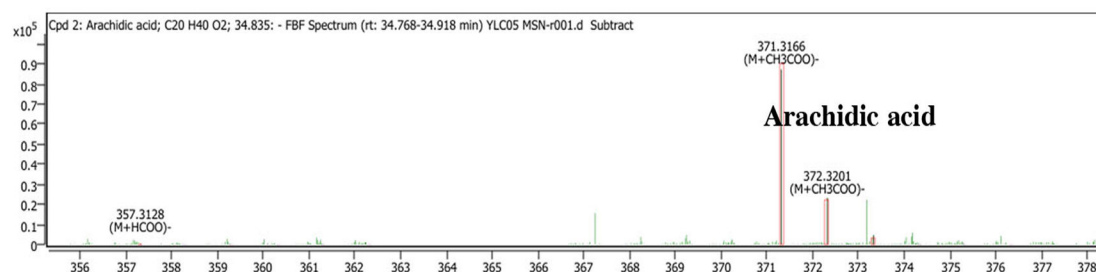
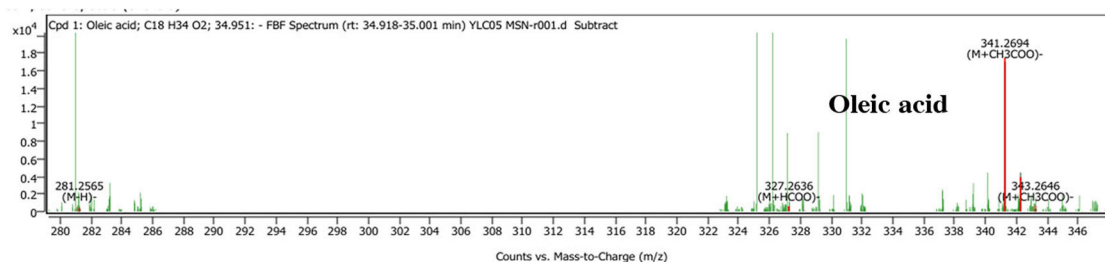
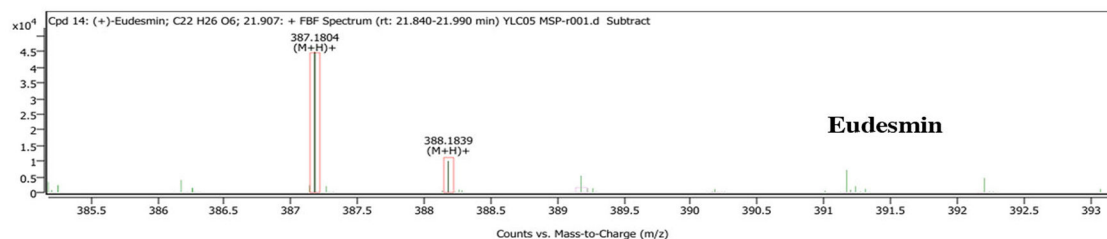
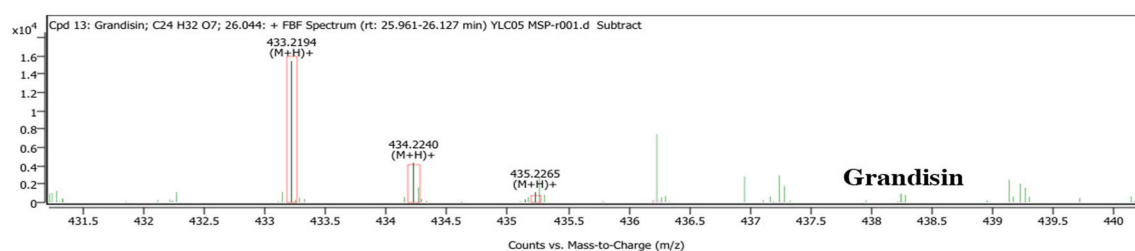


Fig 31(iii) Mass spectra of compounds detected by LC/MS analysis of the methanolic extract of *L.wightiana* leaves

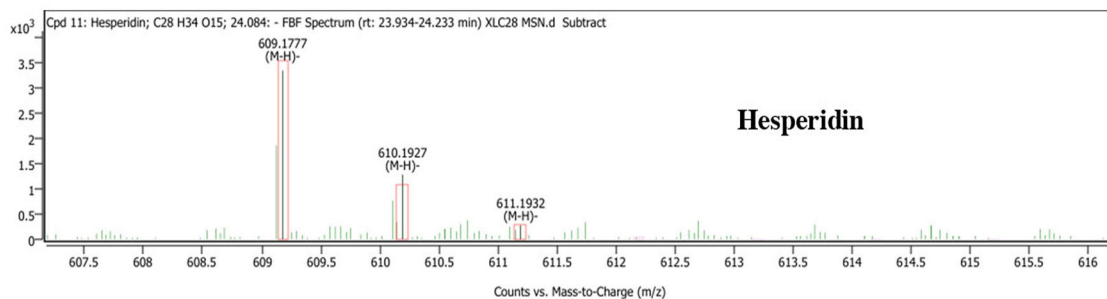
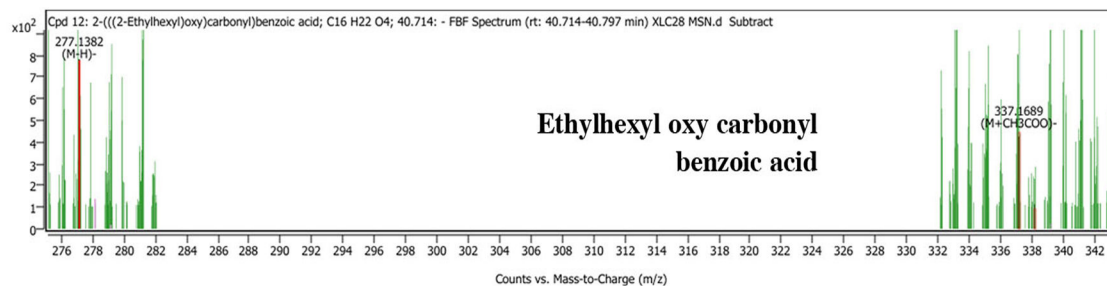
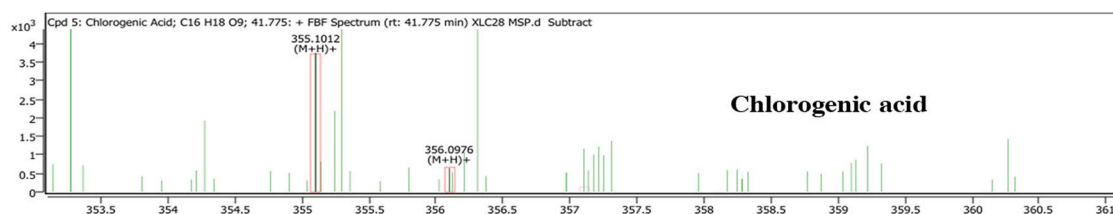
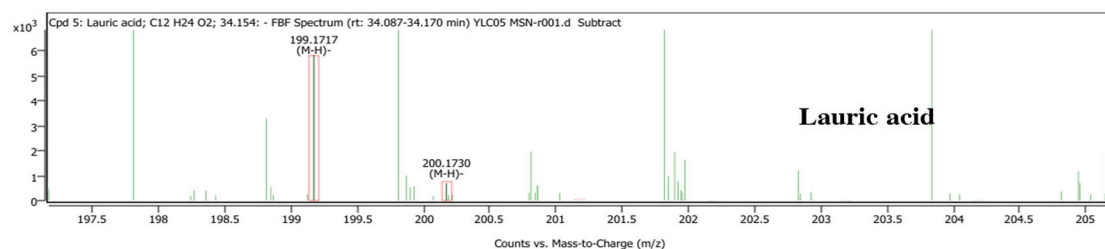
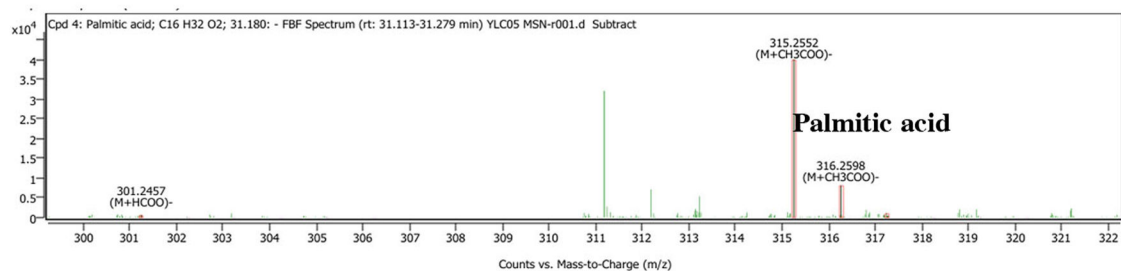


Fig 31(iv) Mass spectra of compounds detected by LC/MS analysis of the methanolic extract of *L.wightiana* leaves

(1 α ,4 $\alpha\alpha$,8 $\alpha\alpha$)(5.99%), spathulenol (2.71%), globulol (5.52%), cubenol (2.70%), tau-cadenol (3.94%), α -Cadinol (2.81%), hexadecanoic acid, methylester (10.49%), n-hexadecanoic acid (100%), 9,12-octadecadienoic acid (Z,Z)-methylester (5.68%), 11-octadecanoic acid, methylester (13.22%), oleic acid (65.16%), decanedioic acid, bis(2-ethylhexyl)ester (5.50%). n-hexadecanoic acid and oleic acid are the compounds found in higher amounts. The GC/MS analysis of *L. bourdillonii* and *L. coriacea* yielded no compounds.

Table 23 GC/MS analysis data of methanolic extract of leaves of *L. wightiana*

Peak	Name of compound	Molecular formula	Retention time (min)	Peak area (%)
1	Copaene	C ₁₅ H ₂₄	31.841	5.68
2	I-H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene	C ₁₅ H ₂₄	33.890	2.04
3	Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-	C ₁₅ H ₂₄	34.991	4.45
4	Naphthalene,1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-(1 α ,4 $\alpha\alpha$,8 $\alpha\alpha$)-	C ₁₅ H ₂₄	35.753	5.99
5	Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-1-(methylethyl)1-(1 α ,4 $\alpha\alpha$,8 $\alpha\alpha$)	C ₁₅ H ₂₄	36.238	3.90
6	Naphthalene,1,2,2,4-tetrahydro-1,6-dimethyl-1-4(1-methylethyl)-(1S-cis)-	C ₁₅ H ₂₄	36.544	7.46
7	Spathulenol	C ₁₅ H ₂₄ O	38.716	2.71
8	9-Isopropyl-1-methyl-2-methylene-5-oxatricyclo[5.4.0.0(3,8)] undecane	C ₁₅ H ₂₄ O	38.901	2.84
9	Globulol	C ₁₅ H ₂₆ O	39.101	5.52
10	2-Naphthalenemethanol, decahydro- α - α ,4a-trimethyl-8-methylene- β -eudesmol	C ₁₅ H ₂₆ O	40.075	1.57
11	Cubenol	C ₁₅ H ₂₆ O	40.900	2.70
12	Carotol	C ₁₅ H ₂₆ O	41.045	2.29
13	tau-Cadinol	C ₁₅ H ₂₆ O	41.879	3.94
14	α -Cadinol	C ₁₅ H ₂₆ O	41.986	2.81
15	Naphthalene,1,6-dimethyl-4-(1-methylethyl)-(Cadalene)	C ₁₅ H ₁₈	43.637	5.57

16	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	53.695	10.49
17	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	55.043	100
18	9,12-Octadecadienoic acid (Z, Z)-methylester	C ₁₉ H ₃₄ O ₂	57.633	5.68
19	11-Octadecanoic acid, methyl ester	C ₁₉ H ₃₆ O ₂	57.790	13.22
20	Octadecanoic acid, methyl ester	C ₁₉ H ₃₈ O ₂	58.371	3.70
21	Oleic acid	C ₁₈ H ₃₄ O ₂	58.837	65.16
22	Decanedioic acid,bis(2-ethylhexyl) ester	C ₂₆ H ₅₀ O ₄	65.559	5.50

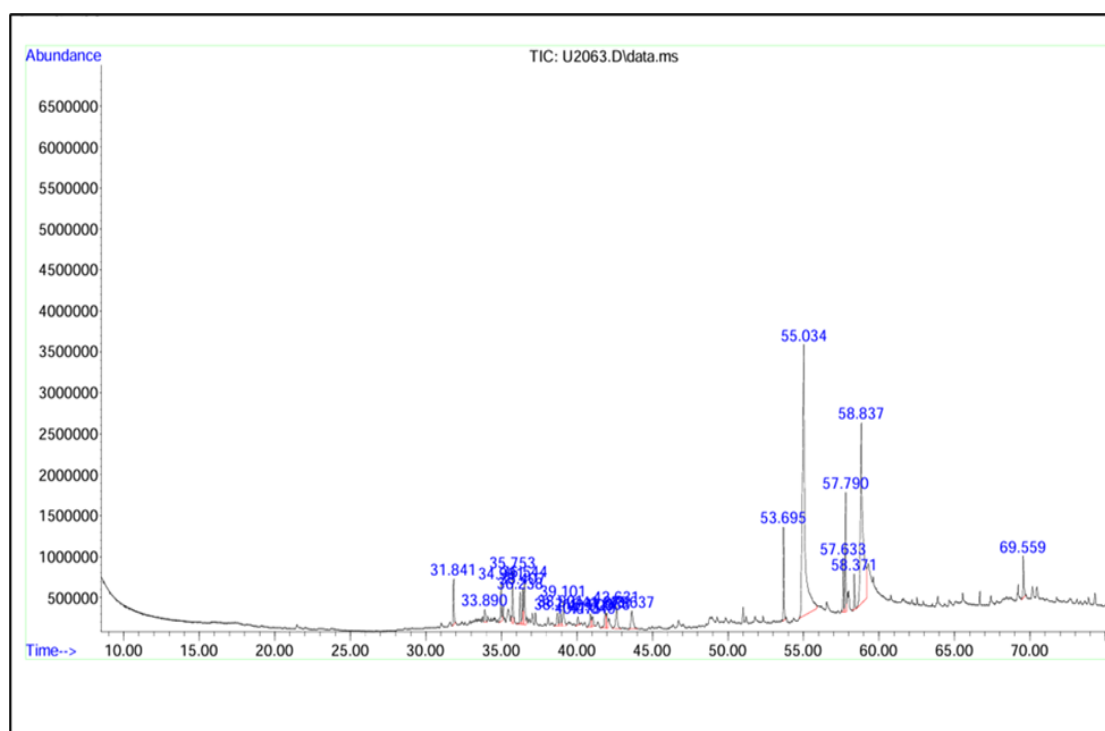
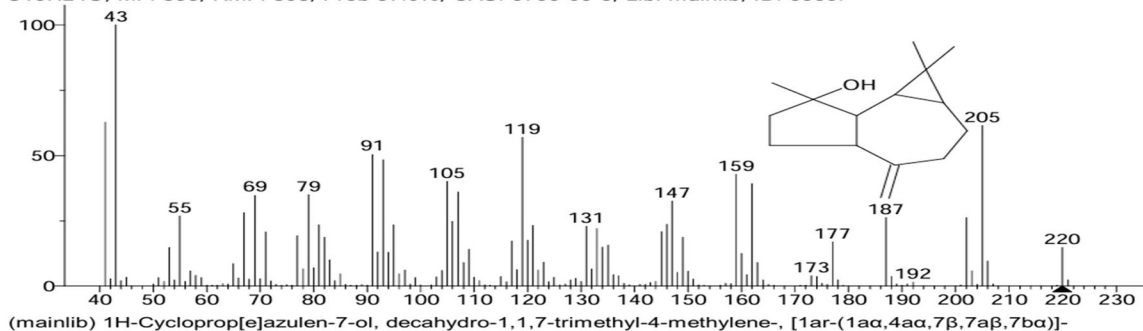


Fig 32 GC/MS chromatogram of *L. wightiana* leaves methanolic extract

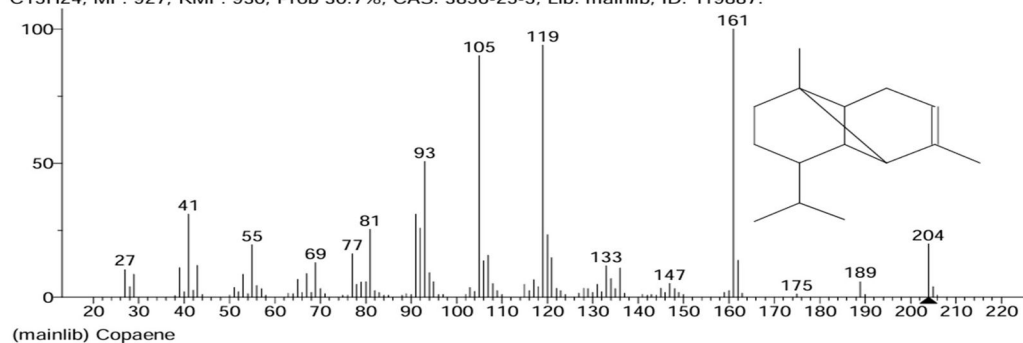
f. Analysis of the essential oil

The essential oil from *L. coriacea* leaves was extracted by the hydro distillation method using the Clevenger apparatus and GC/MS-GC-FID analysis was carried out and the oil components were screened. The descriptions of the compounds are given in Table 24 and chromatogram and mass spectra of compounds depicted in Figures 34 & 35. Various components of essential oil include, α -terpineol (75.81%), linalool (41.06%), bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-(1S)-(6.26%), endo borneol (4.98%), cyclohexanol,5-methyl-2-(1-

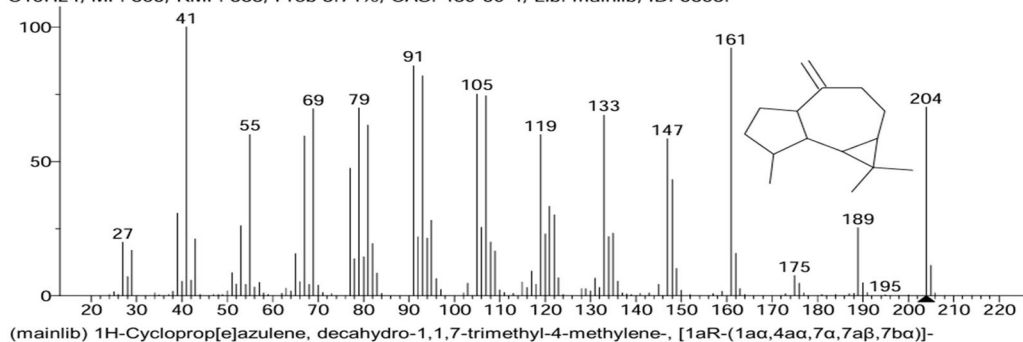
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C15H24; MF: 927; RMF: 930; Prob 30.7%; CAS: 3856-25-5; Lib: mainlib; ID: 119887.



C15H24; MF: 853; RMF: 888; Prob 8.74%; CAS: 489-39-4; Lib: mainlib; ID: 3858.



C15H24; MF: 910; RMF: 916; Prob 31.9%; CAS: 39029-41-9; Lib: replib; ID: 21213.

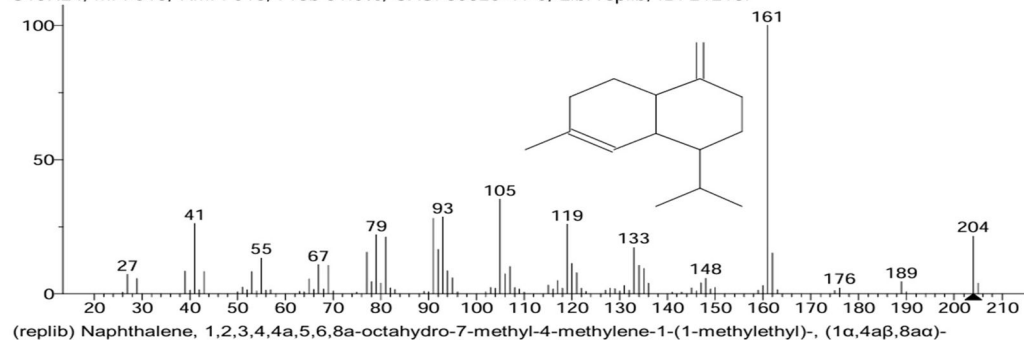
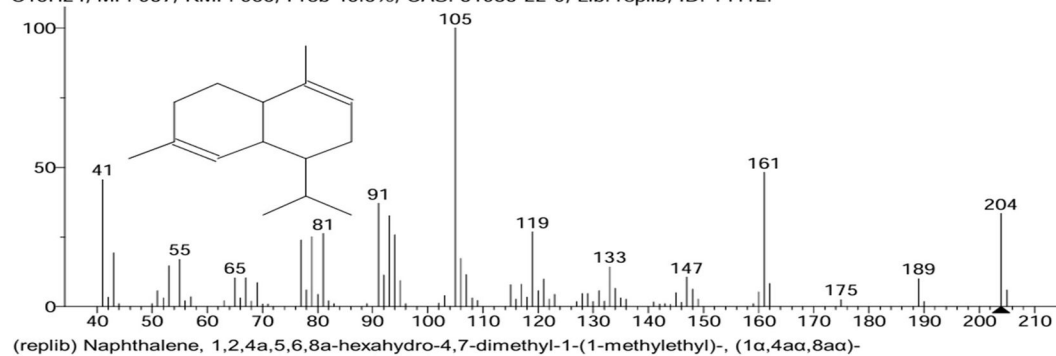
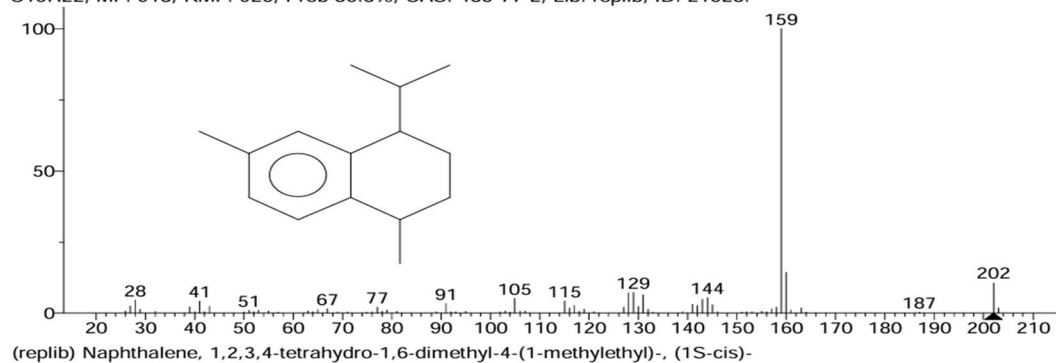


Fig 33(i) Mass spectra of compounds detected by GC/MS analysis of methanolic extract of *L.wightiana* leaves

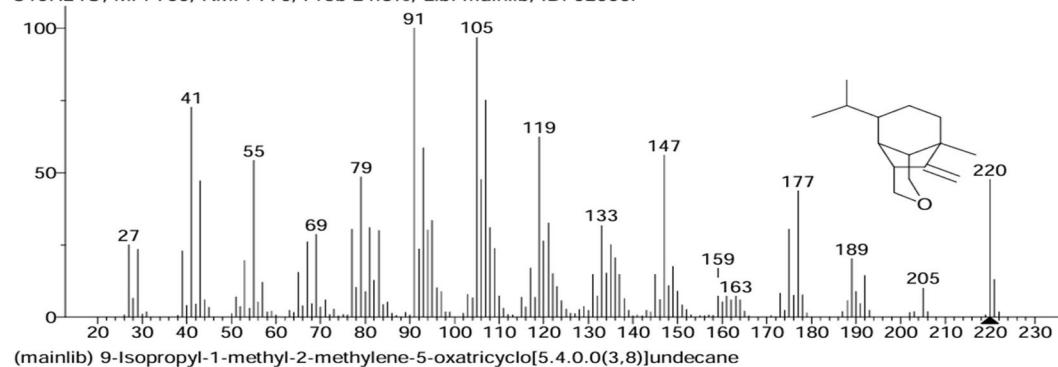
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C15H22; MF: 918; RMF: 920; Prob 85.8%; CAS: 483-77-2; Lib: replib; ID: 21028.



C15H24O; MF: 760; RMF: 776; Prob 24.8%; Lib: mainlib; ID: 52066.



C15H26O; MF: 881; RMF: 892; Prob 29.9%; CAS: 51371-47-2; Lib: mainlib; ID: 5786.

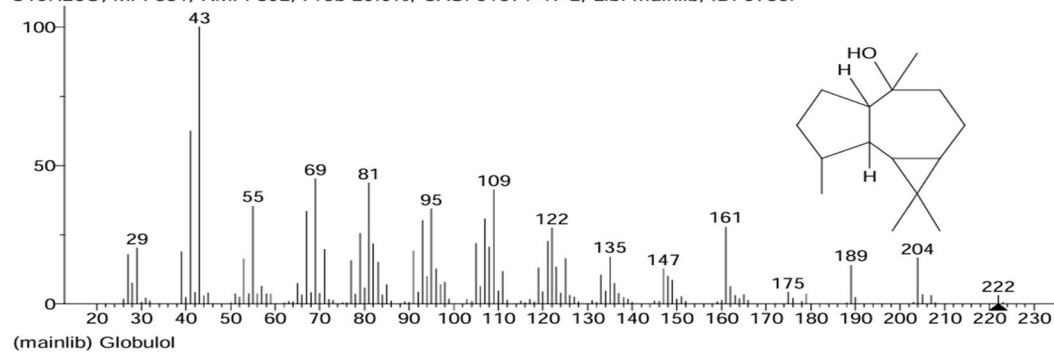
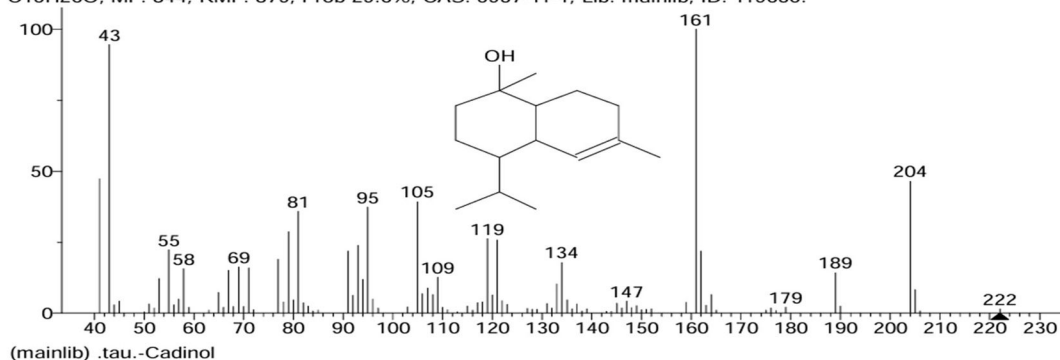
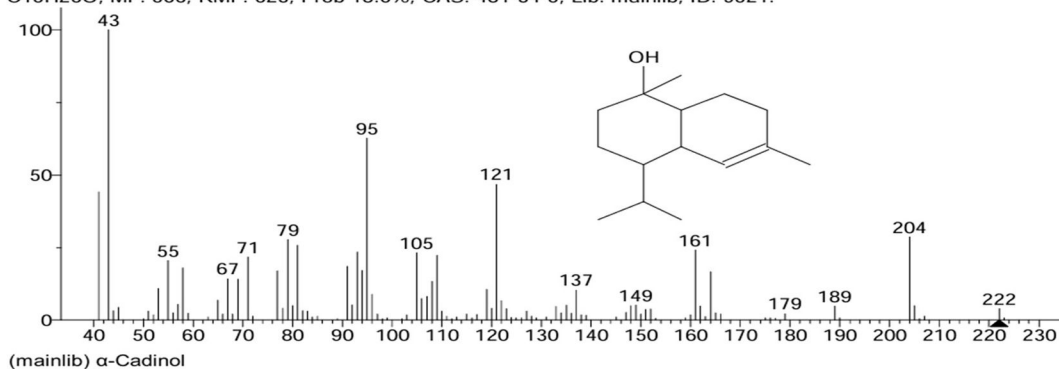


Fig 33(ii) Mass spectra of compounds detected by GC/MS analysis of methanolic extract of *L.wightiana* leaves

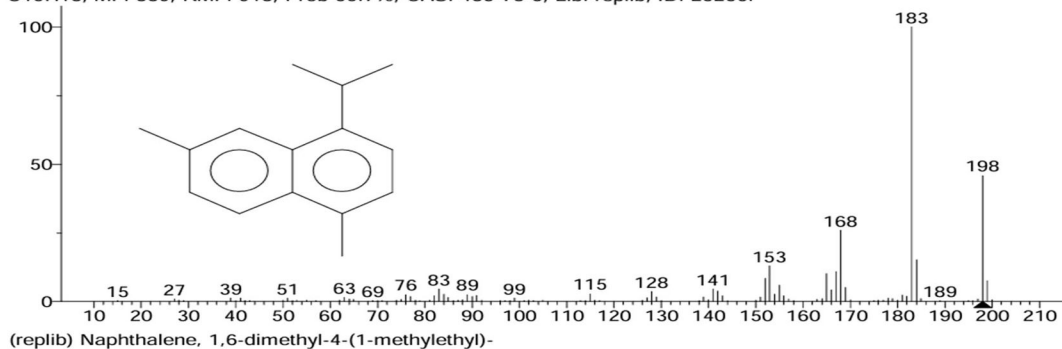
C₁₅H₂₆O; MF: 844; RMF: 879; Prob 29.6%; CAS: 5937-11-1; Lib: mainlib; ID: 119630.



C₁₅H₂₆O; MF: 566; RMF: 623; Prob 18.0%; CAS: 481-34-5; Lib: mainlib; ID: 9521.



C₁₅H₁₈; MF: 889; RMF: 918; Prob 65.7%; CAS: 483-78-3; Lib: replib; ID: 23236.



C₁₇H₃₄O₂; MF: 910; RMF: 942; Prob 73.0%; CAS: 112-39-0; Lib: replib; ID: 9050.

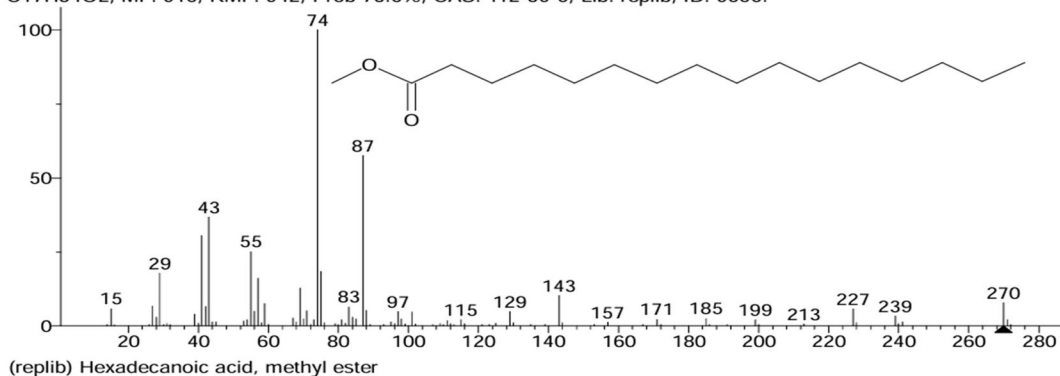
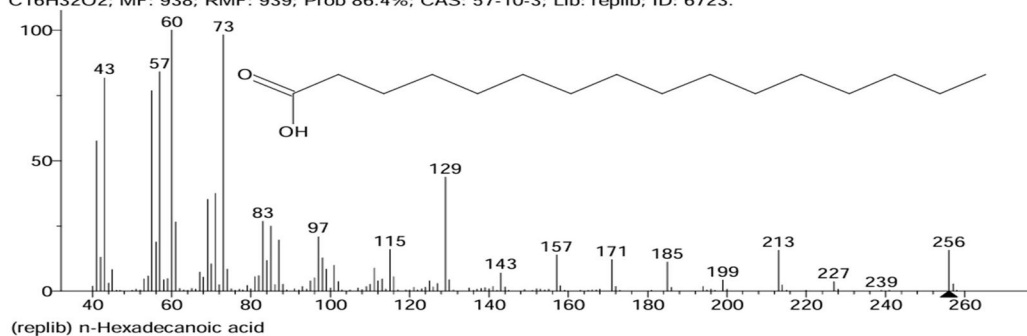
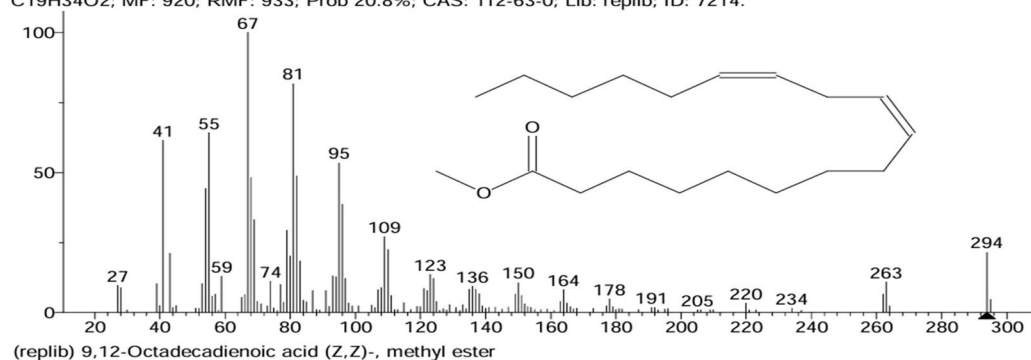


Fig 33(iii) Mass spectra of compounds detected by GC/MS analysis of methanolic extract of *L. wightiana* leaves

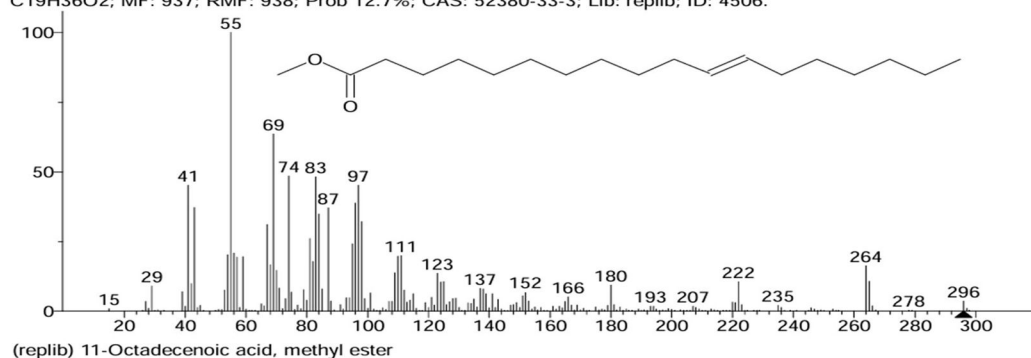
C16H32O2; MF: 938; RMF: 939; Prob 86.4%; CAS: 57-10-3; Lib: replib; ID: 6723.



C19H34O2; MF: 920; RMF: 933; Prob 20.8%; CAS: 112-63-0; Lib: replib; ID: 7214.



C19H36O2; MF: 937; RMF: 938; Prob 12.7%; CAS: 52380-33-3; Lib: replib; ID: 4506.



C19H38O2; MF: 903; RMF: 931; Prob 69.1%; CAS: 112-61-8; Lib: replib; ID: 9086.

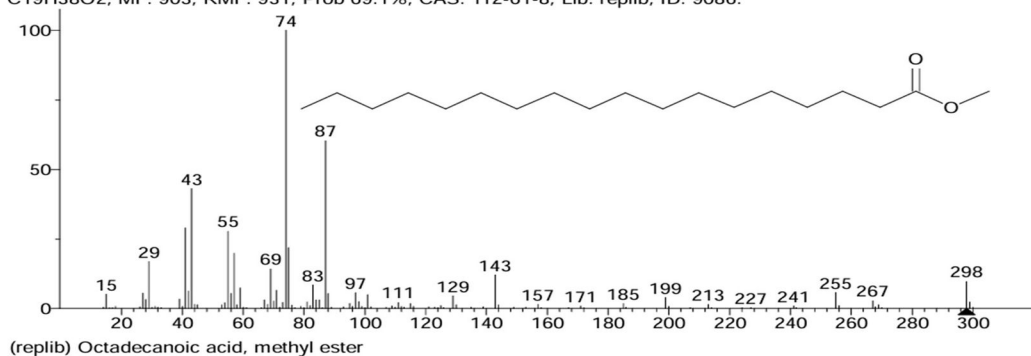


Fig 33(iv) Mass spectra of compounds detected by GC/MS analysis of methanolic extract of *L.wightiana* leaves

methylethyl)-, (1 α ,2 β ,5 β)-(2.58%), eucalyptol (2.12%), linalyl acetate (2.485%), and bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-(1S-endo)-(1.84%).

Table 24 GC/MS with GC-FID analysis data of *L. coriacea* leaves essential oil

Sl.No.	Name of compound	Molecular formula	Retention time (min)	Area %
1	Eucalyptol	C ₁₀ H ₁₈ O	13.819	2.12
2	Linalool	C ₁₀ H ₁₈ O	16.316	41.06
3	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-(1S)-	C ₁₀ H ₁₆ O	17.701	6.26
4	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-(1S-endo)-	C ₁₀ H ₁₈ O	18.364	1.84
5	endo-Borneol	C ₁₀ H ₁₈ O	18.729	4.98
6	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1 α ,2 β ,5 β)-	C ₁₀ H ₂₀ O	19.077	2.58
7	α -Terpineol	C ₁₀ H ₁₈ O	19.782	75.81
8	Cyclohexanol, 2-methyl-5-(1-methylethenyl)-, (1 α ,2 α ,5 β)-	C ₁₀ H ₁₈ O	19.910	41.56
9	Linalyl acetate	C ₁₂ H ₂₀ O ₂	22.408	2.485

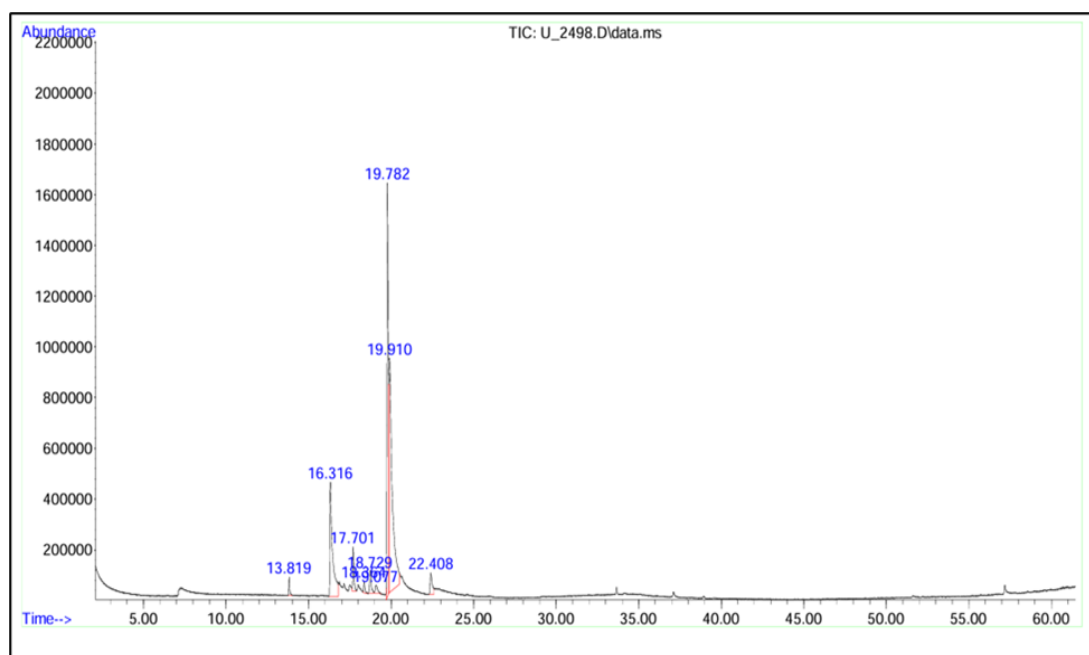


Fig 34 GC/MS chromatogram of *L. coriacea* leaves essential oil

PHASE III- PHARMACOLOGICAL STUDY

Pharmacological evaluation of leaves methanolic extracts and bark extracts of the selected species of *Litsea* were carried out. Bioactivities like antimicrobial, antioxidant, antidiabetic and cytotoxicity studies of three species of *Litsea* namely *L. bourdillonii*, *L. coriacea* and *L. wightiana* were evaluated. Additionally, the anti-inflammatory and wound healing studies of *L. wightiana* and the essential oil analysis of *L. coriacea* were carried out.

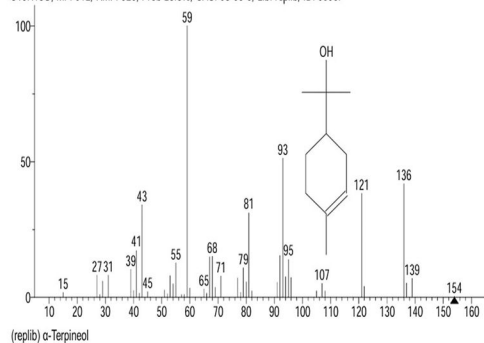
A. ANTIMICROBIAL ACTIVITY

a. Antibacterial study

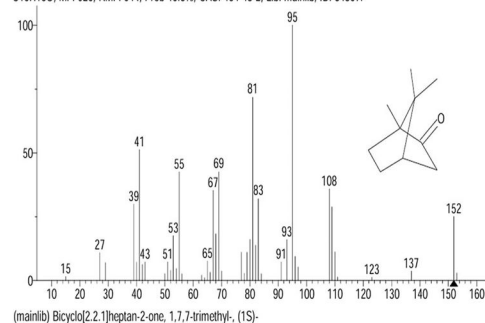
The leaves and bark of the three species of *Litsea* were subjected to antibacterial screening against pathogens such as *S. aureus*, *E. coli*, and *S. aeruginosa* using the agar well diffusion method. The results implicated that the extracts have significant inhibitory activity against gram-negative and less effect towards gram-positive bacteria (Table 25 and Figures 36, 37 & 38). The antibacterial results were observed by measuring the zone of inhibition in mm. The inhibition zone of *E. coli* by the 1000 µg/mL leaf extracts of *L. bourdillonii*, *L. coriacea*, and *L. wightiana* was 14±0.01 mm, 15±0.02 mm, and 14±0.20 mm, respectively, and that of *P. aeruginosa* was 18±0.13 mm, 17±0.43 mm, and 15±0.02 mm each. The bark extracts showed antibacterial activity with inhibition zones such as 13±0.13 mm, 14±0.25 mm, and 16±0.06 mm against *E. coli*, and 17±0.05 mm, 16±0.30 mm, and 17±0.32 mm against *P. aeruginosa*. The bark extract of *L. wightiana* showed maximum inhibition (13±0.80mm) against *S. aureus* at 1000µg/mL when compared to other extracts. The standard antibiotic streptomycin showed antibacterial activity against all selected strains of bacteria with inhibition zones ranging from 27-32mm. The minimum inhibitory concentration (MIC) of the extracts against bacteria was also determined.

The minimum inhibitory concentration of antibacterial activity of leaves and bark methanolic extracts were analyzed and found that the *L. wightiana* leaf extracts showed good activity at minimal concentration against selected strains of bacteria as

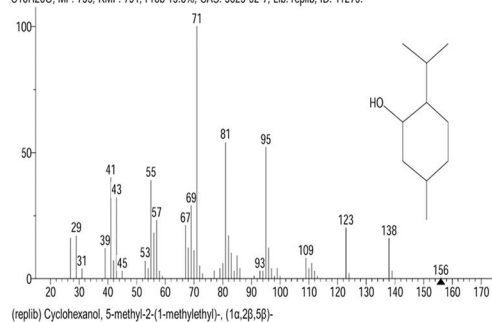
C10H18O; MF: 912; RMF: 929; Prob 28.3%; CAS: 98-55-5; Lib: replib; ID: 9095.



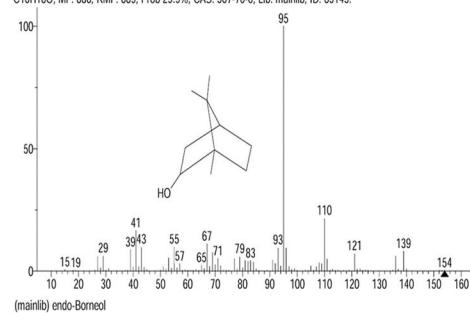
C10H16O; MF: 925; RMF: 944; Prob 46.3%; CAS: 464-48-2; Lib: mainlib; ID: 84807.



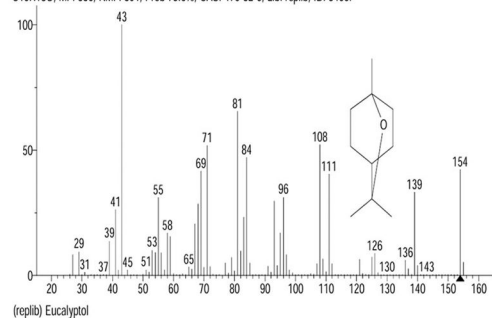
C10H20O; MF: 735; RMF: 791; Prob 13.8%; CAS: 3623-52-7; Lib: replib; ID: 11275.



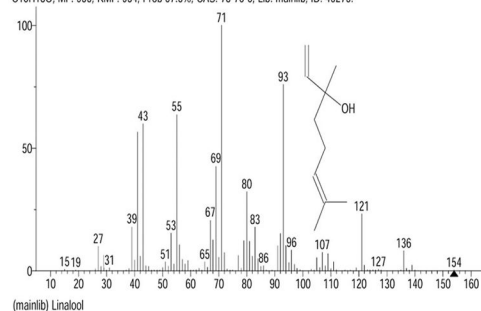
C10H18O; MF: 888; RMF: 889; Prob 29.9%; CAS: 507-70-0; Lib: mainlib; ID: 85143.



C10H18O; MF: 886; RMF: 891; Prob 73.6%; CAS: 470-82-6; Lib: replib; ID: 3400.



C10H18O; MF: 933; RMF: 934; Prob 67.3%; CAS: 78-70-6; Lib: mainlib; ID: 45273.



C12H20O2; MF: 897; RMF: 899; Prob 39.7%; CAS: 115-95-7; Lib: mainlib; ID: 82075.

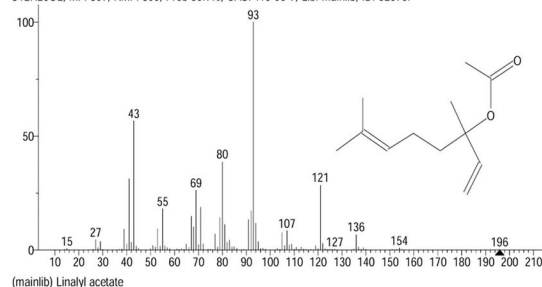


Fig 35 Mass spectra of compounds detected by GC/MS analysis of essential oil of *L.coriacea* leaves

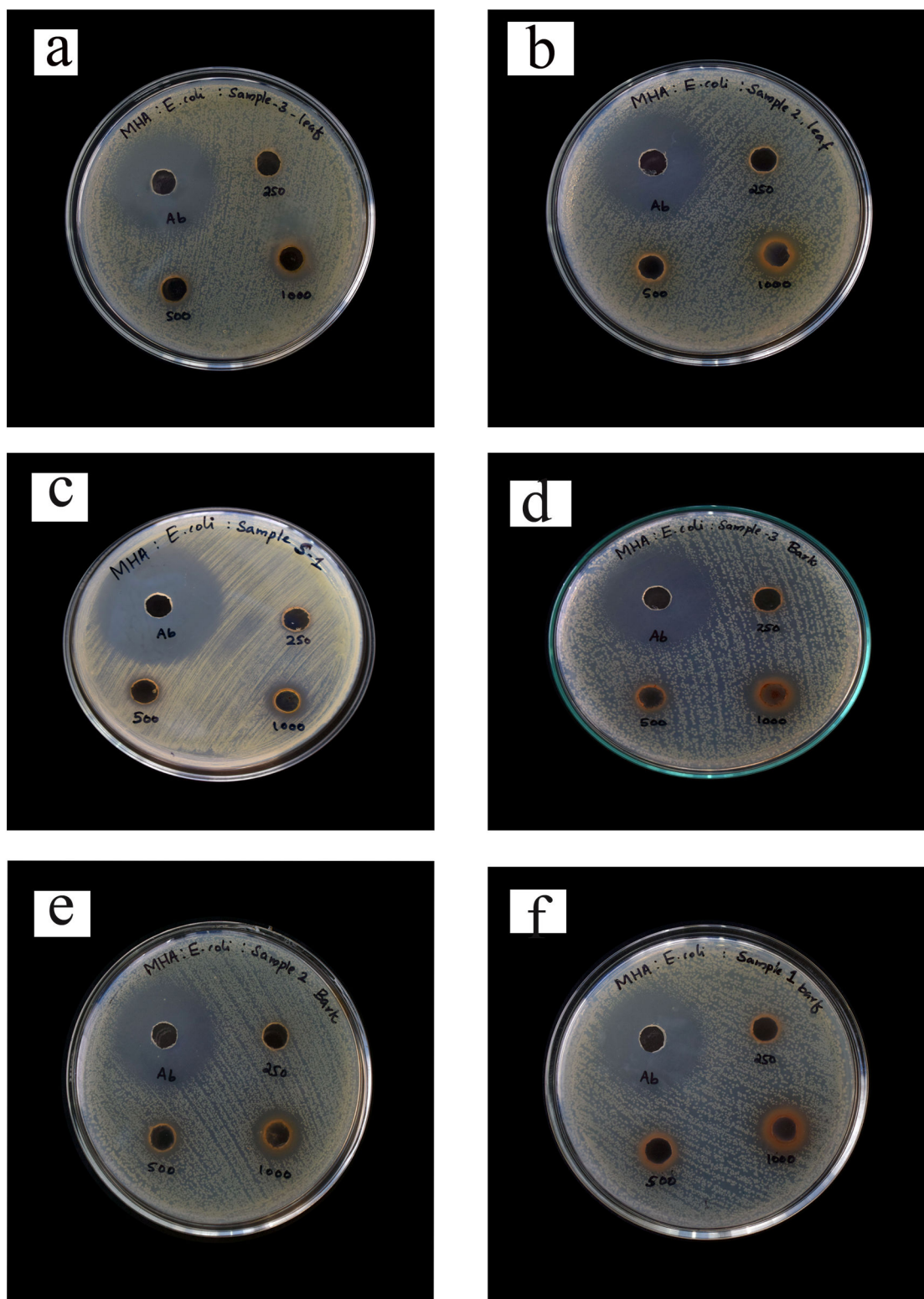


Fig 36 Antibacterial activity of Methanolic leaf & bark extracts. a. Inhibition of *E. coli* by leaf extract of *L. bourdillonii*; b. By leaf extract of *L. coriacea*; c. By leaf extract of *L. wightiana*; d. Inhibition of *E. coli* by bark extract of *L. bourdillonii*; e. By bark extract of *L. coriacea*; f. By bark extract of *L. wightiana*

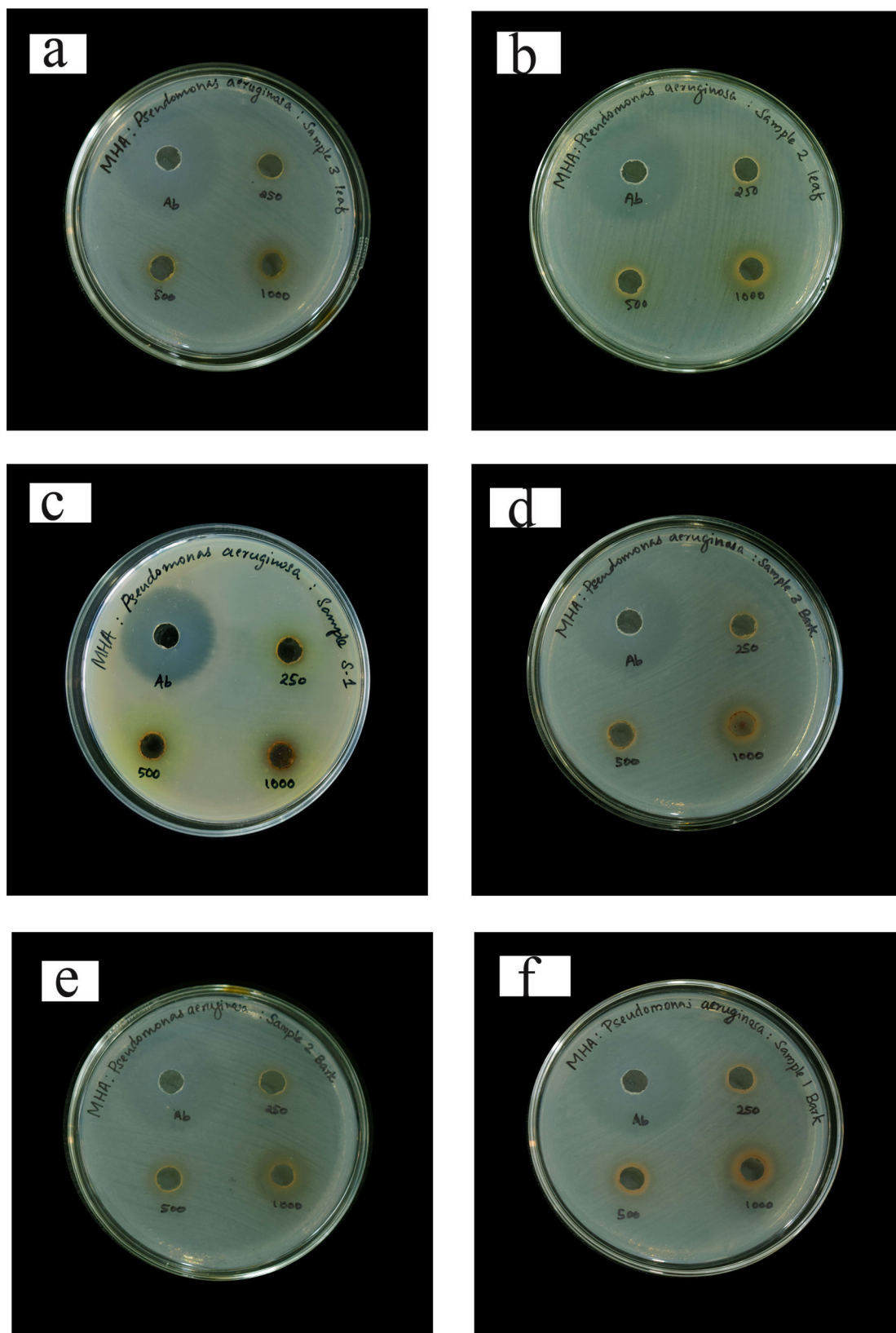


Fig 37 Antibacterial activity of Methanolic leaf & bark extracts. a. Inhibition of *P.aeruginosa* by leaf extract of *L.bourdillonii*; b. By leaf extract of *L.coriacea*; c. By leaf extract of *L.wightiana*; d. Inhibition of *P.aeruginosa* by bark extract of *L.bourdillonii*; e. By bark extract of *L.coriacea*; f. By bark extract of *L.wightiana*

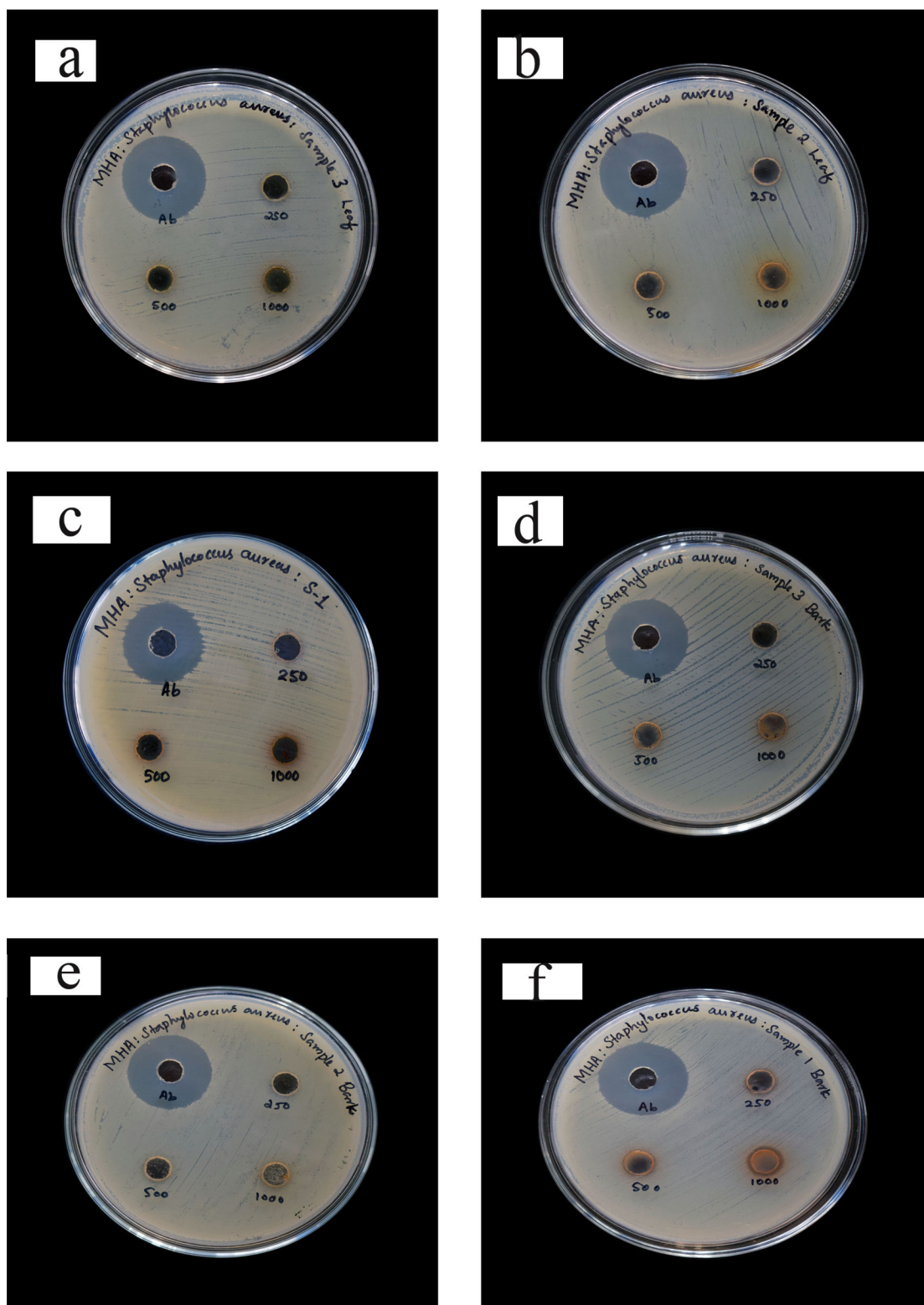


Fig 38 Antibacterial activity of Methanolic leaf & bark extracts. a. Inhibition of *S. aureus* by leaf extract of *L. bourdillonii*; b. By leaf extract of *L. coriacea*; c. By leaf extract of *L. wightiana*; d. Inhibition of *S. aureus* by bark extract of *L. bourdillonii*; e. By bark extract of *L. coriacea*; f. By bark extract of *L. wightiana*

compared to other extracts. The lowest inhibition concentration was 191.401 ± 0.44 $\mu\text{g/mL}$ against *E. coli*, followed by *P. aeruginosa* (488.124 ± 0.23 $\mu\text{g/mL}$) and *S. aureus* (622.666 ± 0.22 $\mu\text{g/mL}$) as shown in Table 26.

Table 25 Antibacterial activity of the leaves and bark extracts of three *Litsea* spp.

Extract	Concentration ($\mu\text{g/mL}$)	Zone of inhibition (mm)& $\pm$ SD		
		<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>
Streptomycin LBL	100	30 ± 0.13	32 ± 0.09	26 ± 0.65
	250	ND	11 ± 0.12	ND
	500	11 ± 0.07	13 ± 0.04	ND
	1000	14 ± 0.01	18 ± 0.13	11 ± 0.07
Streptomycin LCL	100	30 ± 0.05	32 ± 0.05	27 ± 0.43
	250	ND	ND	ND
	500	12 ± 0.12	12 ± 0.09	ND
	1000	15 ± 0.02	17 ± 0.43	11 ± 0.07
Streptomycin LWL	100	30 ± 0.17	30 ± 0.20	27 ± 0.02
	250	11 ± 0.17	11 ± 0.12	ND
	500	12 ± 0.21	12 ± 0.03	ND
	1000	14 ± 0.20	15 ± 0.02	11 ± 0.012
Streptomycin LBB	100	30 ± 0.12	32 ± 0.72	27 ± 0.12
	250	ND	12 ± 0.12	ND
	500	11 ± 0.01	13 ± 0.70	ND
	1000	13 ± 0.13	17 ± 0.05	ND
Streptomycin LCB	100	30 ± 0.15	32 ± 0.06	27 ± 0.02
	250	ND	12 ± 0.11	ND
	500	11 ± 0.30	14 ± 0.18	ND
	1000	14 ± 0.25	16 ± 0.30	11 ± 0.06
Streptomycin LWB	100	30 ± 0.10	32 ± 0.04	27 ± 0.12
	250	ND	11 ± 0.30	11 ± 0.10
	500	12 ± 0.12	13 ± 0.12	12 ± 0.91
	1000	16 ± 0.06	17 ± 0.32	13 ± 0.80

LBL- *L. bourdillonii* leaf, LCL- *L. coriacea* leaf, LWL- *L. wightiana* leaf, LBB- *L. bourdillonii* bark, LCB- *L. coriacea* bark, LWB- *L. wightiana* bark ND-Not Detected

Table 26 Antibacterial activity expressed by minimum inhibitory concentration (MIC) of the leaf and bark extracts of three *Litsea* spp.

Plant sample	Minimum Inhibitory Concentration (MIC) ($\mu\text{g/mL}$)		
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>
LBL	777.012 $\pm$ 0.12	702.40 $\pm$ 0.15	777.012 $\pm$ 0.54
LCL	661.026 $\pm$ 0.22	756.007 $\pm$ 0.15	759.941 $\pm$ 0.23
LWL	191.401 $\pm$ 0.44	488.124 $\pm$ 0.23	622.666 $\pm$ 0.22
L.BB	625.572 $\pm$ 0.23	546.601 $\pm$ 0.20	625.572 $\pm$ 0.25
LCB	643.811 $\pm$ 0.41	533.069 $\pm$ 0.22	643.811 $\pm$ 0.34
LWL	720.355 $\pm$ 1.23	682.387 $\pm$ 0.14	905.62 $\pm$ 0.30

LBL- *L. bourdillonii* leaf, LCL- *L. coriacea* leaf, LWL- *L. wightiana* leaf, LBB- *L. bourdillonii* bark, LCB- *L. coriacea* bark, LWL- *L. wightiana* leaf

b. Antifungal Study

Antifungal activity of studies of leaves and bark samples of *L. bourdillonii*, *L. coriacea*, and *L. wightiana* were carried out. The results (Figures 39 & 40) showed no significant fungal activity by the extracts. The leaves and bark extracts of *L. coriacea* and the leaf extract of *L. wightiana* showed moderate activity with inhibition zones (12 $\pm$ 0.012mm, 11 $\pm$ 0.15mm and 11 $\pm$ 0.01mm) against *C. albicans*. No extracts showed antifungal effect against *A. niger*.

B. ANTIOXIDANT ASSAY

The methanolic leaves extract of selected *Litsea* spp. were subjected to *in vitro* antioxidant activity assays. Four different methods, like DPPH radical scavenging, hydroxyl radical scavenging, superoxide radical scavenging assays and ferrous reducing power assay, were adopted. Each assay results were compared with known antioxidants.

i. DPPH (1,1-Diphenyl-1-Picrylhydrazyl) radical scavenging activity

In phytochemistry the DPPH radical scavenging assay is a commonly used technique to evaluate the antioxidant qualities of different compounds including natural products, pharmaceuticals, *etc.* This test evaluates a compound's ability to donate an electron and a hydrogen atom to neutralize the DPPH radical. For the current study, the methanolic extracts of *L. bourdillonii*, *L. coriacea*, and *L.*

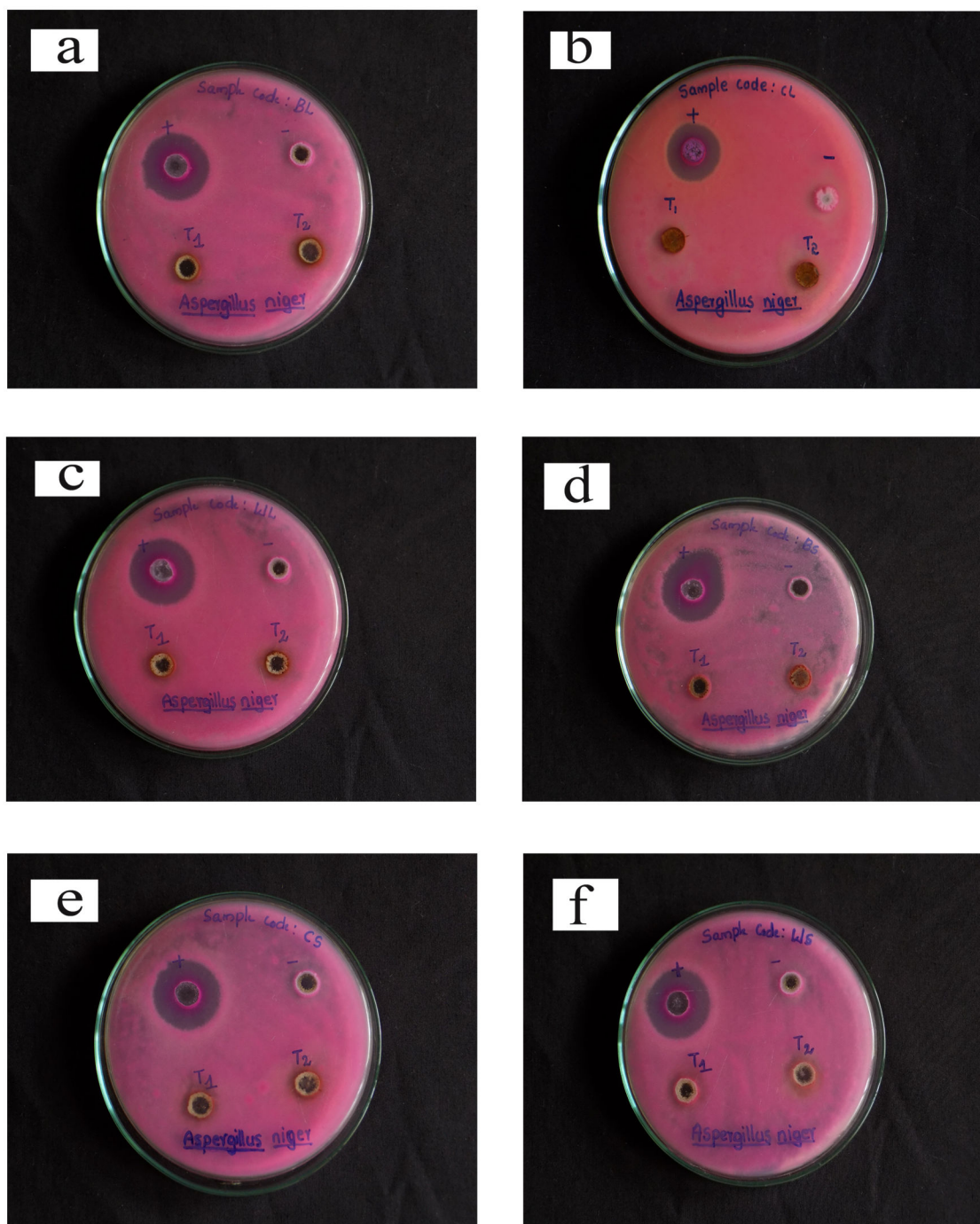


Fig 39 Antifungal activity of Methanolic leaf & bark extracts. a. Inhibition of *A.niger* by leaf extract of *L.bourdillonii*; b. By leaf extract of *L.coriacea*; c. By leaf extract of *L.wightiana*; d. Inhibition of *A.niger* by bark extract of *L.bourdillonii*; e. By bark extract of *L.coriacea*; f. By bark extract of *L.wightiana*

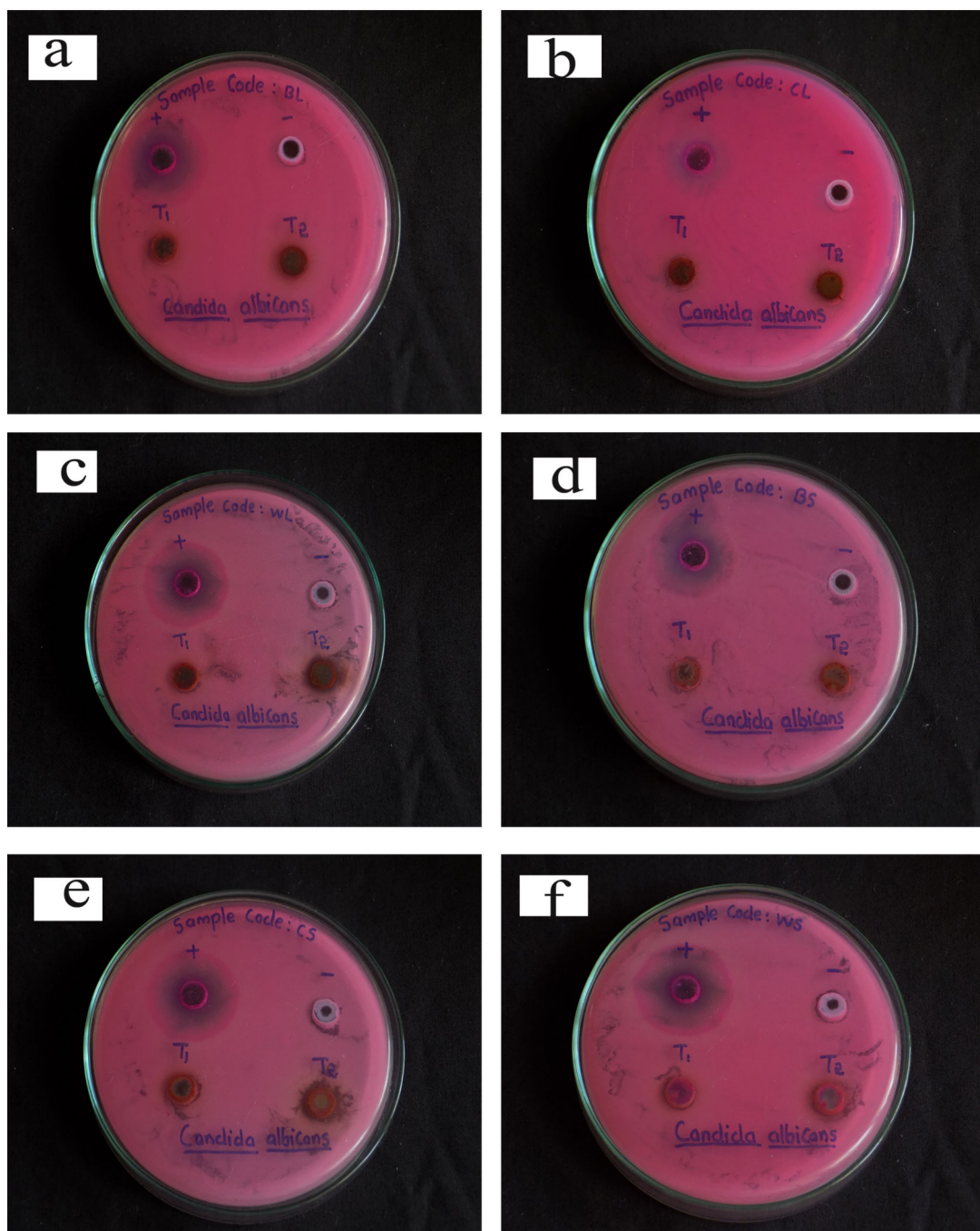


Fig 40 Antifungal activity of Methanolic leaf & bark extracts. a. Inhibition of *C.albicans* by leaf extract of *L.bourdillonii*; b. By leaf extract of *L.coriacea*; c. By leaf extract of *L.wightiana*; d. Inhibition of *C.albicans* by bark extract of *L.bourdillonii*; e. By bark extract of *L.coriacea*; f. By bark extract of *L.wightiana*

wightiana leaves were taken at different concentrations (6.5, 12.5, 25, 50, 100 µg/mL). The results are represented in Table 27 and Fig. 41. From the comparative assessment, it is comprehended that the *L. wightiana* leaves extract showed better antioxidant activity with an IC₅₀ value of 30.32 µg/mL among the remaining extracts. This indicates the high antioxidant potential of *L. wightiana* as compared to the standard antioxidant, ascorbic acid, with an IC₅₀ value of 54.53 µg/mL. Hence, the other methods of antioxidant activity, such as hydroxyl radical scavenging activity, superoxide radical scavenging activity, and ferrous reducing power antioxidant assay studies, were attempted by *L. wightiana* leaf methanolic extracts.

Table 27 DPPH free radical scavenging activity of the leaves of three *Litsea* spp.

Sample	Concentration (µg/mL)	DPPH scavenging activity (%) ±SD	IC ₅₀ (µg/mL)
<i>L.bourdillonii</i>	6.25	5.218±0.01	89.79
	12.5	7.160±0.57	
	25	15.6±0.06	
	50	34.83±0.15	
	100	53.03±0.15	
<i>L.coriacea</i>	6.25	14.05±0.31	67.5
	12.5	17.41±0.20	
	25	25.99±0.52	
	50	44.52±0.11	
	100	66.54±0.73	
<i>L.wightiana</i>	6.25	10.6±0.01	30.32
	12.5	25.15±0.07	
	25	46.63±0.51	
	50	89.45±0.65	
	100	97.58±0.20	
Ascorbic acid	6.25	25.18±0.95	54.53
	12.5	39.80±0.01	
	25	44.93±0.25	
	50	72.67±0.55	
	100	94.98±0.68	

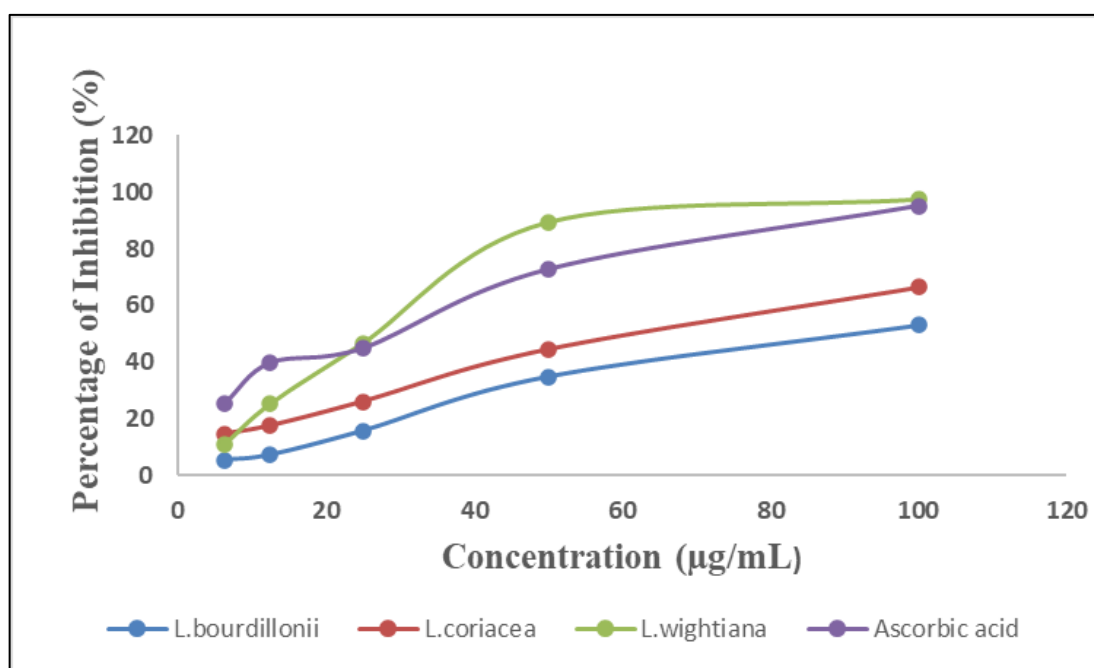


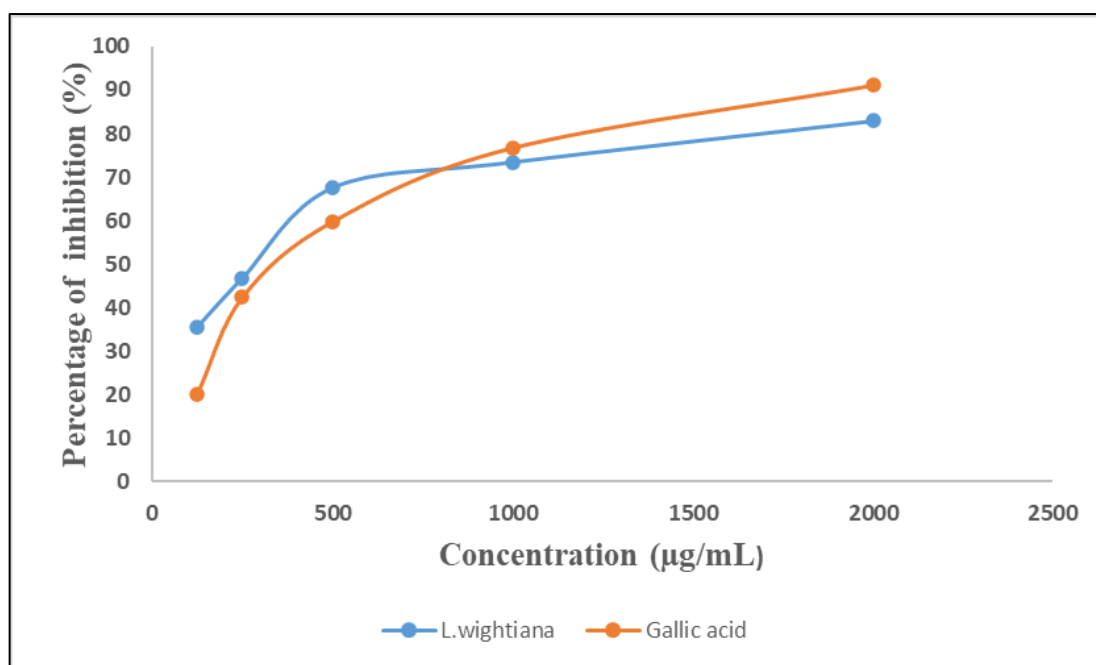
Fig 41 Antioxidant activity of leaves extract of the three *Litsea* spp. using DPPH method

ii. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity is the capability of a substance to nullify hydroxyl radicals, which are highly reactive and damaging free radicals. Hydroxyl radicals are generated within biological systems and can cause oxidative stress, leading to various health issues and cellular damage. Hence, the assessment of hydroxyl radical scavenging activity of compounds is crucial in evaluating their antioxidant properties and potential health benefits. In the present study, the leaf extracts of *L. wightiana* in various concentrations, like (125, 250, 500, 1000, 2000 µg/mL), were used. The antioxidant activity of *L. wightiana* leaves extract was determined with an IC_{50} value of 271.255 µg/mL, and the standard gallic acid, with an IC_{50} value of 533.504 µg/mL. The results are shown in Table 28 & Fig 42.

Table 28 Hydroxyl radical scavenging activity of the leaves extract of *L. wightiana*

Extract	Concentration $\mu\text{g/mL}$	% inhibition	IC ₅₀ Value ($\mu\text{g/mL}$)
<i>L. wightiana</i> leaf	125	35.54 $\pm$ 0.02	271.255
	250	46.76 $\pm$ 0.32	
	500	67.55 $\pm$ 0.15	
	1000	73.43 $\pm$ 0.05	
	2000	82.91 $\pm$ 0.17	
Standard (Gallic Acid)	125	20.17 $\pm$ 0.15	533.504
	250	42.36 $\pm$ 0.72	
	500	59.74 $\pm$ 0.95	
	1000	76.5 $\pm$ 0.26	
	2000	91.11 $\pm$ 0.20	

**Fig 42** Antioxidant activity of *L. wightiana* leaves extract using hydroxyl radical scavenging assay**iii. Superoxide radical scavenging activity**

Superoxide radical scavenging activity refers to the potential of compounds to neutralize and remove superoxide radicals from biological systems. Superoxide radicals are highly reactive and are considered as one of the most significant reactive

oxygen species (ROS) generated in living organisms during various physiological and pathological processes. Their overproduction can lead to oxidative stress, which is associated with numerous health issues, including inflammation, aging, and various diseases. In the present investigation, the methanolic extracts of leaves of *L. wightiana* were subjected to a superoxide radical scavenging activity study. The extract in different concentrations (125, 250, 500, 1000, 2000 µg/mL) were used and encouraging results were obtained. The methanolic extracts of *L. wightiana* leaves showed maximum radical scavenging with an IC₅₀ value of 1052.57 µg/mL compared to ascorbic acid, which was used as the standard with an IC₅₀ value of 567.07 µg/mL (Table 29 & Fig 43).

Table 29 Superoxide radical scavenging activity of the leaves extract of *L. wightiana*

Extract	Concentration(µg/mL)	% inhibition	IC ₅₀ Value (µg/mL)
<i>L. wightiana</i> leaf	125	13.30±0.02	1052.27
	250	25.10±0.16	
	500	34.74±0.01	
	1000	59.71±0.65	
	2000	73.82±0.045	
Standard (Ascorbic Acid)	125	23.13±0.45	567.07
	250	39.80±0.82	
	500	59.21±0.47	
	1000	70.52±0.01	
	2000	91.26±0.05	

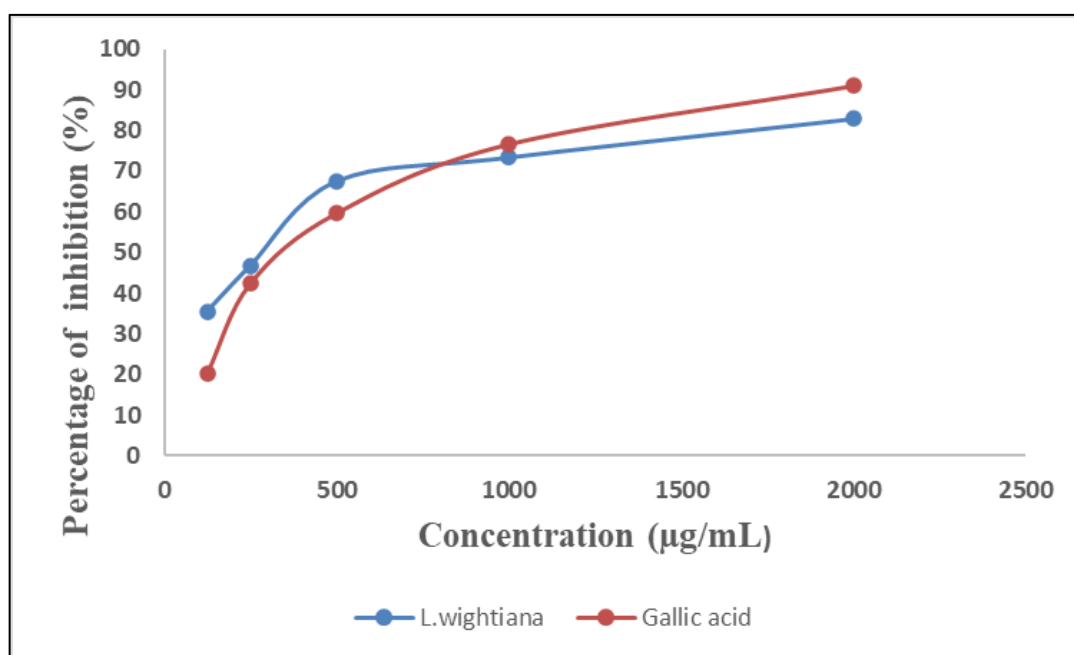


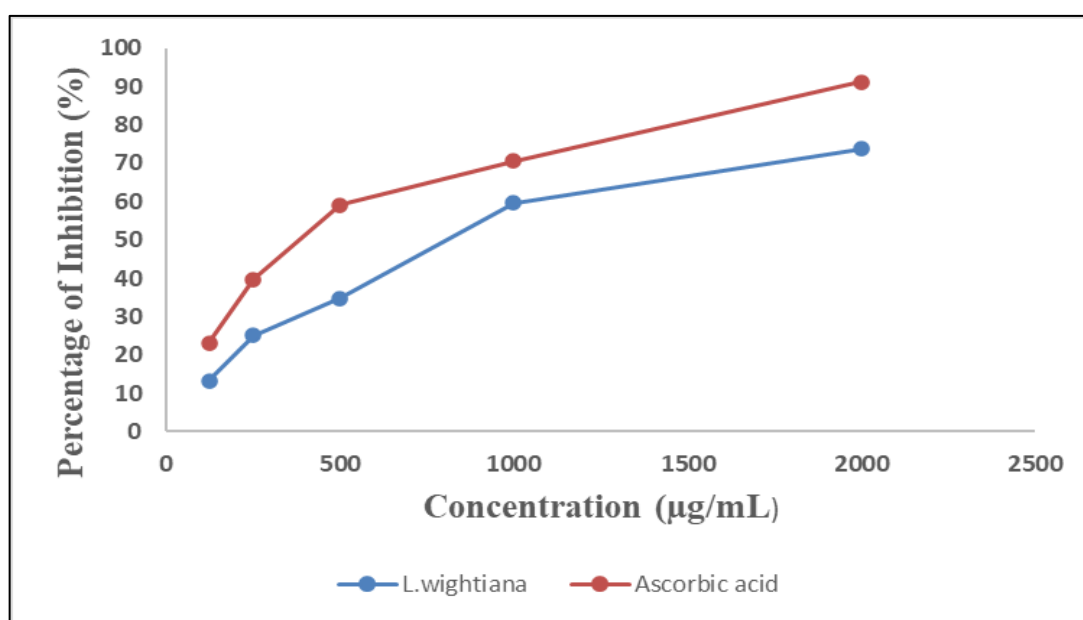
Fig 43 Antioxidant activity of *L. wightiana* leaves extract by Superoxide radical scavenging activity

iv. Ferrous reducing antioxidant power assay (FRAP)

FRAP is a common method used to assess the antioxidant activity of plant extracts. It assesses the ability of a substance to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). This assay is based on the principle that the antioxidants can donate electrons to reduce Fe^{3+} to Fe^{2+} ions, resulting in a color change that can be measured spectrophotometrically. The results of the FRAP assay in *L. wightiana* methanolic leaf extracts indicated an increase in absorbance from 0.135 ± 0.017 to 0.488 ± 0.011 in a dose-dependent manner, and this increase in absorbance was indicated due to increased reducing power of the extracts. The results were compared with the absorbance of standard quercetin in which the absorbance ranged from 0.347 ± 0.05 to 0.878 ± 0.005 (Table 30 and Fig 44).

Table 30. Ferrous reducing antioxidant power assay of the leaves extract of *L. wightiana*

Extract	Concentration($\mu\text{g/mL}$)	Absorbance $\pm$ SD at 700 nm
<i>L. wightiana</i> leaf	125	0.135 $\pm$ 0.17
	250	0.207 $\pm$ 0.26
	500	0.237 $\pm$ 0.20
	1000	0.308 $\pm$ 0.01
	2000	0.488 $\pm$ 0.11
Standard (Quercetin)	125	0.347 $\pm$ 0.05
	250	0.569 $\pm$ 0.01
	500	0.645 $\pm$ 0.02
	1000	0.700 $\pm$ 0.02
	2000	0.878 $\pm$ 0.05

**Fig 44 Antioxidant activity of *L. wightiana* leaves extract by FRAP method****C. ANTIDIABETIC STUDY**

Antidiabetic activity of the leaves and bark extracts of the three *Litsea* species were studied using the alpha amylase and alpha glucosidase enzyme inhibition assays, and the extracts showed remarkable antidiabetic activity. In the alpha amylase assay, the leaves and bark extracts of *L. wightiana* showed significant activity with IC_{50} values, 134.67 $\mu\text{g/mL}$ and 148.83 $\mu\text{g/mL}$. *L. coriacea* leaves and bark showed lower inhibition with IC_{50} 228.95 $\mu\text{g/mL}$ and 412.90 $\mu\text{g/mL}$

respectively. In the alpha glucosidase assay, *L. wightiana* leaf and *L. coriacea* bark exhibited higher inhibition of the enzyme among other extracts with IC₅₀ of 50.30 µg/mL and 88.771 µg/mL. In both assays acarbose, an antidiabetic agent used as standard which showed antidiabetic activity with IC₅₀ values 45.41µg/mL in alpha amylase inhibitory assay and 20.23 µg/mL in alpha glucosidase inhibitory assay. The details of the results are depicted in Table 31& 32 and Figures 45 & 46 below.

Table 31. Antidiabetic activity of the leaves and bark extracts of three *Litsea* spp. by α -amylase inhibition method

Sample	Concentration(µg/mL)	Absorbance at 540 nm	% of inhibition	IC ₅₀ Value µg/mL
LBB	50	0.400± 0.12	34.70	176.20
	100	0.210±0.02	46.37	
	200	0.178±0.23	52.68	
	400	0.135±0.12	70.03	
LBL	50	0.164±0.31	41.28	219.06
	100	0.155±0.01	49.56	
	200	0.141±0.30	58.77	
	400	0.128±0.22	70.97	
LCB	50	0.258±0.54	49.77	412.90
	100	0.249±0.03	58.1	
	200	0.204±0.51	66.91	
	400	0.150±0.43	89.17	
LCL	50	0.235±0.01	27.44	228.95
	100	0.198±0.02	35.45	
	200	0.178±0.76	49.52	
	400	0.123±0.34	68.47	
LWB	50	0.140±0.03	36.85	148.83
	100	0.099±0.12	45.21	
	200	0.086±0.24	59.04	
	400	0.080±0.91	75.69	
LWL	50	0.087±0.02	34.56	134.67
	100	0.081±0.56	48.65	
	200	0.079±0.66	62.21	
	400	0.075±0.30	80.9	
Acarbose (Standard)	50	0.219±0.40	45.32	45.41
	100	0.181±0.38	59.21	
	200	0.156±0.35	74	
	400	0.121±0.36	90.34	
Blank	-	0.515±0.013	-	
Control	-	0.032±0.001	-	

LBB- *L. bourdillonii* bark, LBL- *L. bourdillonii* leaf, LCB- *L. coriacea* bark, LCL- *L. coriacea* leaf, LWB- *L. wightiana* bark, LWL- *L. wightiana* leaf. Absorbance value expressed as ± SEM (n=3)

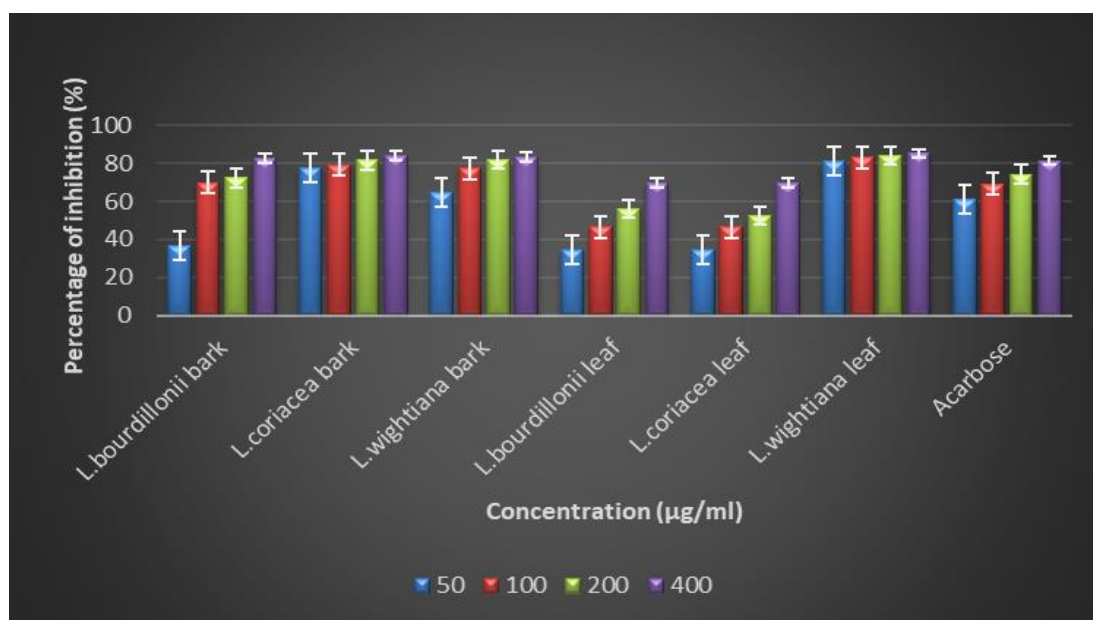


Fig 45 Antidiabetic assay of leaf and bark extracts of *Litsea* spp. using alpha amylase inhibition method

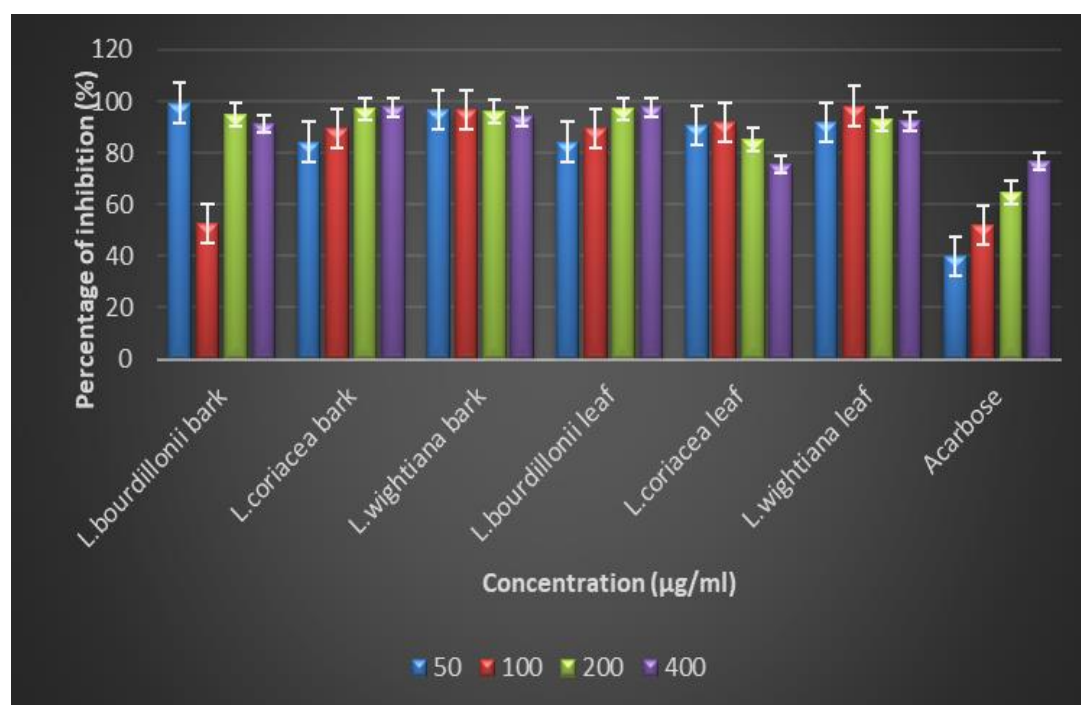


Fig 46 Antidiabetic assay of leaf and bark extracts of *Litsea* spp. by alpha glucosidase inhibition method

Table 32 Antidiabetic activity of leaf and bark extracts of the three *Litsea* spp. by α -glucosidase inhibition method

Extract	Concentration(μ g/mL)	Absorbance at 400 nm	% of inhibition	IC ₅₀ value(μ g/mL)
LBB	50	0.020 $\pm$ 0.16	28.28	217.779
	100	0.276 $\pm$ 0.32	39.43	
	200	0.044 $\pm$ 0.11	48.52	
	400	0.064 $\pm$ 0.65	65.33	
LBL	50	0.040 $\pm$ 0.32	38.23	164.09
	100	0.50 $\pm$ 1.23	44.32	
	200	0.084 $\pm$ 0.93	54.48	
	400	0.116 $\pm$ 0.75	74.82	
LCB	50	0.102 $\pm$ 0.12	44.20	88.771
	100	0.075 $\pm$ 0.54	53.38	
	200	0.033 $\pm$ 0.34	60.08	
	400	0.029 $\pm$ 0.41	75.12	
LCL	50	0.067 $\pm$ 0.11	30.23	176.763
	100	0.062 $\pm$ 0.98	39.28	
	200	0.097 $\pm$ 0.76	58.03	
	400	0.165 $\pm$ 0.09	78.28	
LWB	50	0.036 $\pm$ 0.87	36.40	99.32
	100	0.035 $\pm$ 0.35	54.38	
	200	0.038 $\pm$ 0.65	69.20	
	400	0.049 $\pm$ 0.76	85.43	
LWL	50	0.061 $\pm$ 0.08	40.43	50.30
	100	0.027 $\pm$ 0.023	61.28	
	200	0.055 $\pm$ 0.37	72.30	
	400	0.59 $\pm$ 0.23	81.11	
Standard (Acarbose)	50	0.575 $\pm$ 0.49	48.29	20.23
	100	0.464 $\pm$ 0.65	63.28	
	200	0.348 $\pm$ 0.63	75.82	
	400	0.236 $\pm$ 0.21	86.43	
Blank	-	0.943	-	-
Control	-	0.021		

LBB- *L. bourdillonii* bark, LBL- *L. bourdillonii* leaf, LCB- *L. coriacea* bark, LCL- *L. coriacea* leaf, LWB- *L. wightiana* bark, LWL- *L. wightiana* leaf. Values expressed as $\pm$ SEM (n=3).

D. IN VITRO CYTOTOXICITY

The cytotoxicity of leaf and bark extracts of *L. bourdillonii*, *L. coriacea*, and *L. wightiana* were studied by MTT assay by applying various concentrations such as 6.25 μ g/mL, 12.5 μ g/mL, 25 μ g/mL, 50 μ g/mL, and 100 μ g/mL, and the results

indicated that no extracts showed significant cytotoxicity in SK-MEL cell lines (Table 33 and Figures 47&48). Almost all the treated cell lines showed cell viability greater than or equal to 50% indicating less or no cytotoxicity effect. All experiments were conducted three times as replicates. The *L. coriacea* leaves extract showed moderate cytotoxicity with 40.67% of inhibition at 100 µg/mL concentration.

Table 33 Cytotoxicity effect of leaf and bark extracts of the three *Litsea* spp. in SK-MEL cell lines

Extract	Concentration(µg/mL)	Absorbance at 570 nm	Cell viability (%)
LBB	6.25	0.411±0.12	93.55
	12.5	0.381±0.98	86.87
	25	0.346±0.07	78.90
	50	0.307±0.42	69.87
	100	0.259±0.51	60.92
LBL	6.25	0.381±0.01	86.87
	12.5	0.352±0.025	80.12
	25	0.311±0.65	70.86
	50	0.281±0.32	64.03
	100	0.237±0.33	54.09
LCB	6.25	0.390±0.12	88.92
	12.5	0.353±0.04	80.42
	25	0.305±0.75	69.57
	50	0.283±0.43	64.49
	100	0.239±0.32	54.47
LCL	6.25	0.352±0.04	80.19
	12.5	0.312±0.32	71.01
	25	0.275±0.03	62.59
	50	0.228±0.34	51.97
	100	0.200±0.12	45.67
LWB	6.25	0.369±0.24	84.14
	12.5	0.332±0.07	75.56
	25	0.306±0.23	69.72
	50	0.271±0.46	61.83
	100	0.223±0.11	50.75
LWL	6.25	0.316±0.03	97.21
	12.5	0.293±0.12	92.19
	25	0.271±0.13	87.72
	50	0.227±0.81	83.48
	100	0.165±0.04	77.21
Control	-	0.439±0.12	-

LBB- *L. bourdillonii* bark, LBL- *L. bourdillonii* leaf, LCB- *L. coriacea* bark, LCL- *L. coriacea* leaf, LWB- *L. wightiana* bark, LWL- *L. wightiana* leaf. Experimental results expressed as ± SEM, n=3

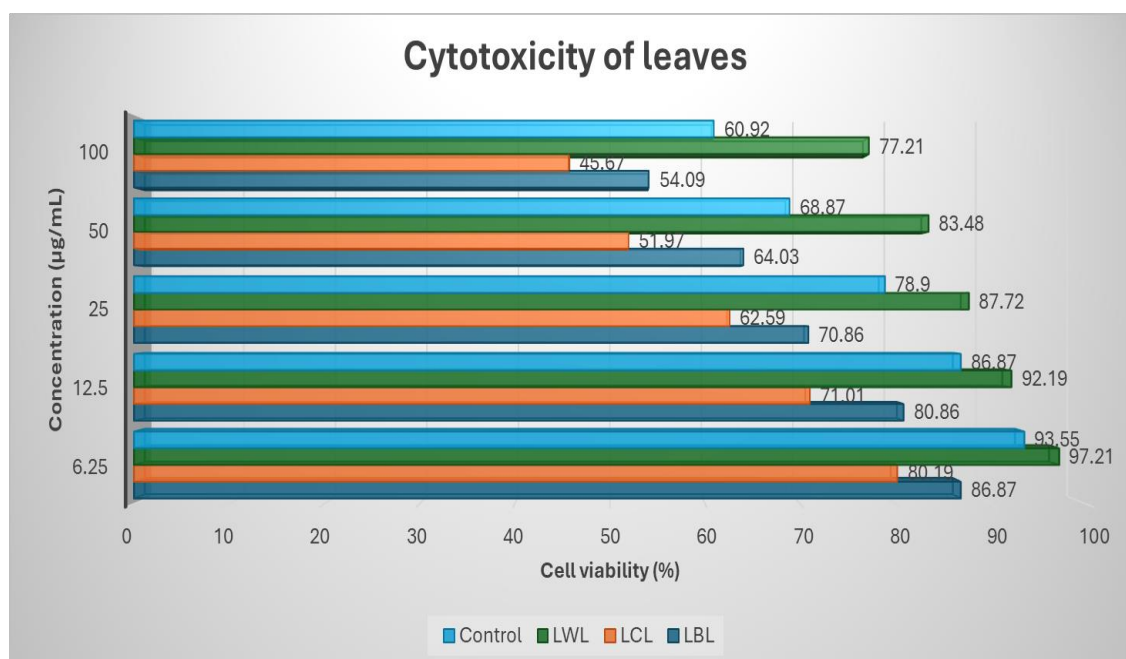


Fig 47 Cytotoxicity of leaf extracts of three *Litsea* spp.

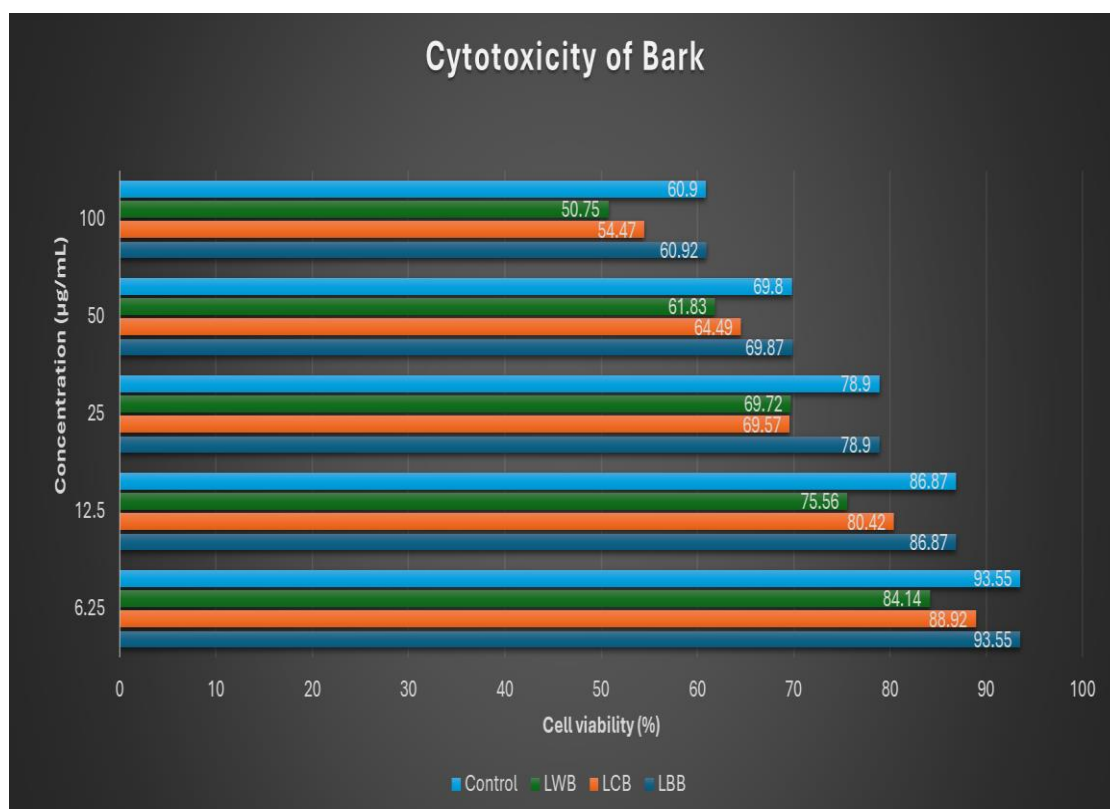


Fig 48 Cytotoxicity of bark extracts of three *Litsea* spp.

From the qualitative phytochemical screening studies, it was found that *L. wightiana* leaves extract contain all the phytochemicals and quantitative assay showed higher content of phenols, flavonoids and tannins when compared to other leaves and bark extracts. Moreover, *L. wightiana* showed significant antioxidant, antibacterial and antidiabetic properties. Hence *L. wightiana* leaves methanolic extract was selected for higher order studies such as anti-inflammatory, gene expression, wound healing and docking studies.

E. ANTIINFLAMMATORY ACTIVITY

Inflammation is a sort of immune response against various types of harmful stimuli. Anti-inflammatory response is a counterbalancing mechanism to regulate and resolve the inflammatory process during an immune response. This study aimed to investigate the anti-inflammatory potential of *L. wightiana* leaves. Both *in vitro* and *in vivo* methods were adopted.

i. *In vitro* anti-inflammatory activity

The anti-inflammatory studies were performed by the *in vitro* method in RAW 264.7 macrophage cell lines. The enzyme inhibition assays were demonstrated by testing the activity of different enzymes like cyclooxygenase, lipoxygenase, myeloperoxidase, and nitric oxide synthase, which are generally involved in the synthesis of proinflammatory mediators. The methanolic extracts of *L. wightiana* leaves were tested for anti-inflammatory activity in selected cell lines. The studies demonstrated dose-dependent inhibition of these enzymes against standard diclofenac sodium as illustrated in Figures 49-52 and Tables 34& 35.

● Cyclooxygenase (COX) activity

Cyclooxygenase enzyme is an important enzyme involved in inflammatory responses. Cyclooxygenase enzyme activity was checked by treating the LPS-induced RAW 264.7 macrophage cells with the methanolic extract of leaves of *L. wightiana*. Diclofenac sodium was used as the standard. The experiment showed a dose-dependent reduction in enzyme activity by the extracts, which indicated the anti-inflammatory potential of the extract. Different concentrations of the sample

(25, 50, 100 $\mu\text{g/mL}$) were taken, the percentage of inhibition of cyclooxygenase function was measured, and the maximum inhibition was found to be $65.44 \pm 0.23\%$ at 100 $\mu\text{g/mL}$. By the same concentration, the standard exhibited a reduction in activity at $85 \pm 0.24\%$.

- **Lipoxygenase (LOX) activity**

Lipoxygenase is a lipid peroxidase enzyme involved in many inflammatory responses and causes various diseases. To check whether *L. wightiana* leaf extracts can act as an anti-inflammatory agent, by inhibiting the LOX, various concentrations of the sample were applied in RAW 264.7 cell lines. The extract inhibited the LOX and the percentage was 17.51 ± 0.005 , 49.88 ± 0.092 , and 64.96 ± 0.43 at varying concentrations like 25, 50 and 100 $\mu\text{g/mL}$. Standard diclofenac sodium exhibited $72 \pm 0.13\%$ of inhibition at 100 $\mu\text{g/mL}$ concentration.

- **Myeloperoxidase (MPO) activity**

MPO is an enzyme which is active in response to pathogenic invasion. In some cases, it produces free radicals and reactive oxygen species that may lead to serious disorders in the cell. The effect of *L. wightiana* leaf extracts was tested for anti-inflammatory activity by checking the inhibition of myeloperoxidase activity using LPS-induced RAW 264.7 macrophage cell lines. Varying concentrations of the sample and the standard diclofenac sodium, like 25, 50, and 100 $\mu\text{g/mL}$ were taken. At 100 $\mu\text{g/mL}$ the MPO enzyme showed reduced activity measured as 0.0129 U/mL of cell lysate. The inhibition of diclofenac was almost like that of the sample.

- **Inducible Nitric oxide synthase (i NOS) activity**

Inhibition of the nitric oxide synthase enzyme using anti-inflammatory agents is widely used in anti-inflammatory treatments. For analyzing the anti-inflammatory effect of *L. wightiana* leaves, the methanolic extracts at the concentrations of 25, 50, and 100 $\mu\text{g/mL}$ were applied in LPS-treated RAW 264.7 cell lines. The standard Diclofenac sodium was also set at the same concentrations to compare the activity. Significant activity was observed, and inhibition of the nitric oxide synthase enzyme was found to be 51.2 % at the concentration of 100 $\mu\text{g/mL}$.

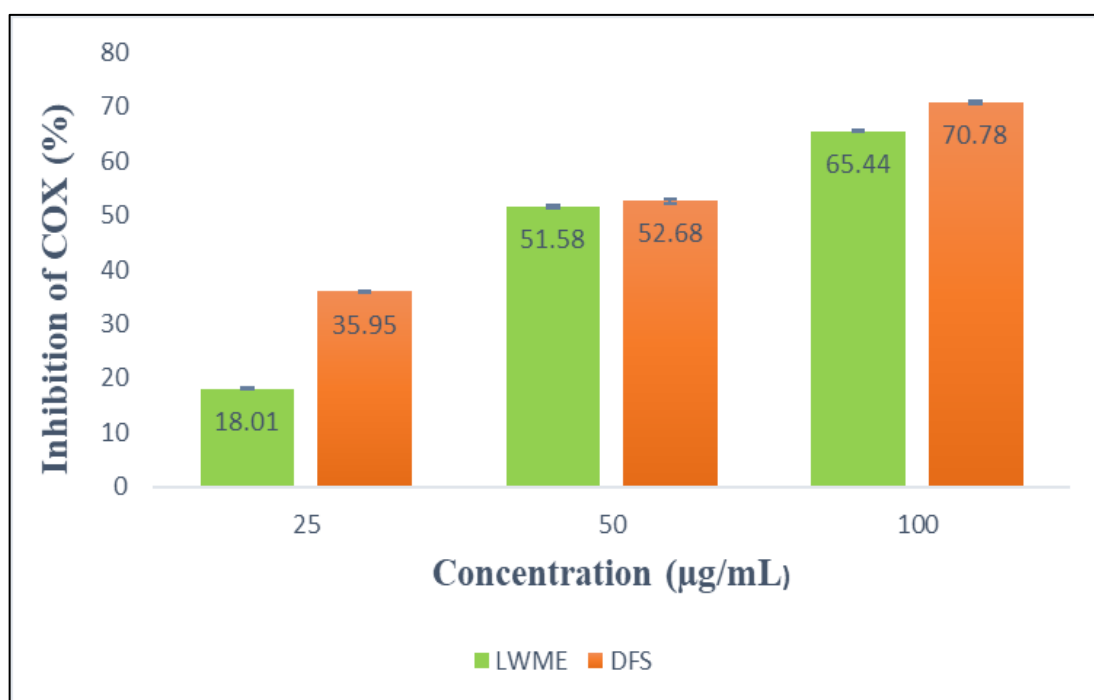


Fig 49 *In vitro* anti-inflammatory activity of LWME by COX inhibition

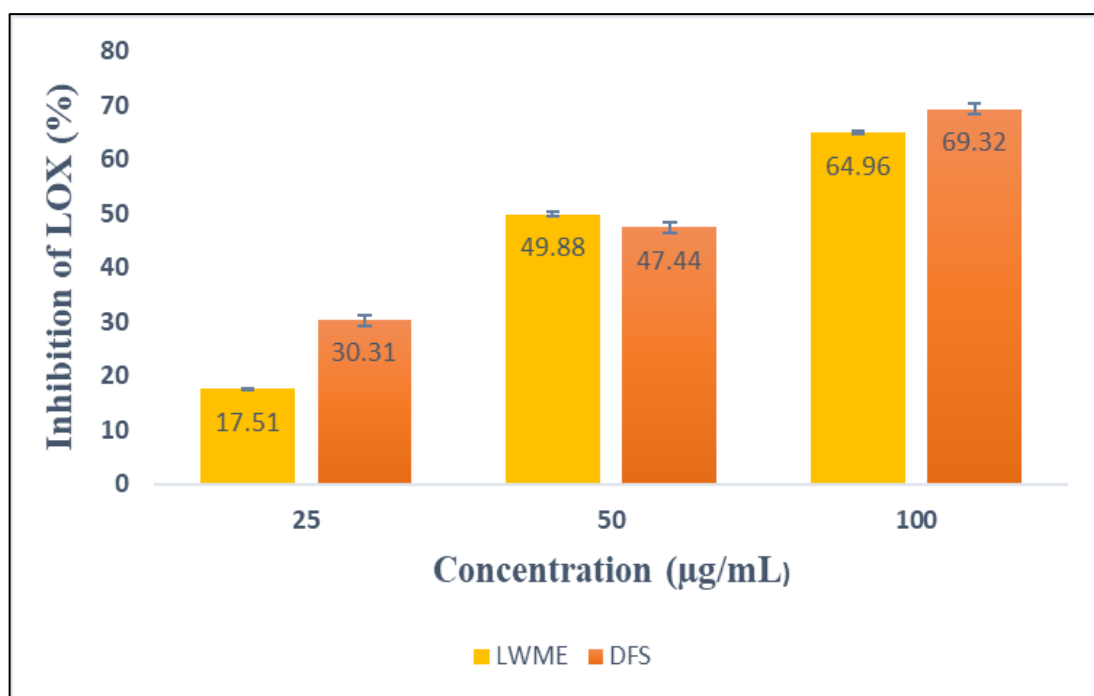


Fig 50 *In vitro* anti-inflammatory activity of LWME by LOX inhibition

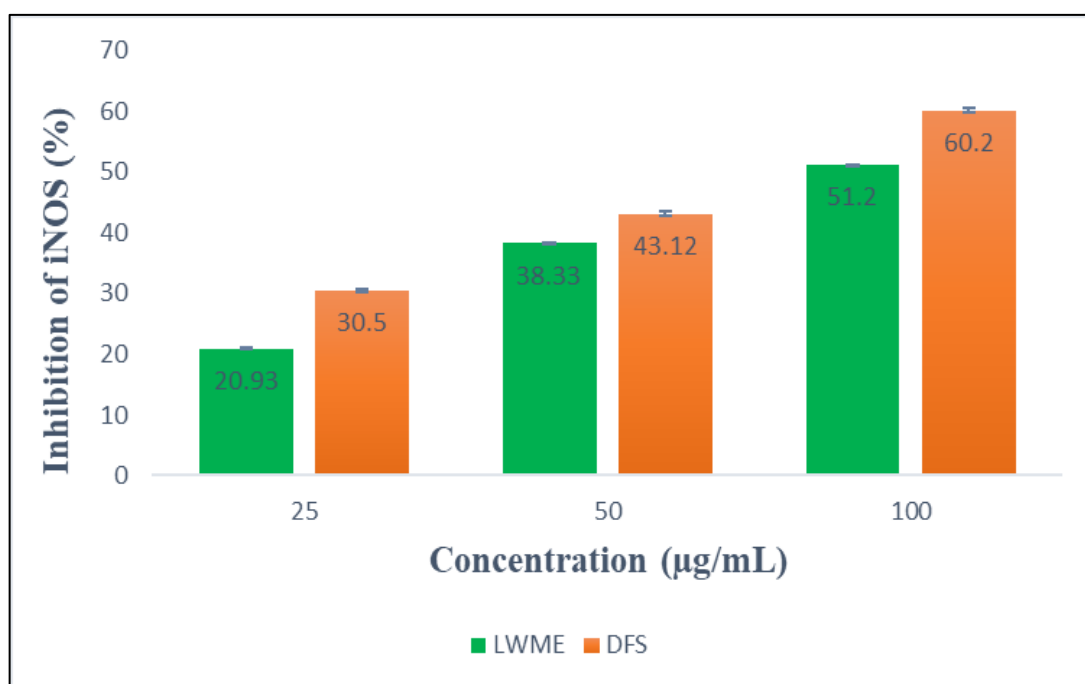


Fig 51 *In vitro* anti-inflammatory activity of LWME by inducible Nitric oxide synthase inhibition

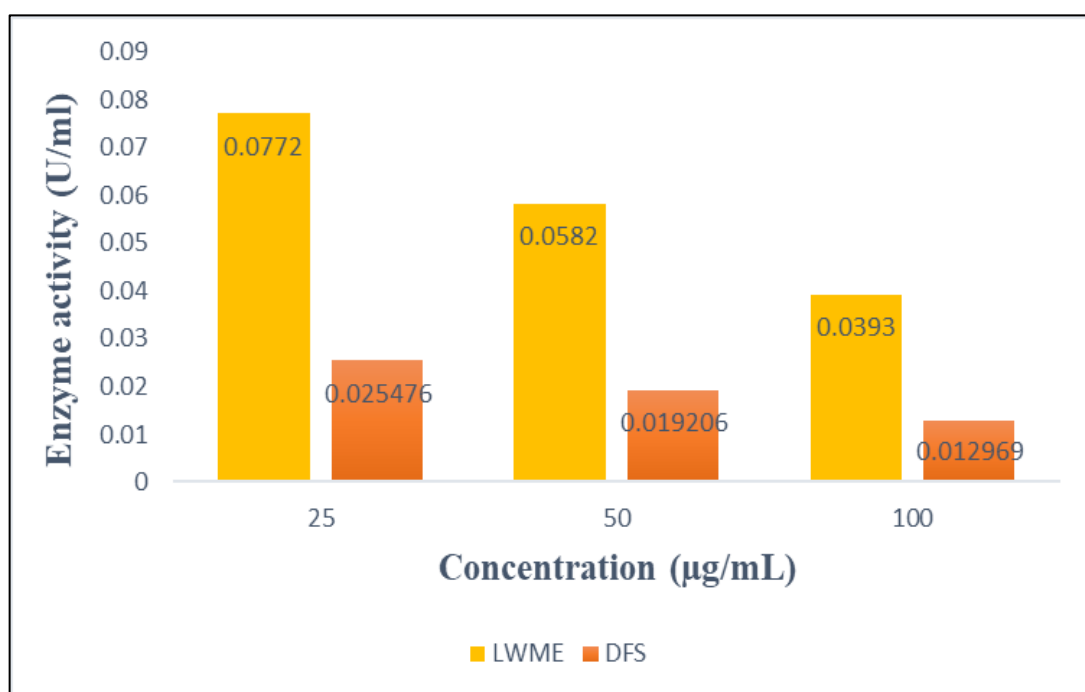


Fig 52 *In vitro* anti-inflammatory activity of LWME by Myeloperoxidase inhibition

- **Cellular Nitrite level**

Evaluation of total cellular nitrite level is utilized as an indicator in inflammatory studies. The higher level of nitrite is always associated with any kind of immune response in cells. Anti-inflammatory agents are commonly used to reduce the nitrite level by inhibiting the nitric oxide synthase enzyme. This experiment studied the potential of *L. wightiana* leaves to reduce the cellular nitrate level. For this, the plant extracts at concentrations of 25, 50, and 100 $\mu\text{g/mL}$ were treated with LPS-stimulated RAW.264.7 macrophage cell lines. The plant extract profoundly reduced the accumulation of NO with respect to increasing concentrations. The extract reduced the amount of NO from 489.55 μg to 208.395 μg (Fig 53).

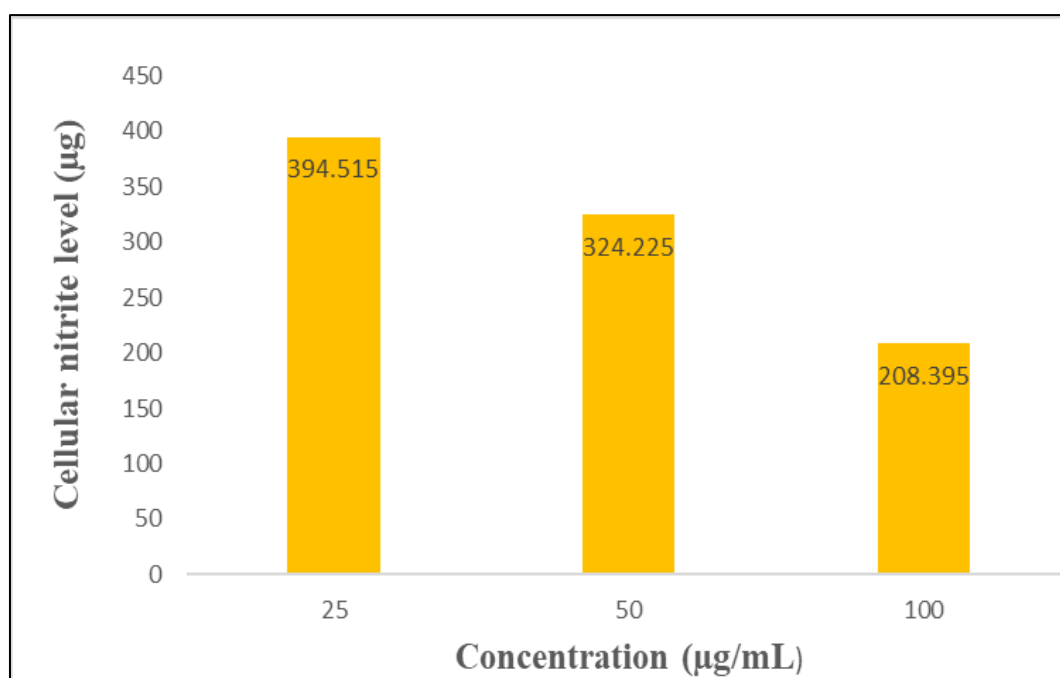


Fig 53 Cellular nitrite level after the treatment with LWME

Table 34 *In vitro* anti-inflammatory activity study by Enzyme inhibition assay

Name of Enzyme	Sample	Concentration (µg/mL)	Enzyme activity /Percentage of Inhibition ± SD
Cyclooxygenase	Extract (LWME)	25	18.01±0.13
		50	51.58±0.23
		100	65.44±0.12
	Standard (DFS)	25	35.95±0.12
		50	52.68±0.43
		100	70.78±0.33
Lipoxygenase	Extract (LWME)	25	17.51±0.15
		50	49.88±0.38
		100	64.96±0.33
	Standard (DFS)	25	30.31±0.29
		50	47.44±0.25
		100	69.32±0.2
Inducible nitric oxide synthase	Extract (LWME)	25	20.93±0.13
		50	38.33±0.15
		100	51.20±0.11
	Standard (DFS)	25	30.50±0.28
		50	43.12±0.34
		100	60.20±0.41

Table 35 *In vitro* anti-inflammatory activity study by Myeloperoxidase inhibition assay

Sample	Concentration (µg/mL)	Enzyme activity ± SD
LWME	25	0.0772±0.13
	50	0.0582±0.55
	100	0.0393±0.34
Standard (DFS)	25	0.0254±0.12
	50	0.0192±0.22
	100	0.0129±0.29

ii. Gene expression studies

To validate the anti-inflammatory effect of *L. wightiana* leaf extracts gene expression studies were carried out. qRT-PCR analysis was used for the study. The results indicated that all the proinflammatory cytokines and transcription factors demonstrated a downhill regulation of genes in LPS-induced RAW 264.7 cells by the extract with increasing concentration, as illustrated in Fig. 54. The extract showed suppression of Tumor Necrosis Factor (TNF)- α , Nuclear Factor kappa B (NFkB), Cyclooxygenase (COX)-2 and expression of Interleukin (IL)-6 (Fig 54).

iii. Docking Study

The molecular docking method was employed to assess the anti-inflammatory potential of the bioactive compounds in *L. wightiana* leaves. Twelve compounds were selected for the study and which were reported with anti-inflammatory activity and these compounds were tested against four target proteins. The overall result showed that, notably, dicentrinone (an alkaloid compound identified by LC/MS analysis) demonstrated favorable binding energy against all target proteins, including Human myeloperoxidase (-8.3), Human inducible Nitric oxide synthase (-9.5), Cyclooxygenase II (-9.2), and 5-Lipoxygenase (-8). These findings position dicentrinone as a promising anti-inflammatory agent. Analysis of interactions revealed that the compound forms a lone hydrogen bond interaction with 1dnu involving GLU102, and 4nos with ASN370. Additionally, it engages van der Waals and π interactions with all other target proteins. Notably, Xanthoplanine, Cubinol, Chalcone, α -cadinol, and similar compounds displayed substantial affinity with the selected proteins. Conversely, molecules such as linalool and hexadecenoic acid exhibited least binding energy and affinity. The binding geometries of the target proteins are illustrated in Figures 55 (i-ii) & 56 (i-ii) and Table 36.

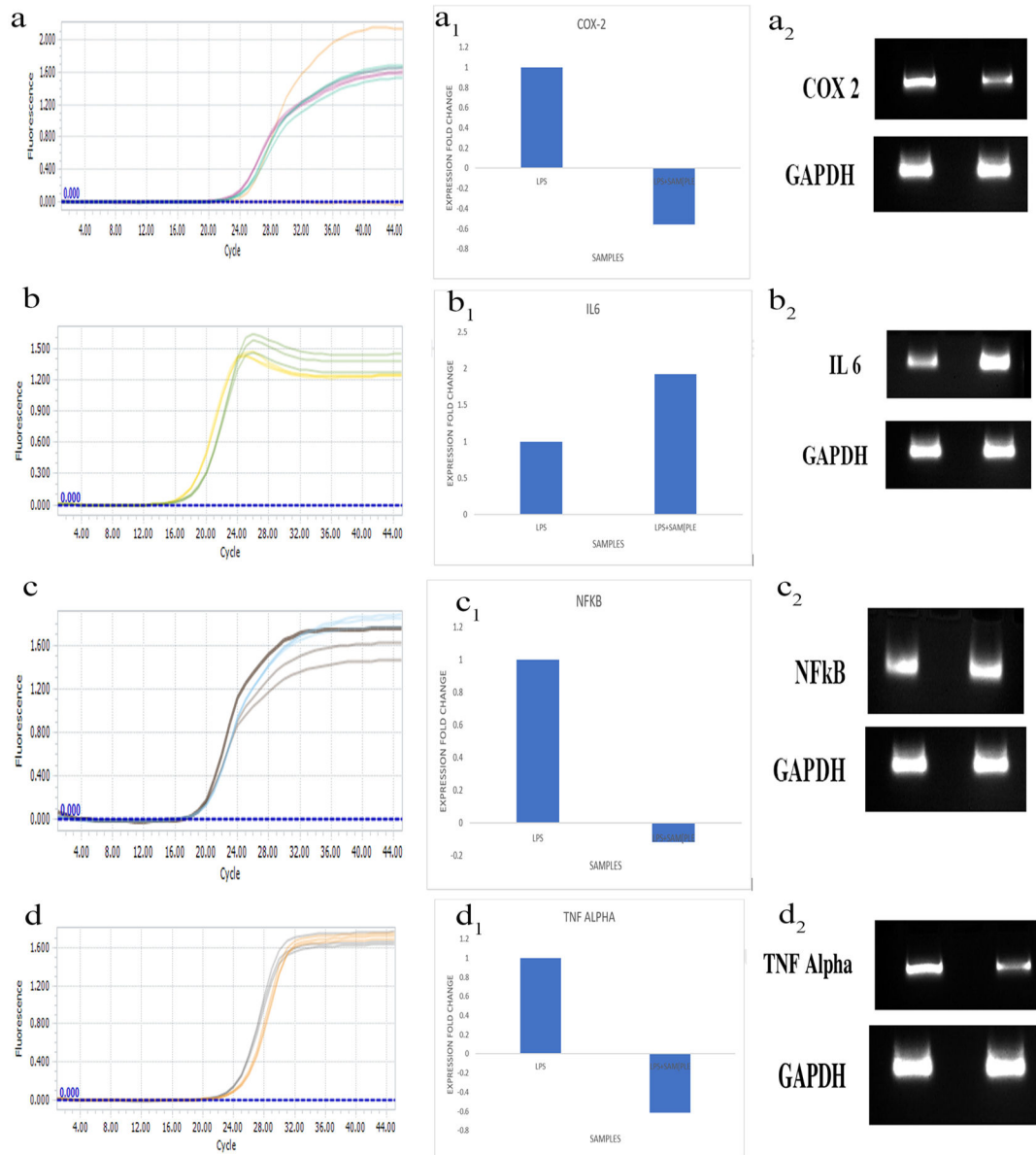


Fig 54 Gene expression analysis of LWME. a, a₁ and a₂. Amplification curve, expression fold change and gel image of COX-2; b, b₁ and b₂. Amplification curve, expression fold change and gel image of IL-6; c, c₁ and c₂. Amplification curve, expression fold change and gel image of NFκB; d, d₁ and d₂. Amplification curve, expression fold change and gel image of TNF Alpha

Table 36 Binding energy of docked proteins with target anti-inflammatory compounds

Compound	Binding Energy (BE) (Kcal/mol)			
	1dnu MPO	4nos iNOS	5ikr COX-2	6n2w LOX
Dicentrinone	-8.3	-9.5	-9.2	-8
Xanthoplanine	-7.8	-9.4	-8.5	-7.3
Chalcone	-7.6	-9.0	-7.5	-6.8
Cubenol	-7.6	-7.1	-7.5	-6.8
α - Cadinol	-7.5	-6.9	-6.9	-6.7
tau-Cadinol	-7.5	-6.9	-6.8	-6.7
1H-Cycloprop[e]azulene,decahydro-1,1,7-trimethyl-4-methylene	-7.3	-6.9	-6.0	-6.2
Carotol	-6.6	-6.6	-5.8	-5.9
9,12-Octadecadienoic acid(Z,Z)-methyl ester	-6.1	-6.5	-5.7	-5.5
Linalool	-5.8	-6.4	-5.3	-5
Decanedioic acid, bis(2-ethylhexyl) ester	-5.7	-6.1	-5.1	-4.7
Hexadecanoic acid-methyl-ester	-5.3	-5.5	-4.9	-4.7

iv. *In vivo* anti-inflammatory study

The *in vivo* anti-inflammatory studies were carried out in animal models using carrageenan induced paw edema model. The *L. wightiana* methanolic leaf extracts showed a remarkable reduction in the size of paw edema, which was treated with carrageenan, an inflammatory substance (Fig 57). The effect of LWME was found to be significant and dose dependent. The drug treatment of 50 mg/kg resulted in a progressive decrease in paw thickness, viz., 3.67 ± 0.04 mm, 3 ± 0.26 mm, 2.33 ± 0.37 mm in the third, fourth and fifth-hour periods, respectively and showed statistically significant at $P < 0.01$ level of significance. Similarly, treatment of the extract with 100 mg/kg exhibited a decline in paw thickness during the second hour after treatment and was significant with a P value < 0.05 . After the fourth hour treatment, the inflammation was reduced to 60.12%, which is higher than the treatment with standard Indomethacin at 58.02% (Table 37 & 38).

Table 37 *In vivo* anti-inflammatory activity of LWME by carrageenan induced paw edema method (Paw thickness)

Groups	Drug Treatment	Paw thickness in mm					
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr
I	Control 0.5%CMC (1 mL/kg)	2.33±0.21	4.50±0.22	5.17±0.31	5.33±0.33	5.17±0.31	4.50±0.22
II	Indomethacin (10 mg/kg)	2.17±0.31	2.83±0.31***	3.17±0.31***	2.50±0.22***	2.17±0.17***	2.17±0.21***
III	LWME (50 mg/kg)	1.83±0.31	3.83±0.31	4.00±0.33	3.67±0.04**	3.00±0.26***	2.33±0.37**
IV	LWME (100 mg/kg)	2.17±0.31	3.17±0.31	3.33±0.17**	3.00±0.26***	2.50±0.22***	2.17±0.21

Values are in Mean±SEM(n=6)

*P<0.05, **P<0.01&***P<0.001 Vs control

Table 38 *In vivo* anti-inflammatory activity of LWME by carrageenan induced paw edema method (% of inhibition)

Groups	% inhibition					
	Drug treatment	1 hr	2 hr	3 hr	4 hr	5 hr
I	Control 0.5% CMC (1 mL/kg)	-	-	-	-	-
II	Indomethacin 10 mg/kg	37.11	38.68	53.09	58.02	51.78
III	LWME 50 mg/kg	14.89	22.63	31.14	41.97	60.12
IV	LWME 100 mg/kg	38.12	42.33	55.43	60.12	65.34

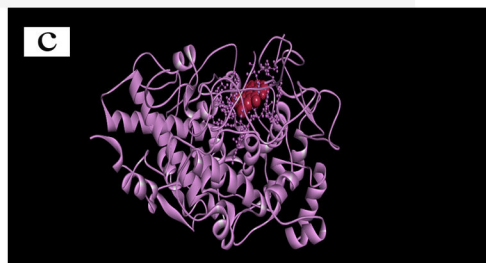
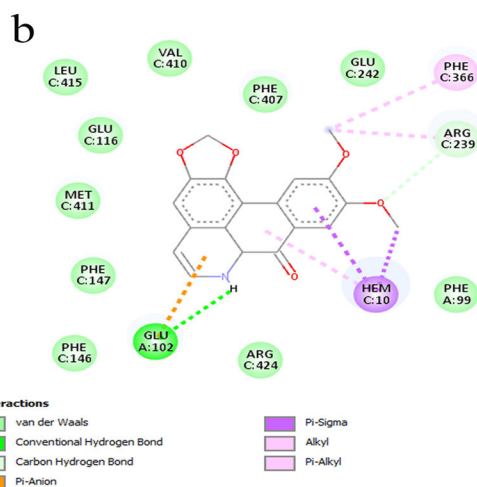
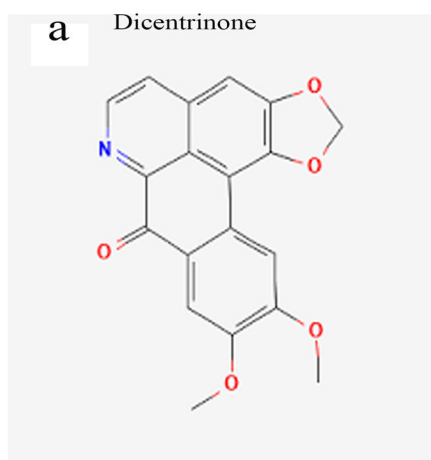


Fig 55(i) Molecular docking of anti-inflammatory compound from LWME with target protein. a. Dicentrinone structure; b. Bound conformation of 1dnu protein and ligand; c. 3D interaction between protein and ligand

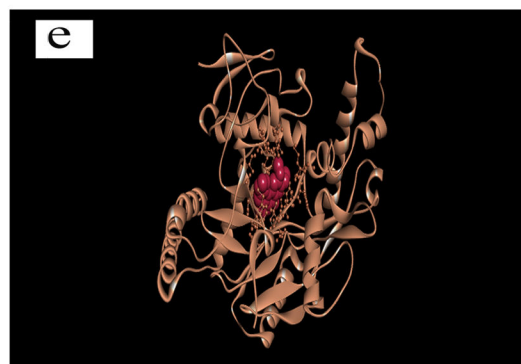
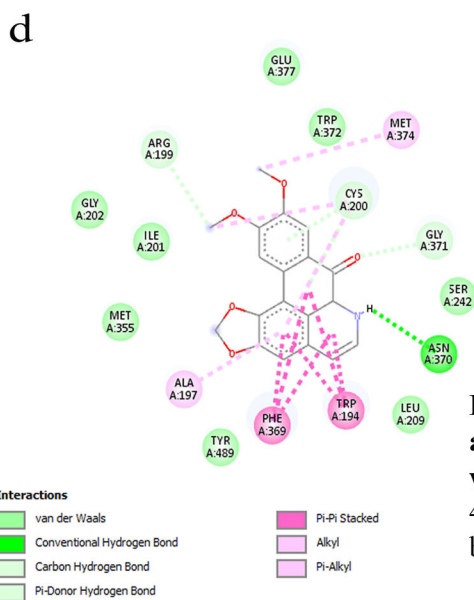


Fig 55 (ii) Molecular docking of anti-inflammatory compound from LWME with target protein. d. Bound conformation of 4nos protein and ligand; e. 3D interaction between protein and ligand

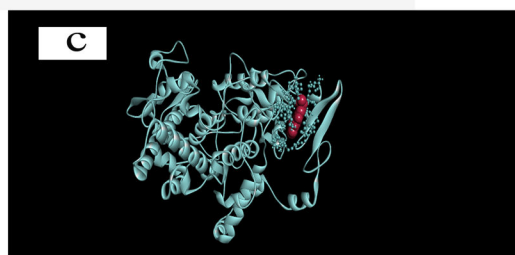
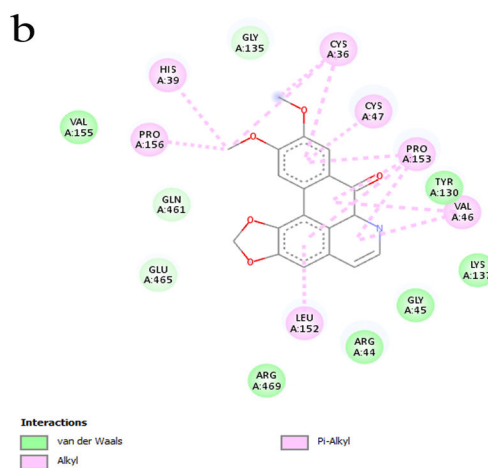
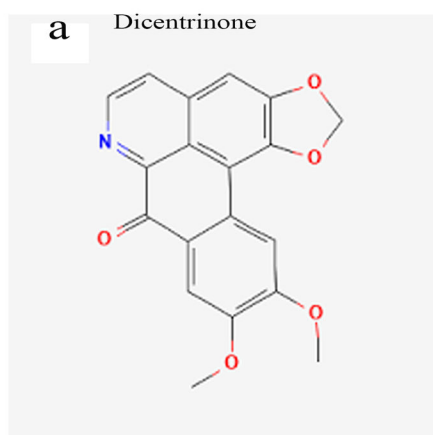


Fig-56(i) Molecular docking of anti-inflammatory compound from LWME with target protein. a. Dicentrinone structure; **b** Bound conformation of 5ikr protein and ligand; **c.** 3D interaction between protein and ligand

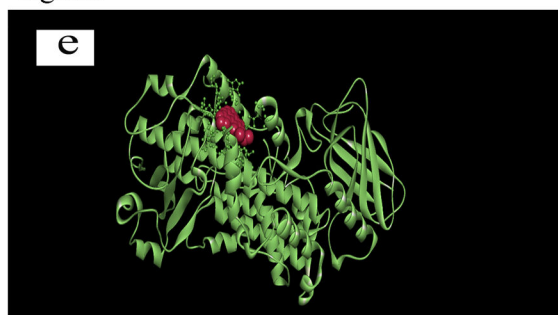
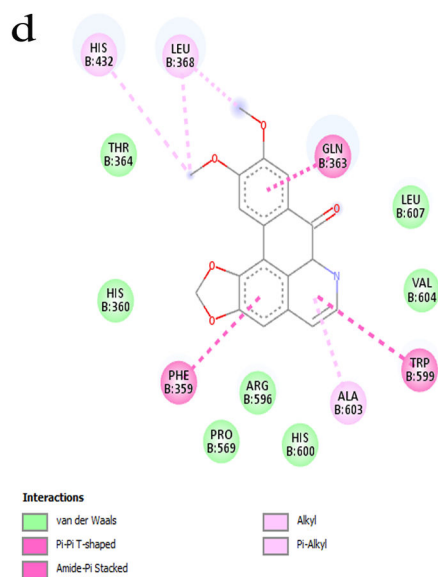


Fig 56(ii) Molecular docking of antiinflammatory compound from LWME with target protein. d. Bound conformation of 6n2w protein and ligand; **e.** 3D interaction between protein and ligand



Fig 57 *In vivo* anti-inflammatory study by carrageenan induced paw edema in wistar albino rats. a. Paw edema induction; b. LWME administration; c. Measurement of paw thickness

F. WOUND HEALING STUDY

i. *In vitro* wound healing assay

The *in vitro* scratch wound assay by *L. wightiana* methanolic leaf extract (25 µg/mL) showed the maximum (100%) wound closure in scratch wounds in L929 cell lines at 72 hr period which is considered to be a significant wound healing effect when compared with the control (untreated cells) which showed 94.4 % wound closure and cell migration at the same period (Fig 58).

ii. *In vivo* wound healing study

● Excision wound model

The excision wound method in the model animals demonstrated that the methanolic extract of *L. wightiana* leaves resulted in the maximum healing of wounds on 21st day post-wounding (Fig 59). The animals treated with 10% concentration of LMWE extract showed remarkable reduction in the wound area (95.04%) and faster rate of epithelialization showed reduction in wound area (19.67 ± 1.05 mm²). In control animals, it is observed that, after the 21st day, the animals treated with reference control povidone iodine ointment showed complete epithelialization and wound contraction (98.08%). The results were statistically significant at $P < 0.01$ and $P < 0.001$ levels. The results are depicted in Tables 39 & 40.

Table 39. Effect of LMWE on excision wounds in rats (Reduction in wound area)

Groups	Drug Treatment	Wound area mm ²				
		0 Day	3 rd Day	7 th Day	14 th Day	21 st Day
I	Vehicle Control (Simple Ointment)	486.17± 3.07	470.00± 0.77	427.66± 3.54	265.33± 2.01	148.17± 5.98
II	Reference Control Povidone Iodine	483.16± 1.94	430.00± 1.46*	297.00± 2.63***	96.33± 1.38***	6.33± 0.76***
III	5% LMWE	485.67± 2.04	452.67± 2.17	358.83± 2.62*	163.17± 2.87***	43.50± 1.28***
IV	10% LMWE	484.33± 2.59	432.66± 1.49*	322.00± 4.25**	144.50± 1.65***	19.67± 1.05***

Values are in Mean ± SEM; (n = 6)

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ Vs Vehicle Control

Table 40. Effect of LMWE on excision wounds in rats (Percentage of reduction in wound size)

Groups	Drug Treatment	Percentage Reduction in Wound Size (%)			
		3 rd Day	7 th Day	14 th Day	21 st Day
I	Vehicle Control (Simple Ointment)	3.33%	12.03%	45.42%	69.52%
II	Reference Control Povidone Iodine	11.00%	38.52%	80.06%	98.68%
III	5% LMWE	6.79%	26.12%	66.40%	91.04%
IV	10% LMWE	10.67%	33.52%	70.16%	95.94%

● Histopathology

Histopathological examinations of the wound sections stained with hematoxylin revealed a greater assemblage of macrophages with few collagen fibers in the control samples. Complete tissue regeneration was seen in the wound sections treated with the standard, there were more collagen fibers, better-organized blood vessels, and fewer macrophages. The 5% methanolic extract-treated animals had minimal macrophages and moderate collagen deposition, and the animals treated with 10% LMWE had improved healing, including thick collagenation, re-epithelialization, and the absence of macrophages (Fig 60).

iii. Docking Study

To assess the wound healing activity by molecular docking of 4 target proteins, like glycogen synthase kinase-3 β (GSK-3 β), transforming growth factor-beta (TGF- β), c-myc, and β -catenin, with 10 ligand molecules, a docking study was conducted. The ligand compounds were dicentrinone, lauroilsine, corytuberine, reticuline, eudesmin, grandisin, dehydroisoeugenol, 9,12-octadecadienoic acid(z,z) methyl ester, n-hexadecanoic acid, and linalool. The compounds selected were based on their anti-inflammatory and antioxidant potential, and these properties were associated with wound healing potential in plants. The binding energy of docked conformations is given in Table 41. In docking experiments with TGF- β , the results showed that, except linalool, all compounds showed better interaction affinity

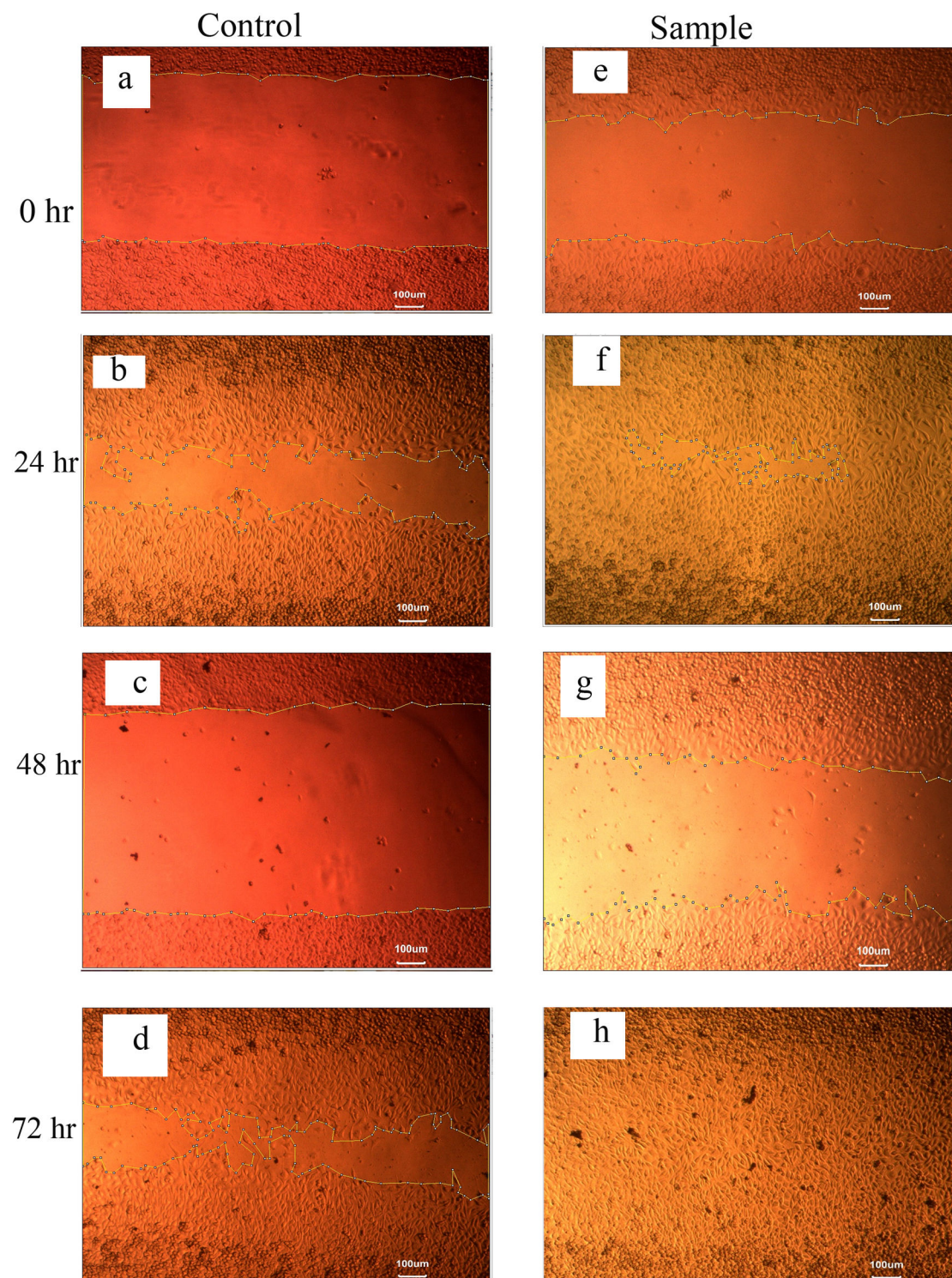


Fig 58 *In vitro* scratch wound healing assay. a-d. Control-cell migration after 72 hr treatment; e-h. Sample- cell migration after treatment with LWME after 72 hours

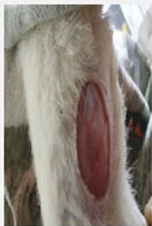
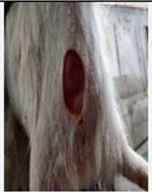
Drug Treatment	0 Day	3 rd Day	7 th Day	14 th Day	21 st Day
Control (Simple Ointment)	 A	 B	 C	 D	 E
Reference Control Povidone Iodine	 F	 G	 H	 I	 J
5% LMWE	 K	 L	 M	 N	 O
10% LMWE	 P	 Q	 R	 S	 T

Fig 59 *In vivo* wound healing study by LWME- wound contraction on different post excision days(1-21 days) A-E. Control; F-J. Reference control; K-O. LWME (5%); P-T. LWME (10%)

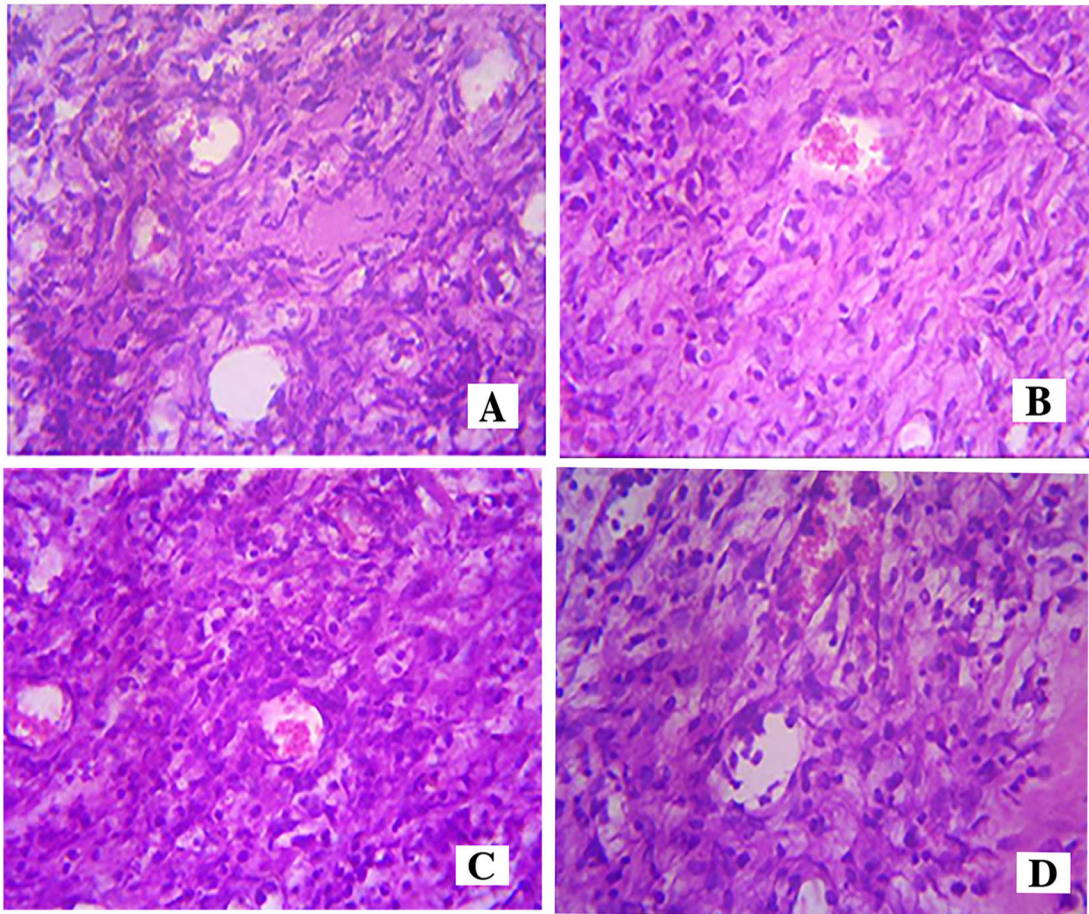


Fig 60 Histopathological study-histological section of granuloma tissue. A. Cells treated with control- incomplete healing; B. Standard-complete healing; C. 5% LWME- moderate healing with increased collagenation and lesser macrophages; D. 10% LWME- better healing with increased collagenation and lesser macrophages

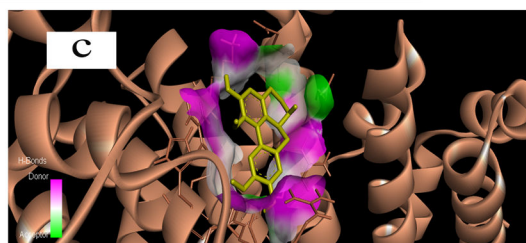
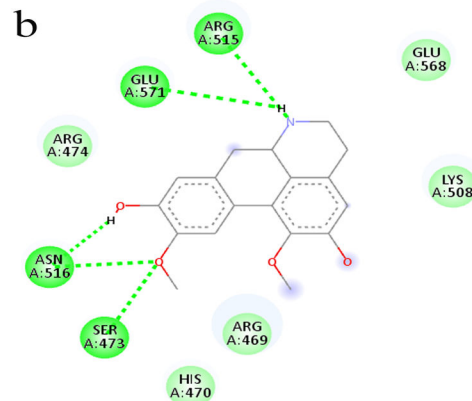
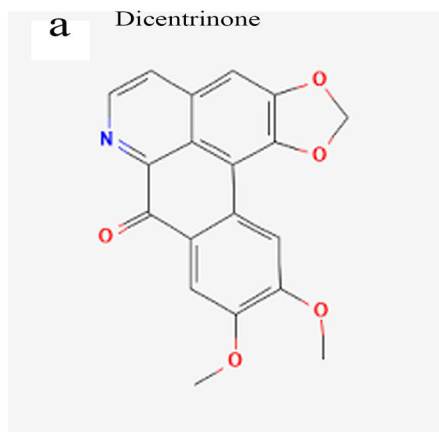


Fig 61(i) Molecular docking of wound healing compound from LWME with target protein. a. Dicentrinone structure; b Bound conformation of 1nkp protein and ligand; c. 3D interaction between protein and ligand

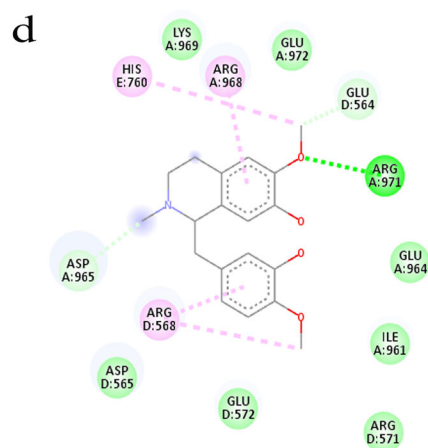
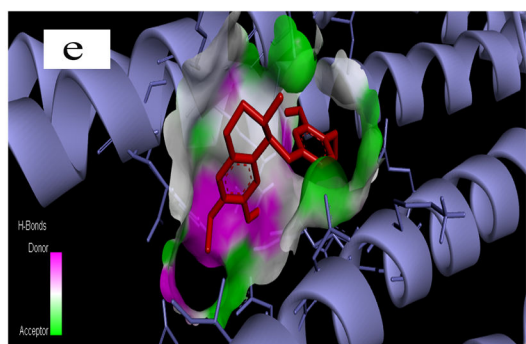


Fig 61(ii) Molecular docking of wound healing compound from LWME with target protein. d. Bound conformation of 1jdh protein and ligand; e. 3D interaction between protein and ligand

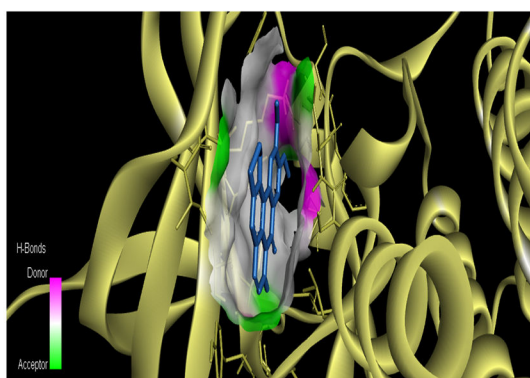
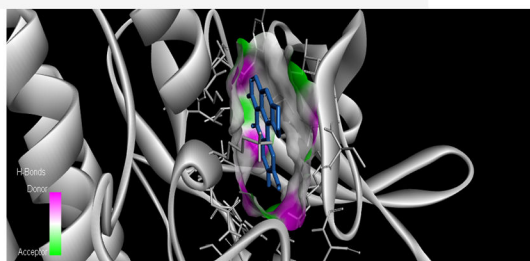
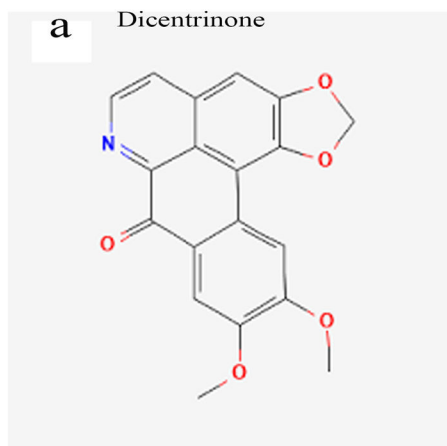


Fig 62(ii) Molecular docking of wound healing compound from LWME with target protein. d. Bound conformation of 5oy4 protein and ligand; c. 3D interaction between protein and ligand

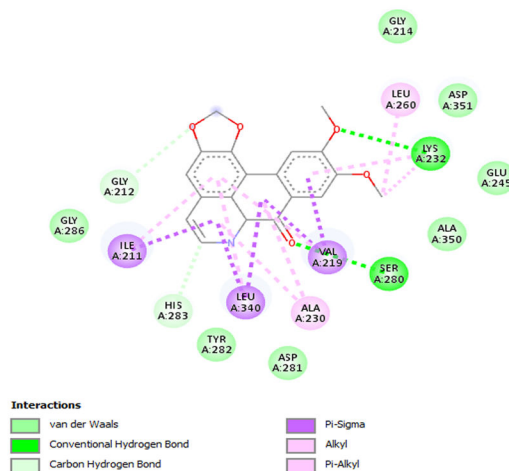
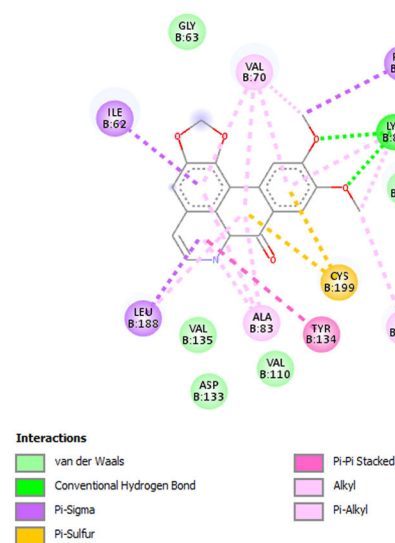


Fig 62(i) Molecular docking of wound healing compound from LWME with target protein. a. Dicentrinone structure; b Bound conformation of 5e8w protein and ligand; c. 3D interaction between protein and ligand



towards the target. From the results, the compound dicentrinone was found to exhibit the highest affinity towards all the selected proteins targets. Docking results with GSK-3 β showed that six among ten molecules showed high affinity towards the target proteins, in which dicentrinone exhibited the highest affinity towards GSK-3 β . Molecular docking with c-myc against ten molecules revealed that six ligands showed high affinity. The docking studies against β -catenine by target compounds showed that six compounds were active against β -catenine, Three-dimensional representation of the binding of compounds with the target proteins and interaction diagrams are given in Figures 61 (i-ii) & 62 (i-ii).

Table 41. Binding energy of docked proteins with target wound healing compounds

Compound	Binding Energy (BE) (Kcal/mol)			
	TGF- β (5e8w)	GSK-3 β (5oy4)	c-myc (1nkp)	β - catenine (1jdh)
Dicentrinone	-10.2	-9.0	-7.7	-6.7
Lauroilsine	-9.6	-8.6	-7.5	-6.6
Reticuline	-9.6	-8.3	-7.3	-6.6
Corytuberine	-9.3	-7.9	-6.9	-6.6
Eudesmin	-8.3	-7.2	-6.7	-6.6
Grandisin	-7.2	-6.8	-6.6	-6.0
Dehydroisoeugenol	-6.8	-5.9	-5.8	-5.2
9,12-octadecadienoic acid (z,z) methyl ester	-6.4	-5.8	-5.4	-5.1
n-hexanoic acid	-6.2	-5.2	-5.3	-4.7
Linalool	-5.6	-5.0	-4.7	-4.7

G. STUDY OF INSECTICIDAL ACTIVITY

The essential oil isolated from *L. coriacea* was exposed to insecticidal activity tests, including contact toxicity, fumigant toxicity, and repellency assays.

i. Contact toxicity

Essential oil isolated from *L. coriacea* leaves was subjected to contact toxicity assay. Different aliquots of oil (5 to 80 ($\mu\text{L}/\text{cm}^2$) prepared in acetone were applied against two stored pests, such as *S. oryzae* and *T. castaneum*, at 24, 48, and 72 hr periods and the efficacy of the oil was evaluated by counting the mortality rate of insects. Maximum mortality of *S. oryzae* insects was observed as $98.8 \pm 0.042\%$ at 72 hrs at maximum concentration. At the same time, the mortality rate of *T. castaneum* was $81.6 \pm 0.063\%$. LD_{50} and LD_{90} and chi-square values were calculated, and the results are depicted in Tables 42 & 43 and Figures 63 & 64.

ii. Fumigant toxicity

Fumigant toxicity assay was conducted to identify the effect of *L. coriacea* essential oil against the target insects *S. oryzae* and *T. castaneum*. It was observed that depending on the different concentrations of essential oil, such as 5, 10, 20, 40, and 80 $\mu\text{L}/\text{L}$ concentrations, *S. oryzae* showed maximum mortality at a rate of $69.2 \pm 0.050\%$, $73.2 \pm 0.95\%$, $83 \pm 0.038\%$, $91.8 \pm 0.085\%$ and $99.2 \pm 0.042\%$, respectively after three days' (72 hr) treatment. At the same concentration and time duration, *T. castaneum* insects showed mortality at a rate of $54.4 \pm 0.077\%$, $62.8 \pm 0.104\%$, $67.8 \pm 0.272\%$, $77.6 \pm 0.095\%$, and $82 \pm 0.129\%$. From the observations, it can be indicated that essential oil showed significant fumigant effects against both the insects, but among them, the maximum effect was towards *S. oryzae*. LC_{50} , LC_{90} , and chi-square values were determined. The results are demonstrated in Tables 44 & 45 and Figures 65 & 66.

iii. Repellency

The repellent activity of *S. oryzae* and *T. castaneum* by the *L. coriacea* essential oil was demonstrated. Essential oils at different concentrations, such as 0.16- 0.63 $\mu\text{L}/\text{cm}^2$ was treated with insects, and after 24 hours it was observed that maximum repellency shown by *S. oryzae* was $88.4 \pm 0.028\%$, $92.4 \pm 0.045\%$, $95.8 \pm 0.055\%$, and 100% and by *T. castaneum* was $71 \pm 0.072\%$, $75 \pm 0.040\%$, $81.4 \pm 0.083\%$, and $85 \pm 0.038\%$, respectively. LC_{50} and LC_{90} values were also recorded. Results are represented in Tables 46 & 47 and Figures 67 & 68.

Table 42 Contact toxicity effect of *L. coriacea* leaves essential oil against *S. oryzae* and *T. castaneum*

Concentration ($\mu\text{L/L}$ air)	% of Mortality (<i>S. oryzae</i>) Mean $\pm$ SD			% of Mortality (<i>T. castaneum</i>) Mean $\pm$ SD		
	24 Hrs	48 Hrs	72 Hrs	24 Hrs	48 Hrs	72 Hrs
5	19.6 $\pm$ 0.09 ^e	40.2 $\pm$ 0.102 ^e	56.8 $\pm$ 0.55 ^e	14 $\pm$ 0.062 ^e	24.6 $\pm$ 0.055 ^e	45.4 $\pm$ 0.040 ^e
10	31.8 $\pm$ 0.085 ^d	54.6 $\pm$ 0.037 ^d	71.6 $\pm$ 0.067 ^d	28.4 $\pm$ 0.116 ^d	32.6 $\pm$ 0.10 ^d	55.8 $\pm$ 0.055 ^d
20	39 $\pm$ 0.089 ^c	67.8 $\pm$ 0.050 ^c	90.2 $\pm$ 0.044 ^c	36 $\pm$ 0.098 ^c	48.8 $\pm$ 0.059 ^c	64 $\pm$ 0.062 ^c
40	44.4 $\pm$ 0.043 ^b	77.4 $\pm$ 0.050 ^b	95.2 $\pm$ 0.042 ^b	40.4 $\pm$ 0.041 ^b	54.2 $\pm$ 0.056 ^b	71.2 $\pm$ 0.049 ^b
80	58 $\pm$ 0.047 ^a	87.2 $\pm$ 0.070 ^a	98.8 $\pm$ 0.042 ^a	54 $\pm$ 0.046 ^a	66.8 $\pm$ 0.079 ^a	81.6 $\pm$ 0.063 ^a

Mean $\pm$ SE of three replications. Mortality of insects was determined by Analysis of Variance (ANOVA), the F value was calculated, followed by LSD at a 0.05% level of significance.

Table 43 Contact toxicity effect of *L. coriacea* leaves essential oil against *S. oryzae* and *T. castaneum* represented by Probit analysis

Time duration (Hrs)	<i>S. oryzae</i> (% mortality)			(X ²)	<i>T. castaneum</i> (% mortality)			(X ²)
	LD ₅₀ (LC ₉₀) (Log Conc.)	LD ₅₀ (LCL-UCL)	LD ₉₀ (LCL-UCL)		LD ₅₀ (LC ₉₀) (Log Conc.)	LD ₅₀ (LCL-UCL)	LD ₉₀ (LCL-UCL)	
24	1.125 (2.589)	(0.962-1.254)	(2.260-1.254)	0.201*	1.683(3.273)	(1.527-1.930)	(2.767-4.283)	0.977*
48	0.908(2.043)	(0.745-1.026)	(1.854-2.344)	0.095*	1.436(2.815)	(1.314-1.580)	(2.459-3.452)	0.992*
72	0.621(1.355)	(0.454-0.735)	(1.261-1.484)	0.973*	0.844(2.433)	(0.568-1.010)	(2.111-3.064)	0.808*

LD₅₀- Lethal dose that kills 50% of treated insects, LD₉₀- Lethal dose that kills 90% of treated insects, LCL- Lower confidence limit, UCL- Upper confidence limit, X² – Chi-square value significant at p< 0.05 level.

Table 44 Fumigant effect of *L. coriacea* leaves essential oil against *S. oryzae* and *T. castaneum*

Concentration ($\mu\text{L/L}$ air)	Mortality % (<i>S. oryzae</i>)			Mortality % (<i>T. castaneum</i>)		
	8 Hrs	12 Hrs	24 Hrs	8 Hrs	12 Hrs	24Hrs
5	35.8 $\pm$ 0.123 ^e	57.2 $\pm$ 0.055 ^e	69.2 $\pm$ 0.050 ^e	32 $\pm$ 0.088 ^e	45.4 $\pm$ 0.040 ^e	54.4 $\pm$ 0.077 ^e
10	47 $\pm$ 0.051 ^d	61.6 $\pm$ 0.072 ^d	73.2 $\pm$ 0.095 ^d	36.6 $\pm$ 0.073 ^d	52.4 $\pm$ 0.115 ^d	62.8 $\pm$ 0.104 ^d
20	54.6 $\pm$ 0.122 ^c	67.2 $\pm$ 0.010 ^c	83 $\pm$ 0.038 ^b	43 $\pm$ 0.143 ^c	57.6 $\pm$ 0.100 ^c	67.8 $\pm$ 0. 272 ^c
40	62.2 $\pm$ 0.113 ^b	76.6 $\pm$ 0.031 ^b	91.8 $\pm$ 0.085 ^b	58.4 $\pm$ 0.074 ^b	67 $\pm$ 0.096 ^b	77.6 $\pm$ 0.095 ^b
80	66.6 $\pm$ 0.092 ^a	89.2 $\pm$ 0.068 ^a	99.2 $\pm$ 0.042 ^a	64.4 $\pm$ 0.083 ^a	82.8 $\pm$ 0.105 ^a	82 $\pm$ 0.129 ^a

Mean $\pm$ SE of three replications. Mortality of insects was determined by Analysis of Variance (ANOVA), the F value was calculated, followed by LSD at a 0.05% level of significance.

Table 45 Fumigant effect of *L. coriacea* leaves essential oil against *S. oryzae* and *T. castaneum* represented by Probit analysis

Time duration (Hrs)	<i>S. oryzae</i> (% mortality)			(X ²)	<i>T. castaneum</i> (% mortality)			(X ²)
	LC50 (LC90) (Log Conc.)	LC ₅₀ (LCL-UCL)	LC ₉₀ (LCL-UCL)		LC50 (LC90) (Log Conc.)	LC ₅₀ (LCL-UCL)	LC ₉₀ (LCL-UCL)	
8	1.042 (2.170)	(0.916-1.141)	(1.356-0.803)	0.227*	2.183(3.24)	(2.091-2.297)	(2.922-3.935)	4.516*
12	0.656(1.709)	(0.356-0.803)	(1.516-2.124)	8.131*	1.883(2.897)	(1.717-1.983)	(2.671-3.367)	7.710*
24	0.507(1.245)	(0.245-0.651)	(1.157-1.380)	11.887*	1.654 (2.810)	(1.306-1.812)	(2.579-3.350)	1.715*

LC₅₀- Lethal concentration that kills 50% of treated insects, LC₉₀- Lethal concentration that kills 90% of treated insects, LCL- Lower confidence limit, UCL- Upper confidence limit, X² – Chi-square value significant at p< 0.05 level.

Table 46 Repellency effect of *L. coriacea* leaves essential oil against *S. oryzae* and *T. castaneum*

Concentration ($\mu\text{L}/\text{cm}^2$)	<i>S. oryzae</i> Percentage Repellency (PR)					<i>T. castaneum</i> Percentage Repellency (PR)				
	2 Hrs	4 Hrs	8 Hrs	12 Hrs	24 Hrs	2 Hrs	4 Hrs	8 Hrs	12 Hrs	24 Hrs
0.16	59.8 $\pm$ 0.054 ^d	64 $\pm$ 0.078 ^c	75.2 $\pm$ 0.065 ^a	79 $\pm$ 0.050 ^b	88.4 $\pm$ 0.028 ^b	40.6 $\pm$ 0.069 ^d	55 $\pm$ 0.047 ^d	69.8 $\pm$ 0.050 ^d	75.6 $\pm$ 0.195 ^c	71 $\pm$ 0.072 ^d
0.31	67.8 $\pm$ 0.082 ^c	70.2 $\pm$ 0.042 ^b	77 $\pm$ 0.050 ^a	84.2 $\pm$ 0.042 ^a	92.4 $\pm$ 0.045 ^a	62.6 $\pm$ 0.056 ^c	70.6 $\pm$ 0.053 ^c	84.6 $\pm$ 0.029 ^c	85.8 $\pm$ 0.070 ^b	75 $\pm$ 0.040 ^c
0.47	74.2 $\pm$ 0.058 ^b	79.8 $\pm$ 0.042 ^a	82 $\pm$ 0.027 ^a	89 $\pm$ 0.00 ^a	95.8 $\pm$ 0.055 ^a	75.6 $\pm$ 0.031 ^b	82 $\pm$ 0.067 ^b	96.6 $\pm$ 0.027 ^b	94.4 $\pm$ 0.028 ^a	81.4 $\pm$ 0.083 ^b
0.63	87.8 $\pm$ 0.022 ^a	92.8 $\pm$ 0.022 ^a	97 $\pm$ 0.012 ^a	98 $\pm$ 0.00 ^a	100.0.000 ^a	93.8 $\pm$ 0.011 ^a	90.6 $\pm$ 0.028 ^a	97.2 $\pm$ 0.00a	93.8 $\pm$ 0.011 ^a	90.6 $\pm$ 0.028 ^a

Mean $\pm$ SE of three replications. Repellency of insects was determined by Analysis of Variance (ANOVA), the F value was calculated, followed by LSD at a 0.05% level of significance

Table 47 Repellency effect of *L. coriacea* leaves essential oil against *S. oryzae* and *T. castaneum* represented by Probit analysis

Time duration (Hrs)	<i>S. oryzae</i> (% mortality)			(X ²)	<i>T. castaneum</i> (% mortality)			(X ²)
	LC50 (LC90) (Log Conc.)	LC ₅₀ (LCL- UCL)	LC ₉₀ (LCL- UCL)		LC50 (LC90) (Log Conc.)	LC ₅₀ (LCL- UCL)	LC ₉₀ (LCL- UCL)	
2	-0.877(-0.324)	(-1.037—0.780)	(-0.400- -0.209)	0.105*	-0.671(0.169)	(-0.753- 0.611)	(-0.250—0.046)	0.062*
4	-1.085(-0.656)	(-1.369-0.950)	(0.-737- -0.578)	0.908*	-0.839(0.159)	(-1.0240-734)	(-0.266—0.031)	0.599*
8	-3.481(-1.986)	(-4.931-- 0.259)	(-2.120- -0.832)	0.700*	-0.947(0.501)	(-1.111-0.850)	(-0.563—0.421)	0.092*
12	-1.527(-1.022)	(-2.062- -0.458)	(-2.812- -0.096)	0.832*	-1.130(0.468)	(-1.5180.951)	(-0.555—0.351)	0.584*
24	-4.377(-1.691)	(-5.126- -0.288)	(-2.112- -0.053)	0.878*	-1.445(0.160)	(-4.6161.007)	(-0.148—2.287)	0.803*

LC₅₀- Lethal concentration that kills 50% of treated insects, LC₉₀- Lethal concentration that kills 90% of treated insects, LCL- Lower confidence limit, UCL- Upper confidence limit, X²– Chi-square value significant at p< 0.05 level.

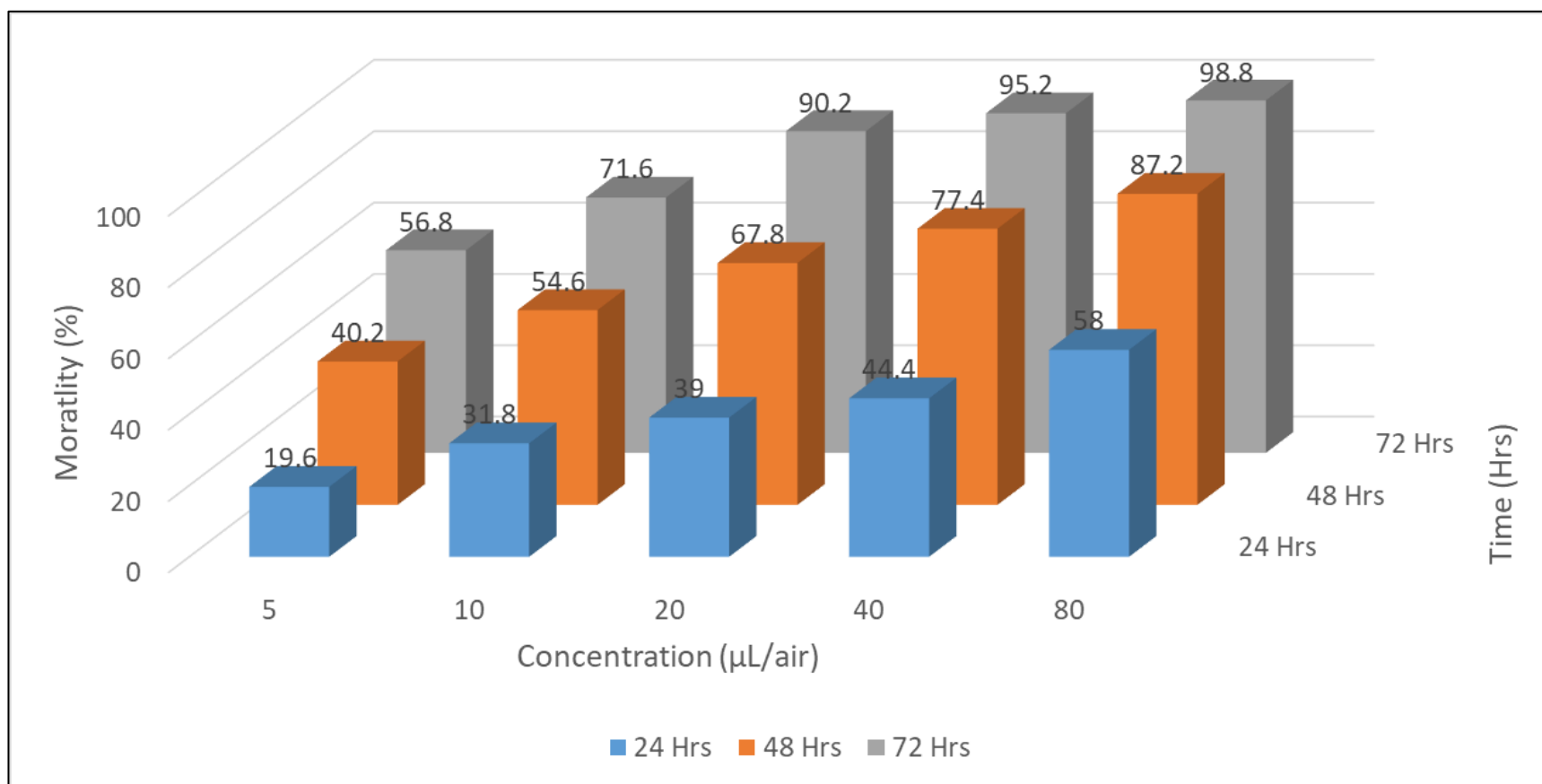


Fig 63 Contact toxicity effect of *L. coriacea* leaves essential oil against *S. oryzae*

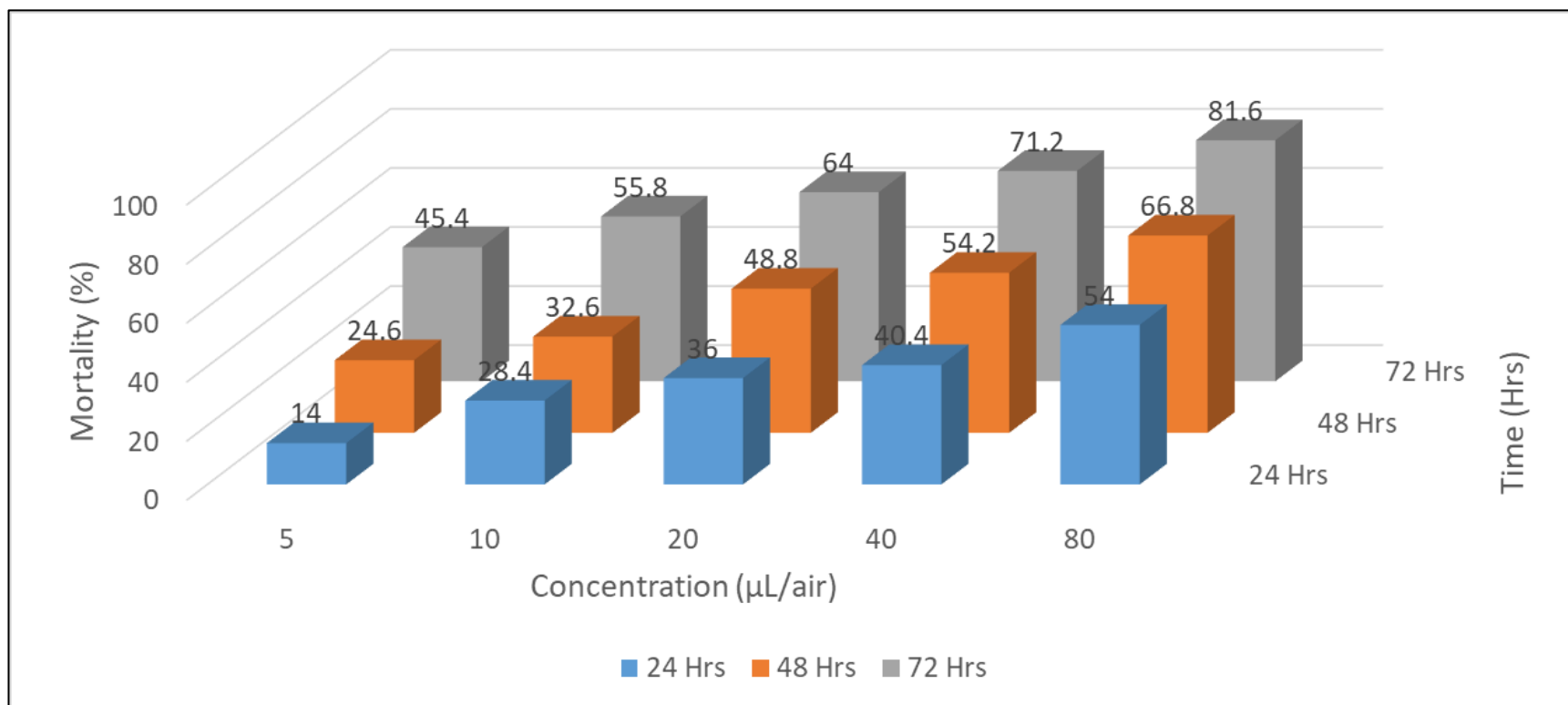


Fig 64 Contact toxicity effect of *L. coriacea* leaves essential oil against *T. castaneum*

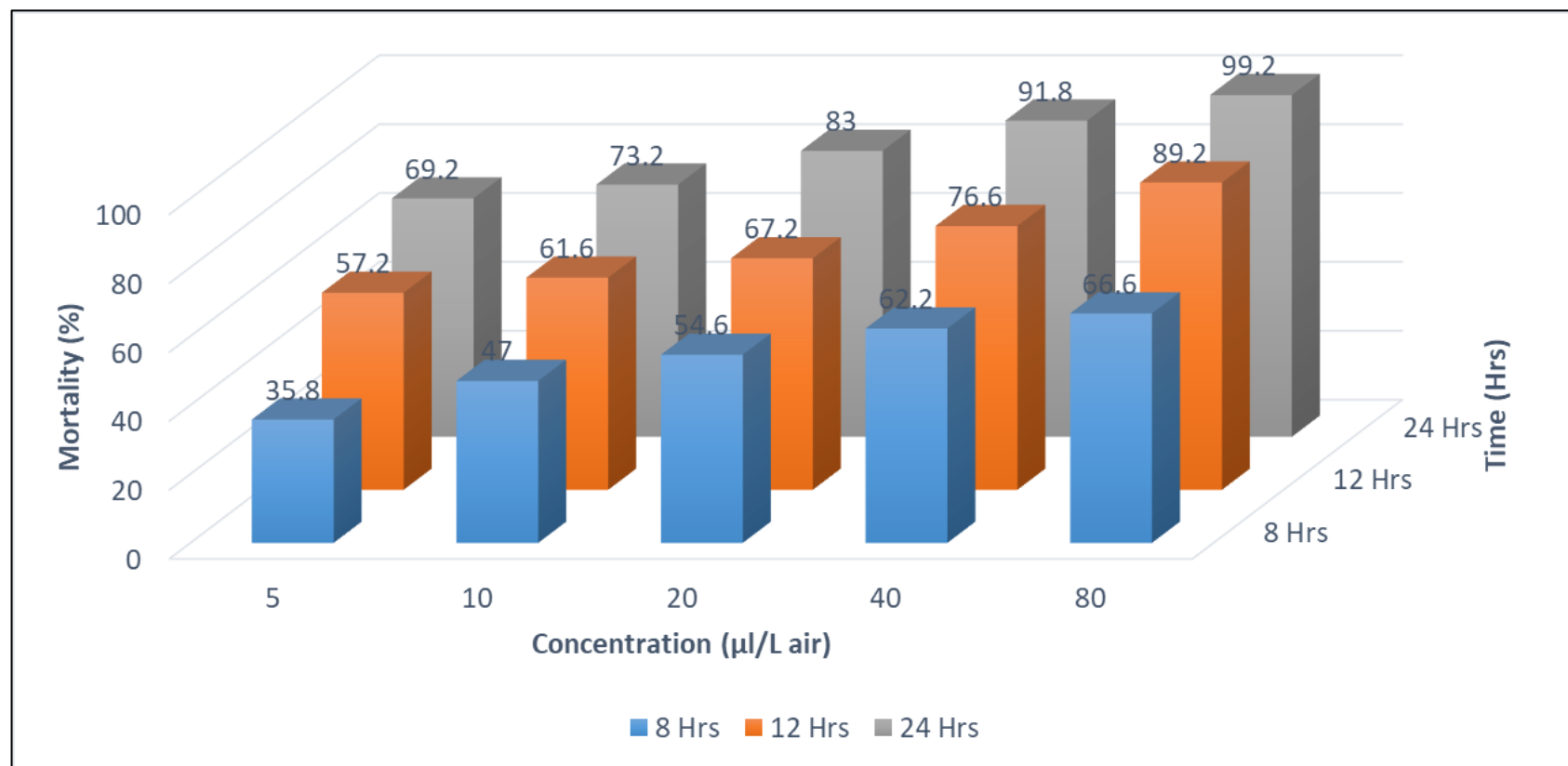


Fig 65 Fumigant toxicity of *L. coriacea* leaves essential oil against *S. oryzae*

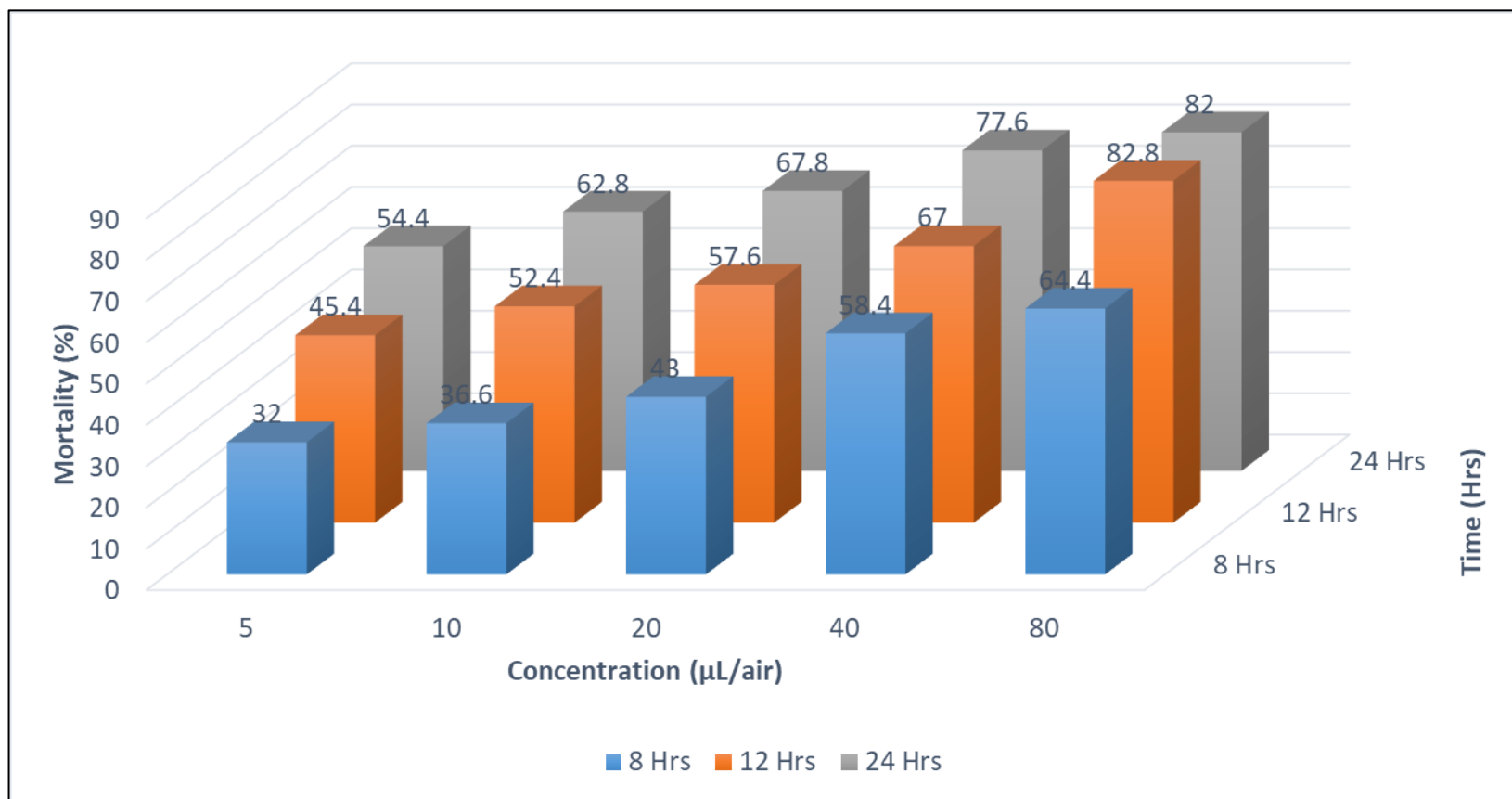


Fig 66 Fumigant toxicity of *L. coriacea* leaves essential oil against *T. castaneum*

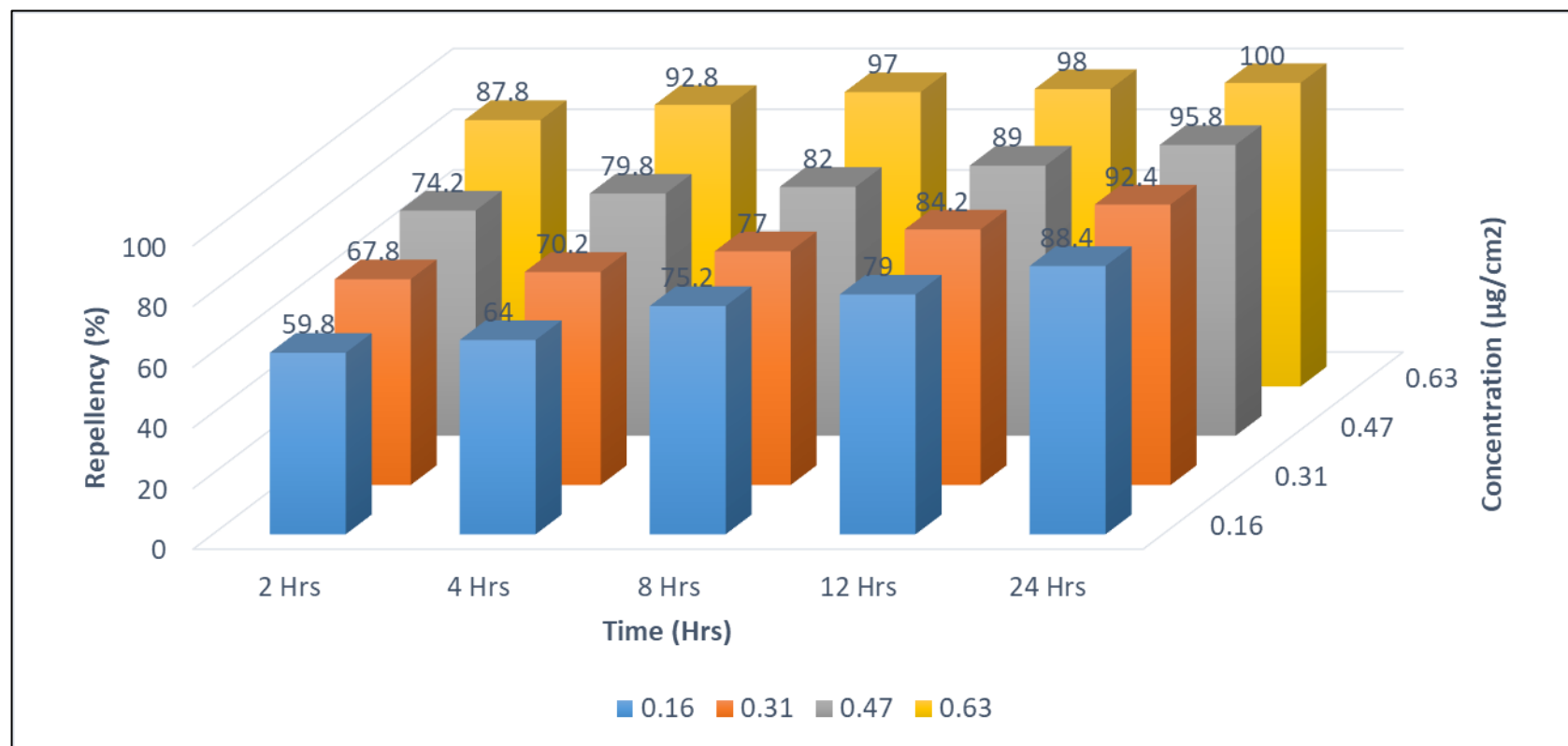


Fig 67 Repellency effect of *L. coriacea* leaves essential oil against *S. oryzae*

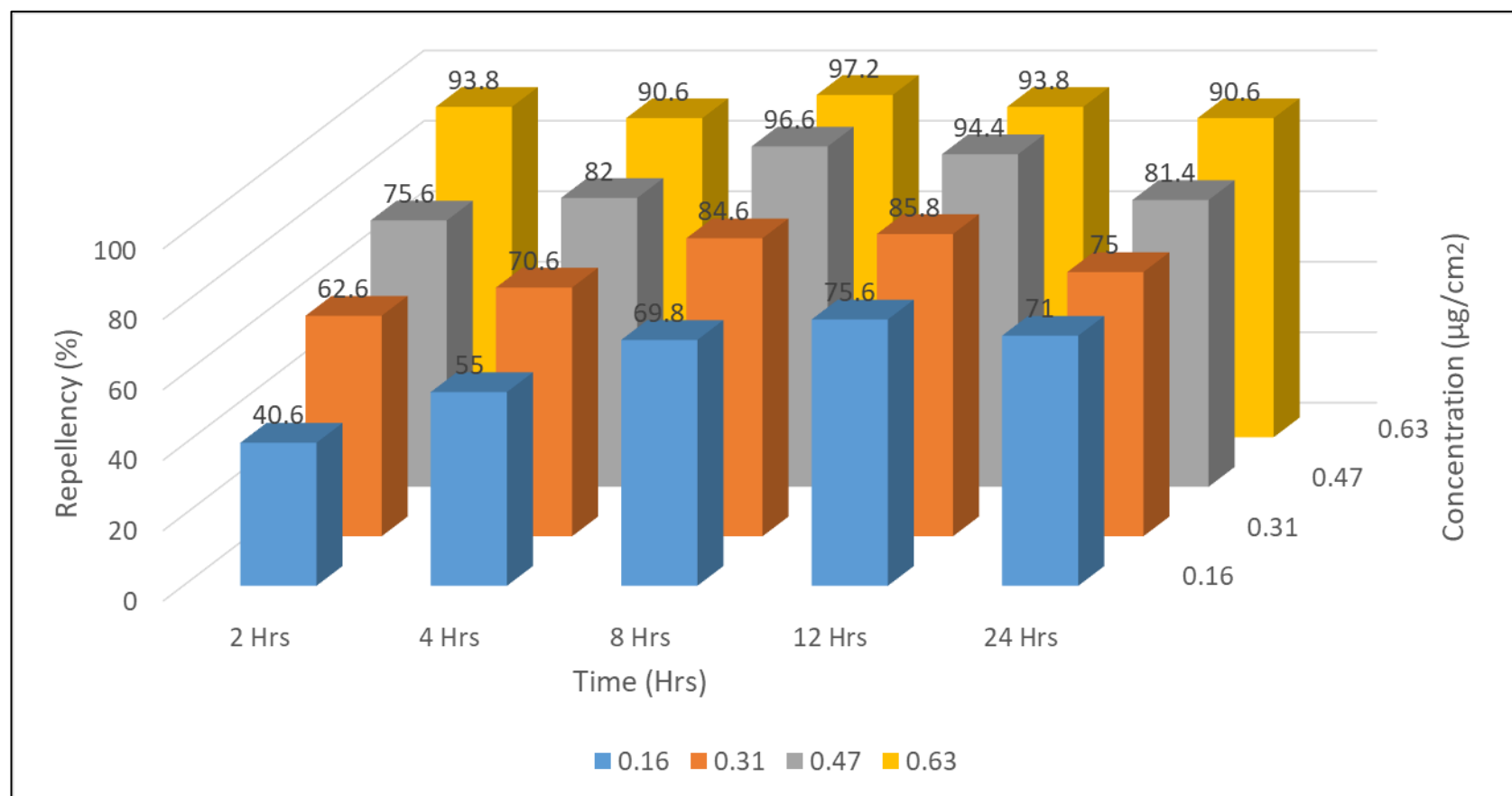


Fig 68 Repellency effect of *L. coriacea* leaves essential oil against *T. castaneum*

CHAPTER IV

RESULTS

The current study examined the pharmacognostic, phytochemical and pharmacological characteristics of three *Litsea* species, namely *L. bourdillonii*, *L. coriacea*, and *L. wightiana*. Pharmacognostic features like morphology, anatomy, histochemistry, physicochemical, and powder characters were analyzed. Preliminary qualitative and quantitative phytochemical profiling and pharmacological properties, including antimicrobial, antioxidant, antidiabetic, and cytotoxicity potentials, were evaluated. The most active and efficient *Litsea* species was selected for anti-inflammatory, wound healing and docking studies. The research revealed the bioactivity potential of the studied *Litsea* spp.

PHASE I. PHARMACOGNOSTIC CHARACTERIZATION

a. Morphological study

The macromorphological features of the three selected species of *Litsea* were studied. The details are listed in Table 9 and Figures 5, 6 & 7. The three plants showed similarities in leaf, stem, inflorescence and fruit characteristics and exhibited differences in some morphological features like the length of leaves, leaf lamina, petiole, fruit size, shape, and the perianth tube. Among the three species, *L. bourdillonii* and *L. wightiana* exhibited more resemblances when compared to *L. coriacea*.

Table 9 Comparative morphological characterization of three *Litsea* spp.

Morphological characters	<i>L. bourdillonii</i>	<i>L. coriacea</i>	<i>L. wightiana</i>
Habit	Tree	Tree	Tree
Height of the plant	8-10 m	10-18 m	10-15 m
Leaf shape	Elliptical	Elliptical lanceolate	Elliptical
Leaf length (cm)	20.16 cm	12.5 cm	18 cm
Leaf tip	Acute	Acute	Acute
Leaf margin	Entire	Entire	Entire
Leaf lamina	Hairy	Fleshy	Hairy
Leaf Petiole	Hairy	Smooth	Hairy
Phyllotaxy	Alternate	Alternate	Alternate
Stem	Woody	Woody	Woody
Bark color	Pale Brown	Pale Brown	Brown
Inflorescence	Umbel	Umbel	Umbel
Number of flowers	4-6	4-5	5
Male flowers	12 stamens	12 stamens	12 stamens
Female flowers staminodes	12	12	12
Flower colour	Brown	White	White
Fruit type	Berry	Berry	Berry
Fruit size	10-12 mm	8 mm	1.5 mm
Fruit colour	Reddish to dark purple	Reddish to dark purple	Reddish to dark purple
Fruit shape	Oblong or obovoid	Oblong or obovoid	Ellipsoid
Perianth tube	Enlarged obconic	Enlarged cup-shaped	Cup-shaped

b. Anatomical study. The anatomical characteristics of the leaf, petiole and stem parts of three *Litsea* plants were studied and compared the results with each other.

- ***L. bourdillonii* stem**

The outline of the stem was circular. The outermost layer was the epidermis, which was uniseriate, irregularly arranged, squarish cells. Epidermal cells give rise to

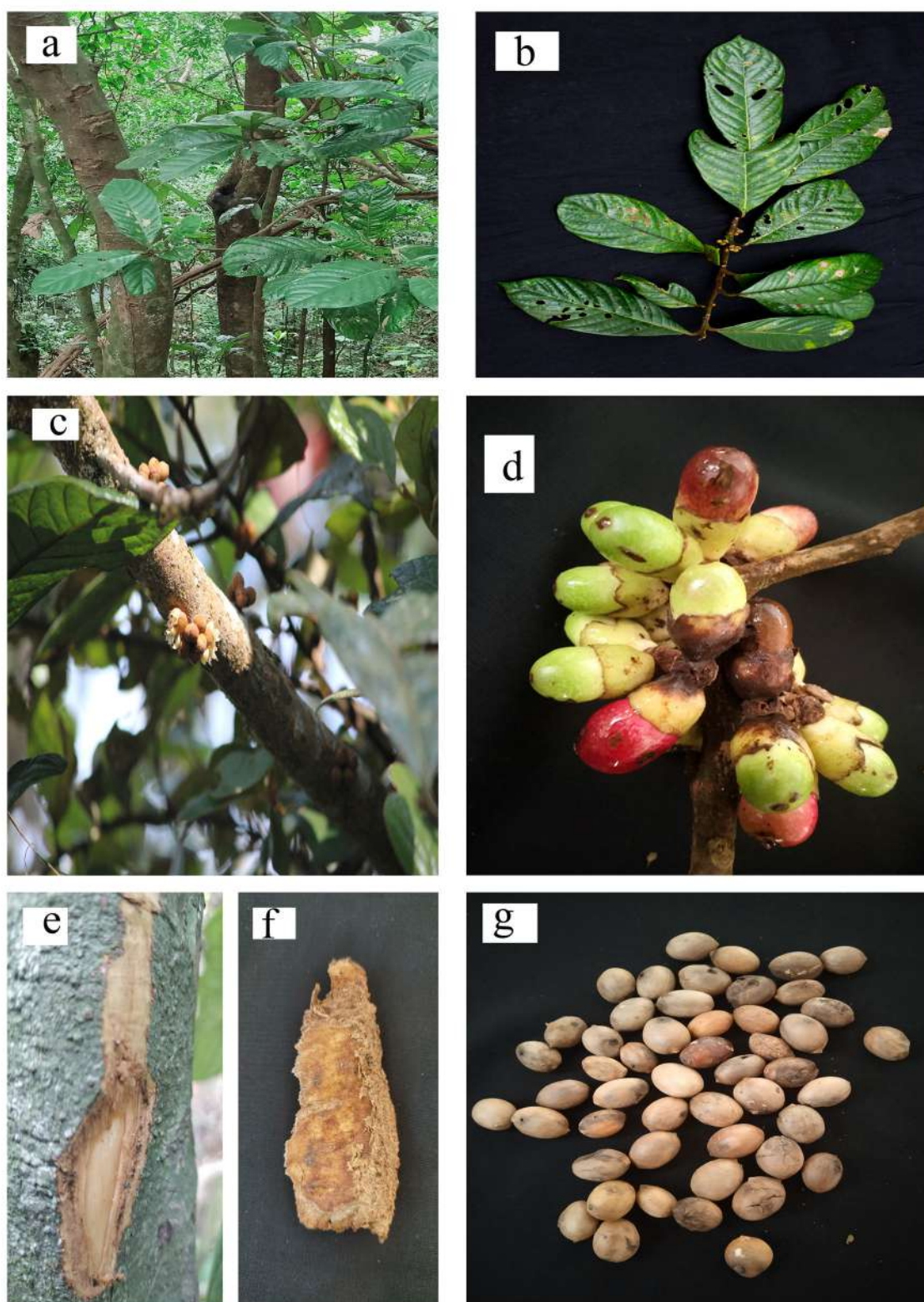


Fig 5 Macromorphological characters of *L.bourdillonii*. a.Habit; b. Twig with leaf and bud; c. Twig with flower; d. Young and mature fruits; d & e. Bark; g. Seeds

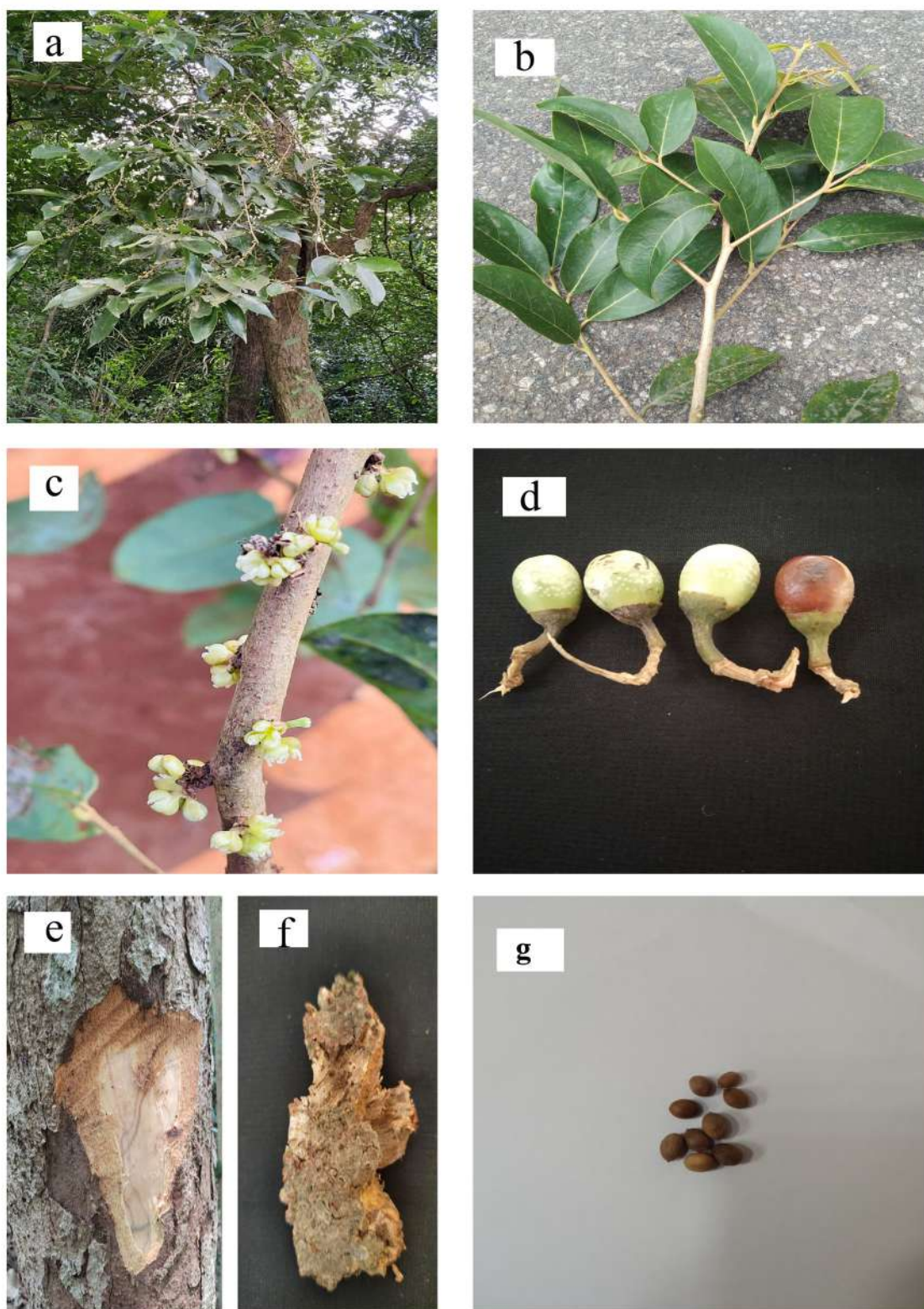


Fig 6 Macromorphological characters of *L. coriacea*. a. Habit; b. Twig with leaf; c. Twig with flower; d. Young and mature fruits; e & f. Bark;g. Seeds



Fig 7 Macromorphological characters of *L.wightiana*. a. Habit;b. Twig with leaves and bud; c. Twig with flower;d. Young & mature fruits. e & f. Bark; g. Seeds

elongated unicellular trichomes. The cortex was multilayered and composed of parenchyma cells intermingled with a few sclerenchyma cells. A few cells in the inner cortex contained starch grains. Sclerenchymatous pericyclic fibers were present. The vascular bundles showed xylem strands with numerous tracheids mixed with large vessels. The phloem faced towards the outside with phloem parenchyma and fibers. The central part of the stem was occupied by a large pith with loosely arranged polygonal parenchyma cells (Table 10 & Fig 8).

- ***L. coriacea* stem**

The structure was circular in outline. Epidermis was single layered with squarish cells. The outer cortex was multilayered with simple polygonal parenchymatous cells with angular thickening. The inner cortex was multilayered with parenchyma cells, which were loosely arranged. One layer of sclerenchyma cells was present between the outer and inner cortex. Continuous patches of pericyclic fibers were also present. The secondary phloem contained fibers and parenchyma. The xylem has parallel rows of tracheids mixed with vessel elements. The pith consisted of polygonal parenchymatous cells (Table 10 & Fig 9).

- ***L. wightiana* stem**

The internal structure of the stem showed a wavy outline. The epidermis was found to be single layered with small squarish type cells. Some cells gave rise to unicellular glandular trichomes. The outer cortex is multilayered with compactly arranged parenchyma cells intermingled with a few sclerenchyma cells. The inner cortex was multilayered with loosely arranged thin-walled parenchyma cells. Discontinuous patches of pericyclic fibers were present. In the xylem, numerous rows of tracheids were intermingled with large vessel elements. External to the xylem, the phloem consisted of phloem parenchyma and fibers. A large pith was present in the center with compactly arranged polygonal parenchyma cells (Table 10 & Fig 10).

Table 10 Comparative anatomical characterization of the stem of three *Litsea* spp.

Microscopic characters	<i>L. bourdillonii</i>	<i>L. coriacea</i>	<i>L. wightiana</i>
Epidermis	Uniseriate, squarish cells with cuticle	Uniseriate, squarish cells with cuticle	Uniseriate, small, squarish cells, with cuticle
Trichomes	Unbranched, long unicellular	Unicellular, broad, small and glandular	Small, unicellular, and glandular
Cortex	The outer and inner cortex-multilayered parenchyma cells; the inner cortex-1-2-layered sclerenchyma cells	The outer cortex-multilayered, with compactly arranged parenchyma cells; the inner cortex-loosely arranged parenchyma cells; the middle cortex single-layered with sclerenchyma cells	The outer cortex-multilayered with compactly arranged parenchyma cells intermingled with a few sclerenchyma cells; the inner cortex-multilayered with loosely arranged thin-walled parenchyma cells
Pericyclic fibres	Continuous patch of pericyclic fibers	Continuous patch of pericyclic fibres	Discontinuous patch of pericyclic fibers
Xylem	Groups of tracheids intermingled with varying sizes of vessels and parenchyma	Elongated patches of xylem tracheids with varying size of vessels	Elongated patches of xylem tracheids intermingled with large vessels
Phloem	Patches of condensed phloem with parenchyma and fibres	Continuous patch of phloem cells with phloem fibers and parenchyma	Continuous patch of phloem cells with fibers and parenchyma
Medullary rays	Less prominent	Prominent with uniseriate parenchyma cells	Narrow and less prominent
Vessels	Large, single or in groups	Varying sizes in groups	Large varying sizes
Pith	Polygonal parenchyma cells	Polygonal parenchyma cells	Polygonal, compactly arranged parenchyma cells

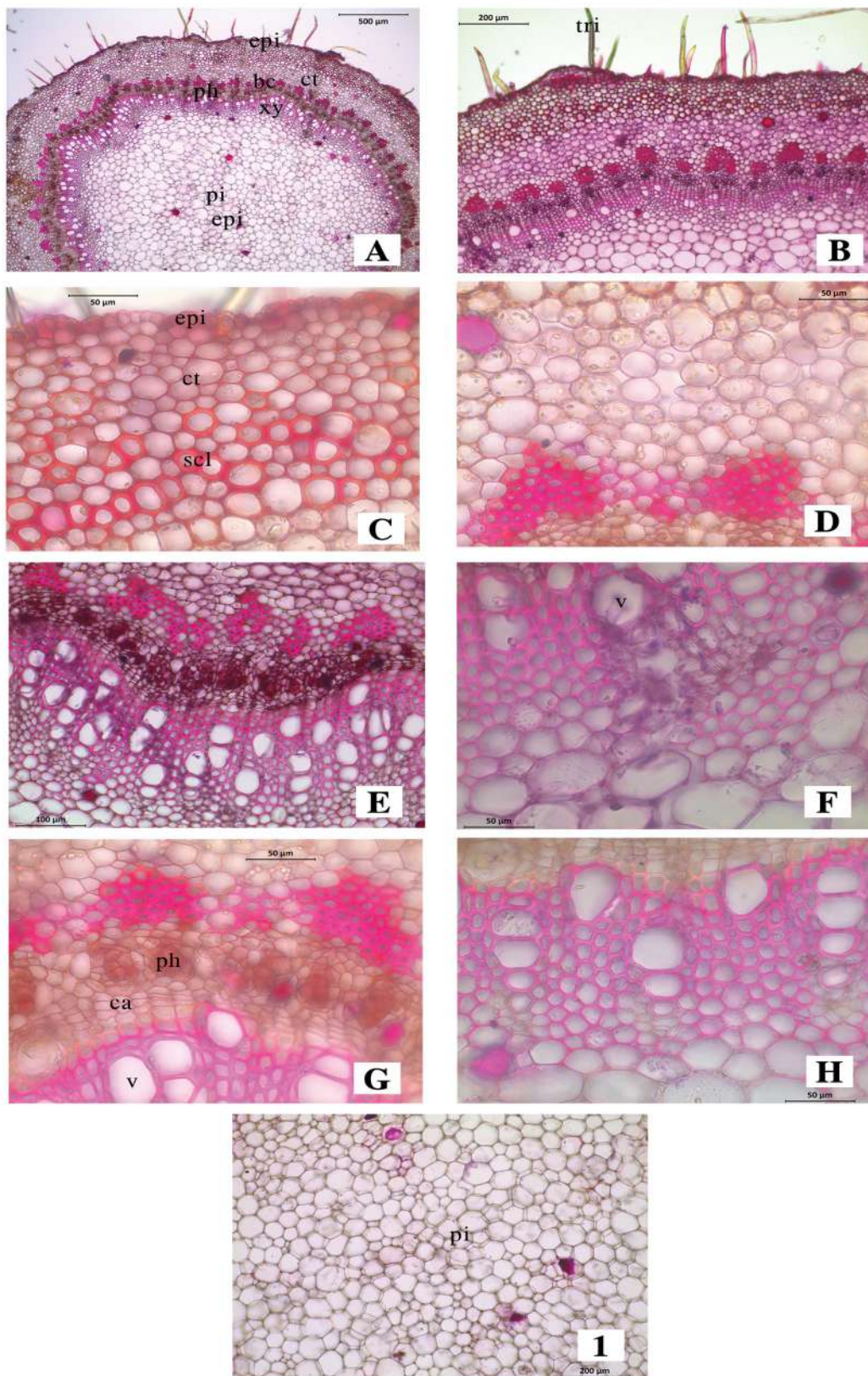


Fig 8 Microscopic characters of *L.bourdillonii* stem A. Ground plan; B. upper portion of stem; C. hypodermis parenchyma & middle cortex sclerenchyma; D. Inner cortex parenchyma and pericyclic fibres; E, F, G, & H- Xylem with large vessels, phloem and cambium; I. Pith parenchyma

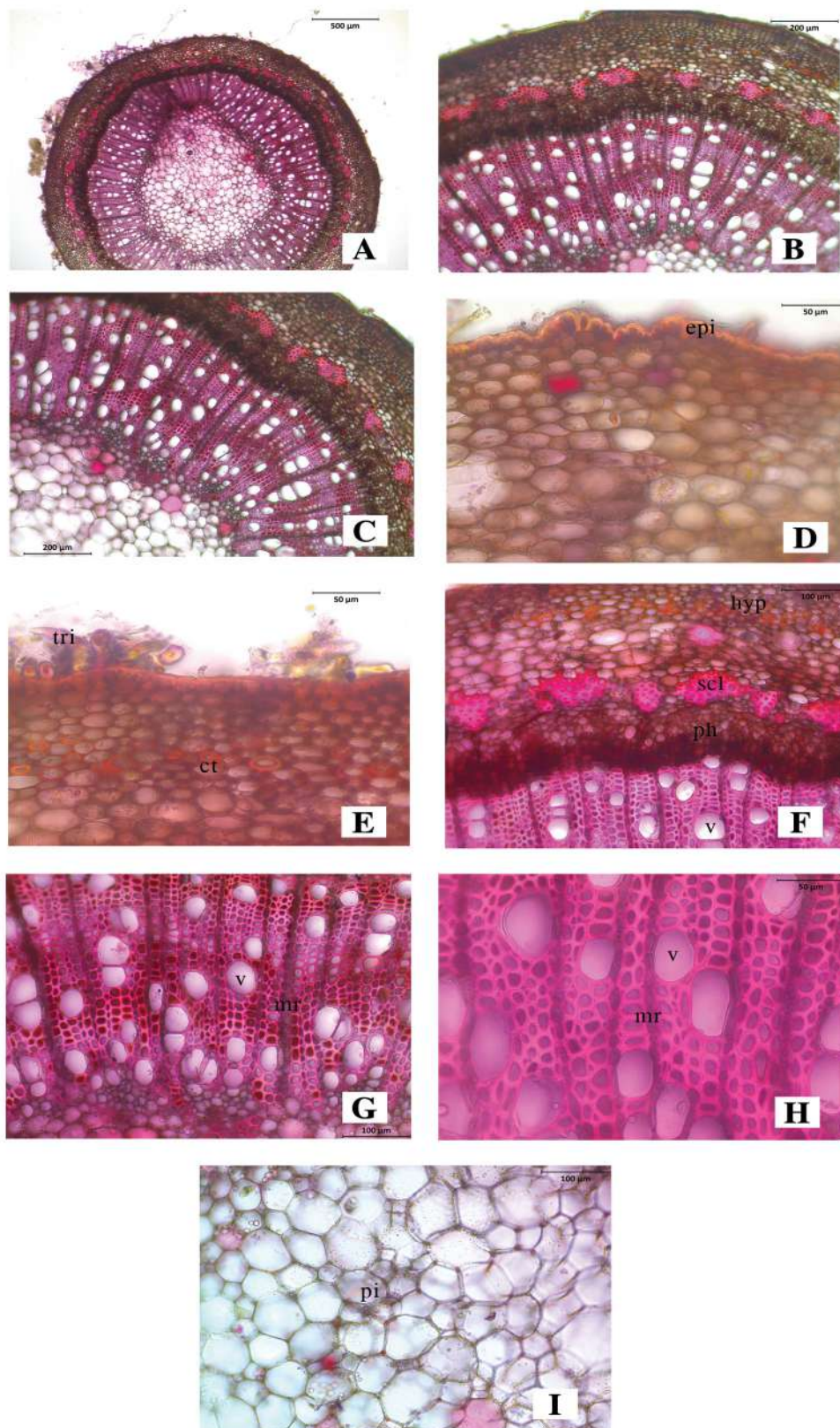


Fig 9 Microscopic characters of *L. coriacea* stem. A-C. Ground plan full view & portion enlarged; D&E-Epidermis with trichomes, outer and inner cortex parenchyma and middle sclerenchyma layer; F-H-Stelar region, xylem with large vessels and tracheids impregnated with medullary ray, phloem, cambium and pericyclic fibres; I. pith parenchyma

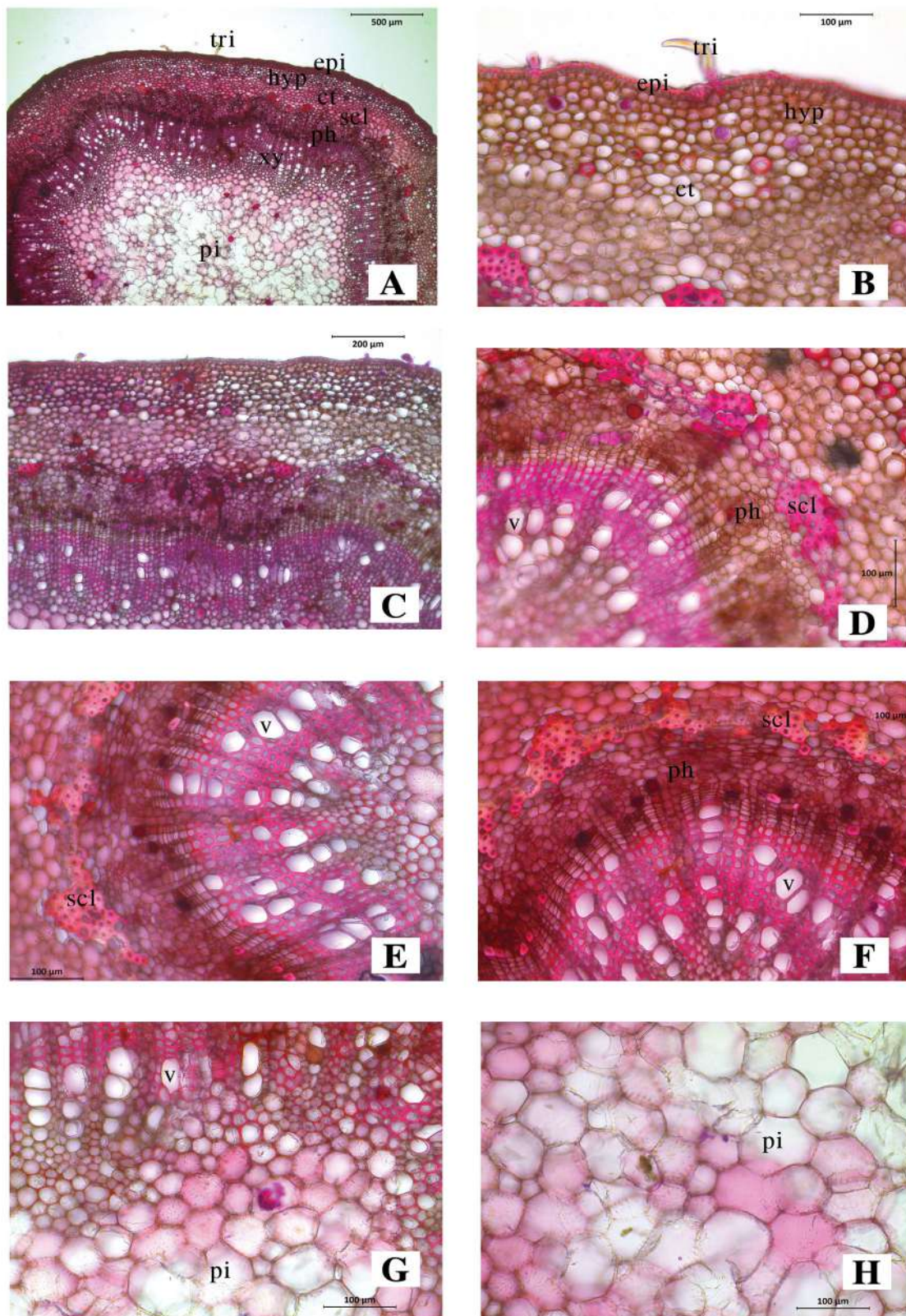


Fig 10 Microscopic characters of *L.wightiana* stem. A. Ground plan; B.Hypodermis and cortex parenchyma,C,D,E&F.Pericyclic fibres,thick patch of phloem,cambium, xylem with large vessels and tracheids intermingled with medullary rays G&H.pith parenchyma

- ***L. bourdillonii* leaf**

The internal structure showed a prominent midrib, lateral veins and uniformly thin lamina. The upper epidermis in the midrib consisted of a uniseriate layer of squarish, thin-walled parenchyma cells. The upper epidermal cells gave rise to a few unicellular trichomes in the midrib region. The lower epidermal cells produced a few narrow, unicellular elongated trichomes from the midrib and numerous from the laminar regions. The cuticle cells in the upper epidermis were uniformly distributed, and the cuticle in the lower epidermis were undulate and irregularly arranged.

In the midrib region below the epidermis, multilayered ground tissue with thin-walled round parenchyma cells were present. A major portion of the midrib was occupied by wide vasculature with collateral bundles consisting of continuous parallel rows of xylem with two large, distinct bundles, somewhat fused, in the upper part. The phloem was seen as a continuous horizontal band below the xylem. A regularly arranged sclerenchymatous bundle cap was present below the phloem patch. Lateral veins with distinct collateral bundles surrounded by the sclerenchyma bands.

In the laminar region, the adaxial epidermis with squarish cells was evenly distributed with a thin cuticle. Abaxial surface cells with short, irregularly arranged, somewhat columnar and undulate cells, covered by a thick cuticle. There were no stomata in the adaxial epidermis, but a few were in the abaxial epidermis. Below the epidermis, mesophyll consisted of two layers of thick, columnar, pillar-like palisade tissue interspersed with a few secretory idioblasts. Lower spongy mesophyll was composed of loosely and irregularly arranged, thin-walled round, and some elongated parenchyma cells (Table 11 & Fig 11).

- ***L. coriacea* leaf**

The section showed a prominent midrib with lateral veins and uniformly thin lamina. The upper epidermal cells were squarish, covered with a thin cuticle and the lower layer with columnar cells with an undulated cuticle. The upper and lower epidermis did not bear trichomes. In the midrib region, the ground tissue comprised 1-2 irregular layers of sclerenchyma, followed by a broad zone of round, thin-walled

parenchyma cells. Below, a broad vascular strand covering the central part of the midrib appeared as two fused half rings, with conjoint collateral vascular bundles with parallel rows of xylem. Two distinct xylem bundles were visible in the upper part, while the phloem forms a continuous horizontal band below the xylem strands. Phloem was surrounded by a thin band of regularly arranged sclerenchyma. The lateral veins had collateral bundles surrounding sclerenchyma bands.

The adaxial epidermal cells were squarish in the laminar region, evenly distributed and covered with thin cuticle. The abaxial epidermal cells were columnar, unevenly distributed, with an undulate cuticular layer. A few stomata exist in the abaxial side and no stomata exist in the adaxial side. Below the epidermis appear mesophyll tissue with 2-3 layers of thick, elongated, pillar-like palisade cells and a lower spongy layer with loosely arranged spherical parenchyma cells. The mesophyll cells were intervened with secretory idioblasts (Table 11 & Fig 12).

- ***L. wightiana* leaf**

The section of the leaf showed a prominent midrib with lateral veins and uniformly thin lamina. The upper epidermis consisted of squarish cells, evenly distributed and covered with a thin cuticle. The lower epidermis had irregularly distributed, small columnar cells with papillose and undulate cuticle. The entire leaf epidermis had numerous elongated, unicellular, and narrow trichomes. In the midrib, the ground tissue consisted of multilayered parenchyma cells. The central part of the midrib was occupied by a wide vasculature that consisted of collateral bundles arranged as two half rings, of which the lower was broader and the upper narrower. Phloem was seen as a continuous horizontal band below the xylem strands. Surrounding the phloem, there was a thin band of regularly arranged sclerenchyma. The lateral veins had distinct collateral bundles surrounding sclerenchyma bands.

In the laminar region, the upper epidermis had thick-walled, regular, squarish-type cells. The lower epidermis was composed of irregularly arranged parenchyma cells with a thick cuticle covering. Below the upper epidermis, mesophyll tissue with 2-3 layers of elongated palisade cells, interspersed with large idioblasts, was seen (Table 11& Fig 13).

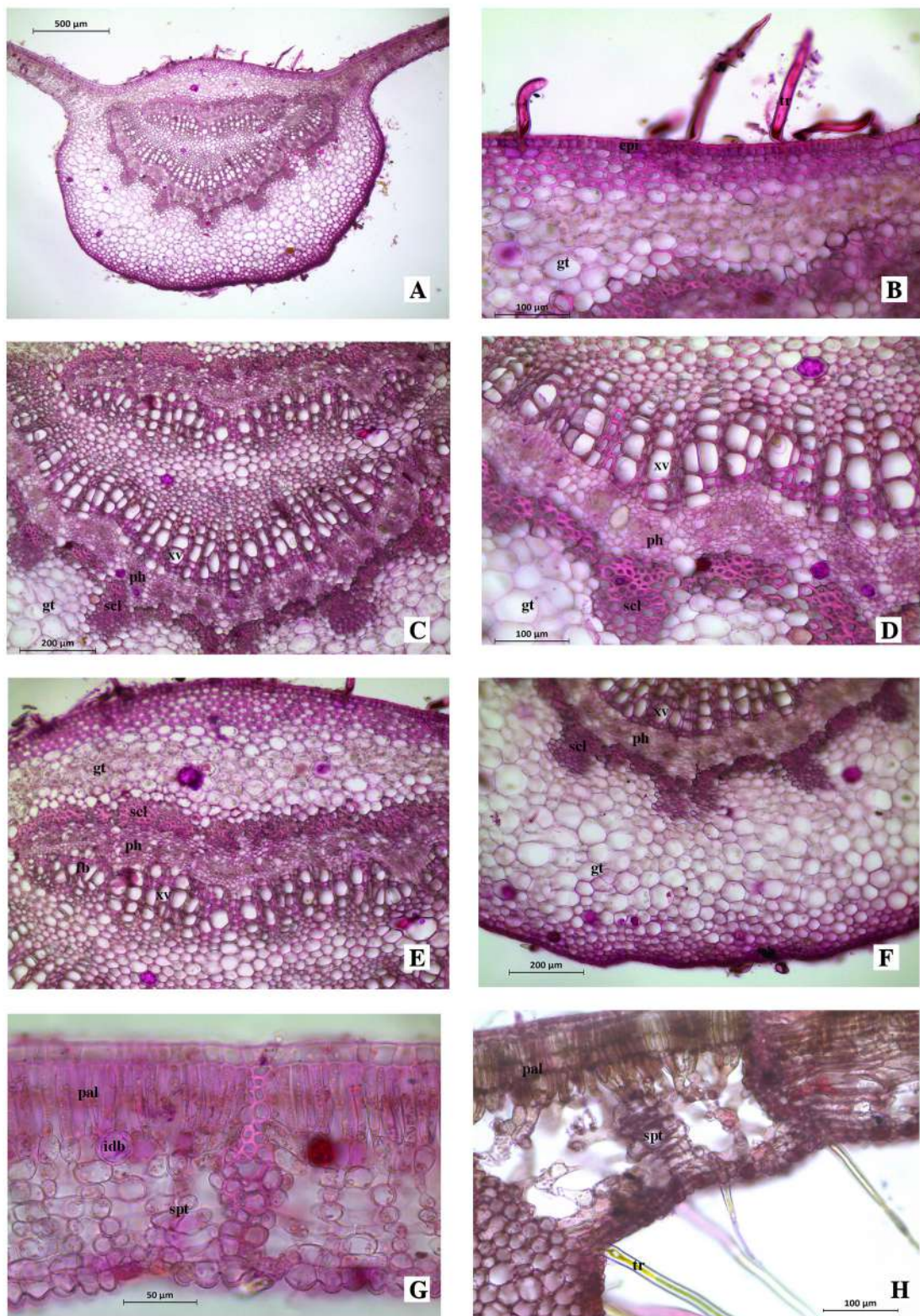


Fig 11 Microscopic characters of *L.bourdillonii* leaf A. ground plan, B. trichome, C,D & E. mid rib& vasclature, F. ground tissue, G. palisade & spongy tissue, H. lower epidermis with trichomes.

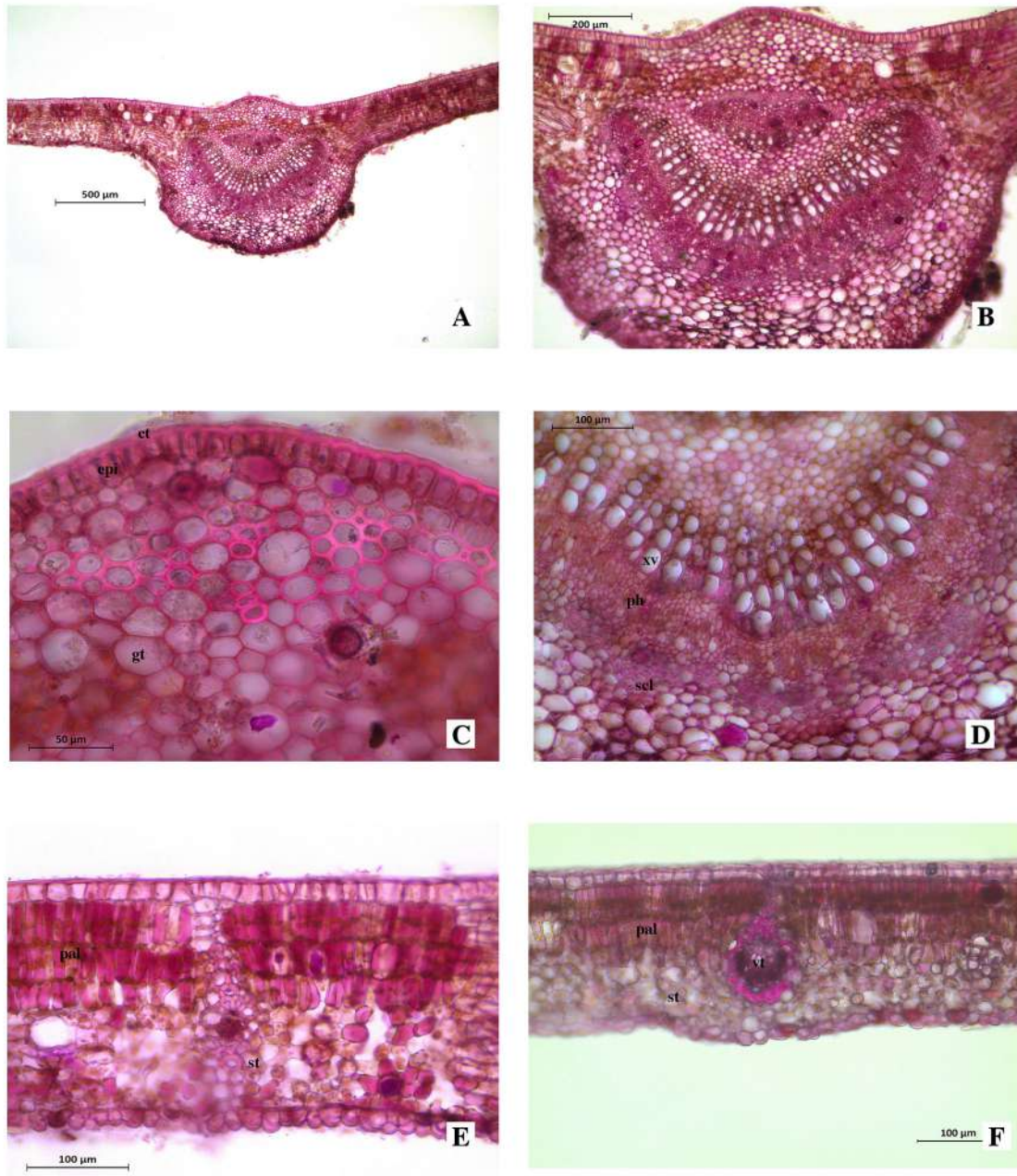


Fig 12 Microscopic characters of *L. coriacea* leaf. A. ground plan, B. mid rib, C. ground tissue, D. Vascular tissue E&F. lamina with palisade & spongy tissue

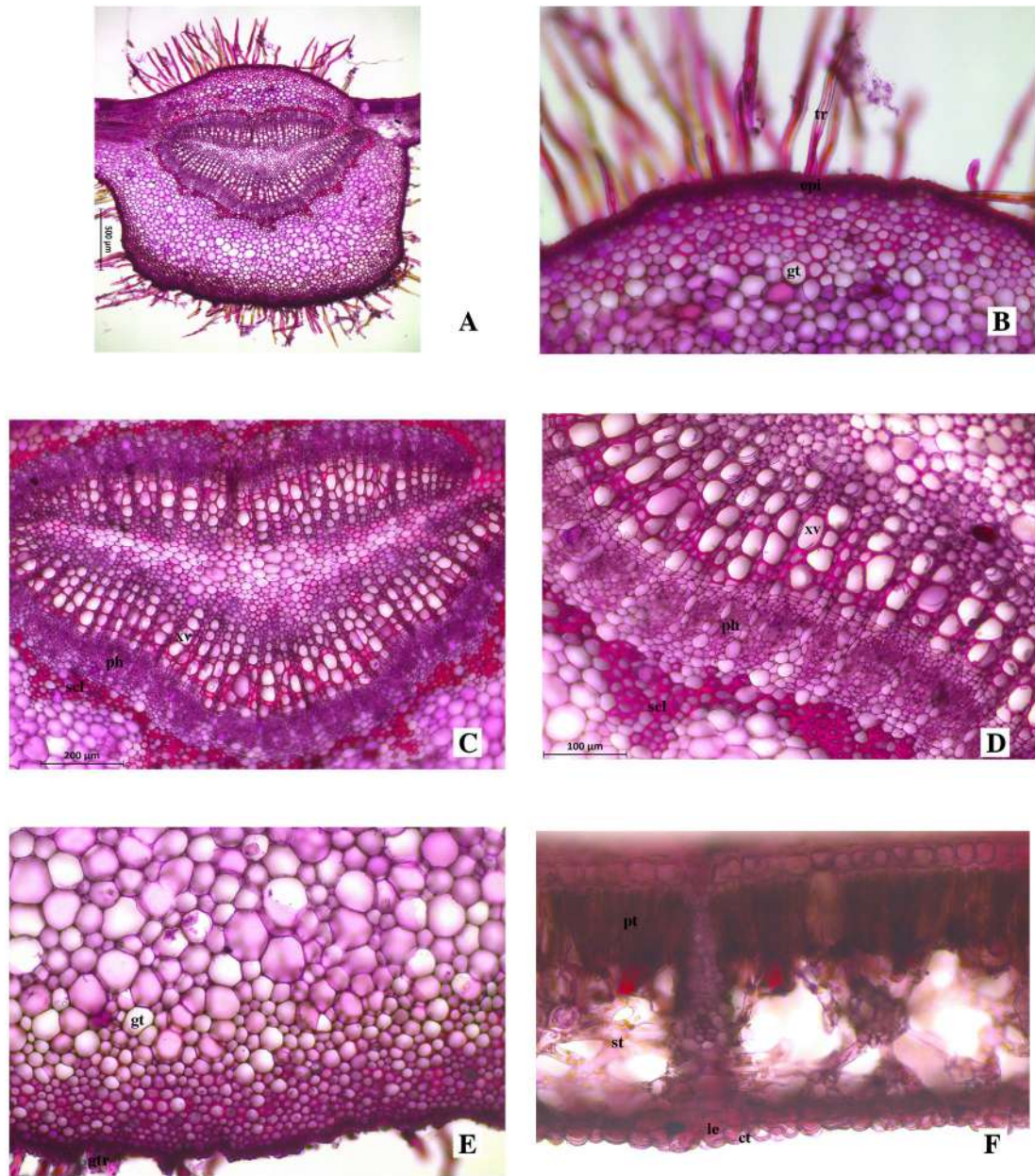


Fig 13 Microscopic characters of *L. wightiana* - A. ground plan, B. trichome, C&D. mid rib vasculature E. ground tissue, F.lamina with palisade & spongy tissue.

Table 11 Comparative anatomical characterization of the leaves of three *Litsea* spp.

Microscopic characters	<i>L. bourdillonii</i>	<i>L. coriacea</i>	<i>L. wightiana</i>
Epidermis	Upper uniseriate, small, narrow, thin-walled, and squarish parenchyma cells, lower columnar cells	Upper uniseriate, small, narrow, thin-walled, and squarish parenchyma cells, lower columnar cells	Upper uniseriate, small, squarish, and thin-walled parenchyma cells, lower columnar cells
Cuticle	Upper: thin-walled and uniformly arranged Lower: undulate and irregularly arranged	Upper: thin-walled and uniformly arranged Lower: undulate and irregularly arranged	Upper: thin-walled and uniformly arranged Lower: undulate and irregularly arranged
Trichomes	Unicellular and elongated	Absent	Numerous, elongated, and unicellular
Ground tissue	Multilayered and round parenchyma in the midrib region	Multilayered parenchyma and 1-2 irregular sclerenchyma	Multilayered parenchyma
Vasculature	Wide vasculature with collateral bundles, with two large, fused bundles on the upper side	Wide vasculature with two fused half rings of bundles, the upper small and the lower large	Wide vasculature consists of collateral bundles arranged as two half rings, the lower broader and the upper narrower ring.
Palisade tissue	Two layers of elongated, compactly arranged, pillar-like columnar cells interspersed with a few secretory idioblasts	Thick, elongated, pillar-like columnar cells interspersed with secretory idioblasts	2-3 layers of elongated palisade cells interspersed with large secretory idioblasts
Spongy mesophyll	Loosely arranged parenchyma cells	Loosely arranged parenchyma cells	Loosely arranged parenchyma cells
Bundle sheath	Continuous sclerenchymatous layer	Discontinuous sclerenchymatous layer	Continuous sclerenchymatous layer

● ***L. bourdillonii* petiole**

The section of the petiole showed a crescent outline. The epidermis was single-layered with thin-walled parenchyma cells covered by a thick cuticle. The entire epidermis gave rise to numerous short and narrow unicellular trichomes. The ground tissue was wide with thin-walled parenchyma cells. The center of the petiole consisted of crescent-shaped vasculature that contained numerous narrow bundles.

The xylem tissue had tracheids and vessels, and the phloem tissue had phloem parenchyma and a few fibers. The centre of the petiole was occupied by pith that consisted of loosely arranged polygonal parenchyma cells (Table 12 & Fig 14).

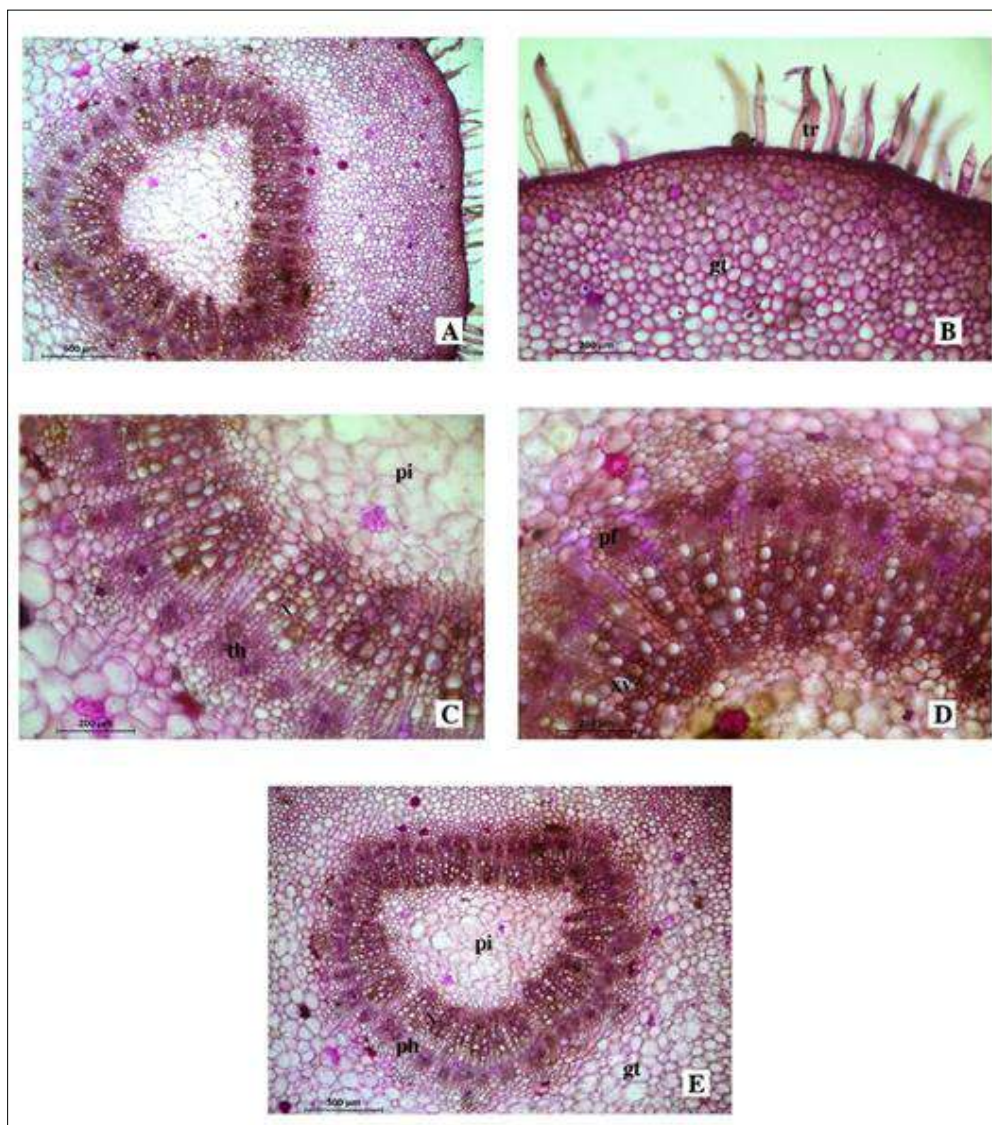


Fig. 14 Petiole anatomy of *L. bourdillonii*. A. Ground plan; B. Trichome; C&D Vasculature; E. Pith

- ***L. coriacea* petiole**

The section showed a crescent-shaped outline. The epidermis was uniseriate with small, squarish-type cells covered with a thin cuticle. The epidermis did not give rise to trichomes. The ground tissue was wide and consisted of thin-walled parenchyma cells. Numerous stone cells were scattered in the cortex. Crescent-

shaped vasculature had a hooked upper end. Extensive xylem strands had tracheids, vessels and parenchyma. Outside the xylem, the phloem cells contained parenchyma and a few fibers. A single layer of sclerenchyma was seen outside the phloem above the hook. The centre of the petiole was occupied with a narrow and condensed pith with a few polygonal parenchyma cells (Table 12 & Fig 15).

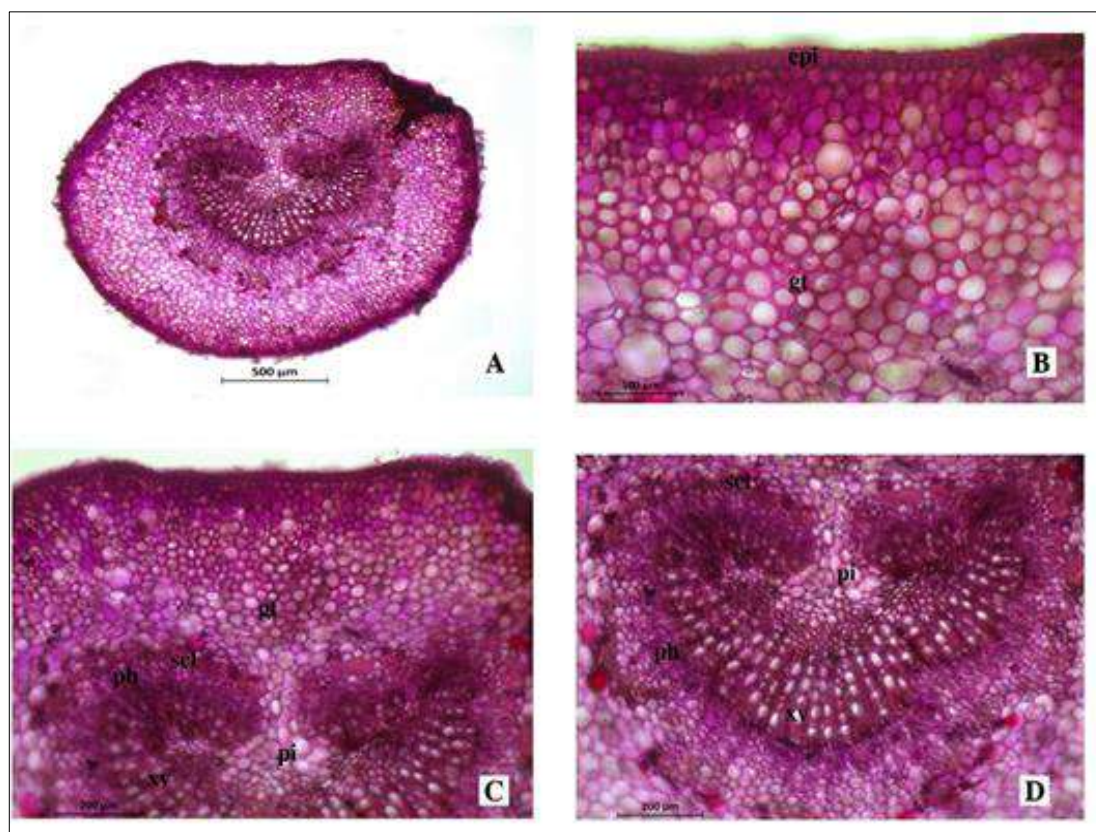


Fig 15 Petiole anatomy of *L. coriacea*. A. Ground plan; B. Ground tissue; C&D. Vasculature

- ***L. wightiana* petiole**

The section showed a horizontally flattened crescent-shaped outline. The outer epidermis contained small parenchyma cells covered by a thick cuticle. The epidermis gave rise to numerous long unicellular trichomes. The ground tissue was wide with thin-walled parenchyma cells and a few lignified cells. Numerous stone cells were seen scattered in the cortex. Vasculature was horizontal and crescent shaped. Numerous long, narrow xylem strands encompassed with uniformly distributed vessels and tracheids. Outer to the xylem, a band of uniseriate phloem

was present. The centre of the petiole was occupied by a crescent-shaped pith that consisted of loosely arranged polygonal parenchyma cells (Table 12 & Fig 16). The comparative anatomical features of petiole of three species of *Litsea* represented in Table 12.

Table 12 Comparative anatomical characterization of the petiole of three *Litsea* spp.

Microscopic characters	<i>L. bourdillonii</i>	<i>L. coriacea</i>	<i>L. wightiana</i>
Shape	Crescent-shaped	Crescent-shaped	Horizontal crescent-shaped
Epidermis	Uniseriate with thin-walled parenchyma covered by a thick cuticle	Uniseriate with thin-walled and squarish parenchyma covered by a thin cuticle	Uniseriate with thin-walled parenchyma covered by a thick cuticle
Trichome	Short, narrow, and unicellular	Absent	Numerous, long, and unicellular
Ground tissue	Wide with thin-walled parenchyma	Wide with thin-walled parenchyma	Wide with thin-walled parenchyma and numerous scattered stone cells
Shape of Vasculature	Crescent-shaped	Crescent-shaped with two hooks at the end	Horizontal and crescent-shaped
Xylem	Tracheids and vessels	Tracheids, vessels and parenchyma	Vessels and tracheids
Phloem	Parenchyma and a few fibres	Parenchyma and a few fibres	Parenchyma
Bundle cap	Absent	Sclerenchymatous above the hooks	Absent
Pith	Crescent-shaped with loosely arranged polygonal parenchyma	Narrow and condensed with few polygonal parenchyma	Crescent-shaped with loosely arranged parenchyma

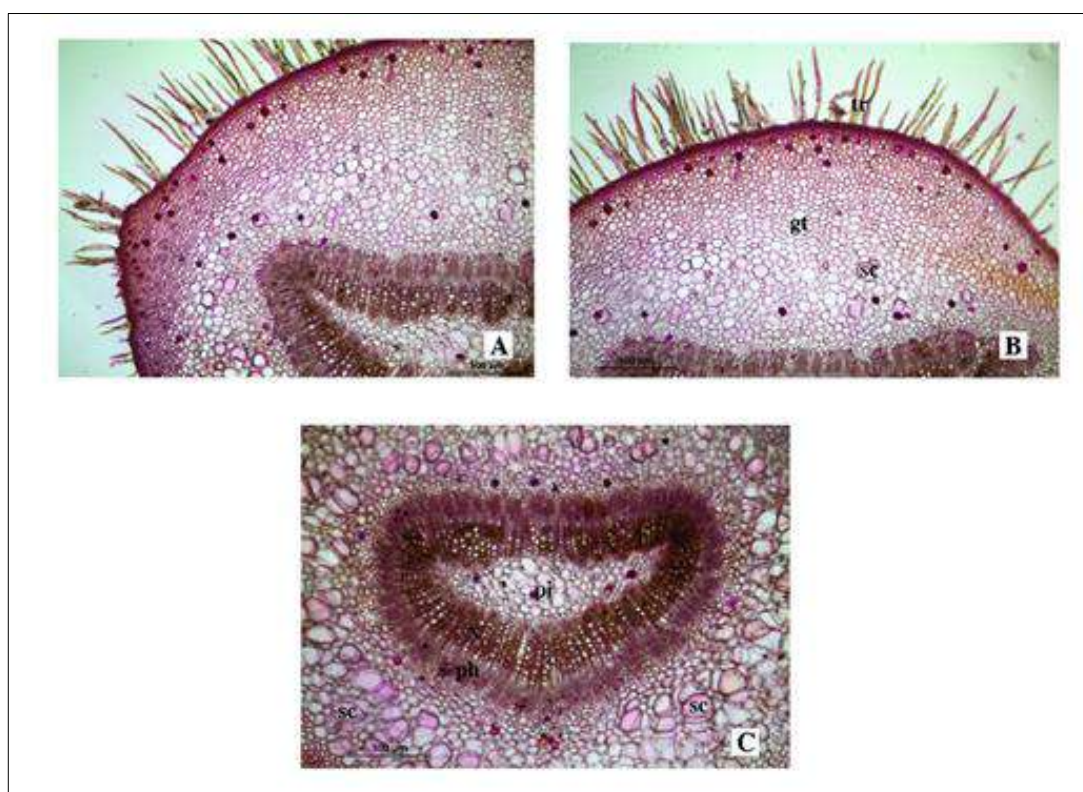


Fig 16 Petiole anatomy of *L. wightiana*. A. Ground plan; B. Ground tissue; C. Vasculature & pith

c. Histochemical study

The results of the histochemical study revealed significant differences in the localization of compounds such as starch, tannin, lignin and fixed oils in various cells of the leaf and stem of selected *Litsea* species. In stem, starch is found abundantly in the stele and cortical regions of *L. coriacea* than that of *L. wightiana*. In *L. bourdillonii*, starch deposition was observed very little. Lignin deposition was mainly located in pericyclic fibers and in the wall of xylem tracheids and tannin was present in cortical regions of all the three species. Fixed oil content was observed to be higher in the hypodermal, cortical and pith region of *L. coriacea* compared to *L. wightiana* and *L. bourdillonii* (Table 13 and Figures 17, 18 & 19).

Table 13 Comparative histochemical characterization of the stem of three *Litsea* spp.

Test for histochemicals	<i>L. bourdillonii</i>	<i>L. coriacea</i>	<i>L. wightiana</i>
Starch	Present in a few cells in the inner cortex	Abundant in stele and cortex	Present in inner cortex, phloem parenchyma than <i>L.bourdillonii</i>
Lignin	Present in bundle cap, xylem tracheids	Present in bundle cap, abundant in xylem tracheids and vessels	Abundant in the outer and the inner cortex and the secondary xylem
Tannins	Present in hypodermis, cortex, phloem and pith	Present abundantly in the cortex, pericyclic fibres and xylem	Abundant in hypodermis, cortex and less in pith
Fixed oils	Small deposition in hypodermal cells	Increased deposition in the hypodermis, cortex and pith	More in the hypodermis, and fewer in the inner cortex and pith

Histochemical studies in the leaves indicated various histochemicals in three *Litsea* plants. Starch was present in *L. coriacea* and *L. wightiana* and absent in *L. bourdillonii*. Lignin was located mainly in the bundle sheath and xylem wall of *L. coriacea* and *L. wightiana* compared to *L. bourdillonii*. Tannin was found to be present in the midrib region and was abundant in *L. wightiana* than *L. bourdillonii* and *L. coriacea*. Oil content was localized more in cortical cells of *L. coriacea* and scarcely present in *L. wightiana* and absent in *L. bourdillonii* (Table 14 & Fig 20).

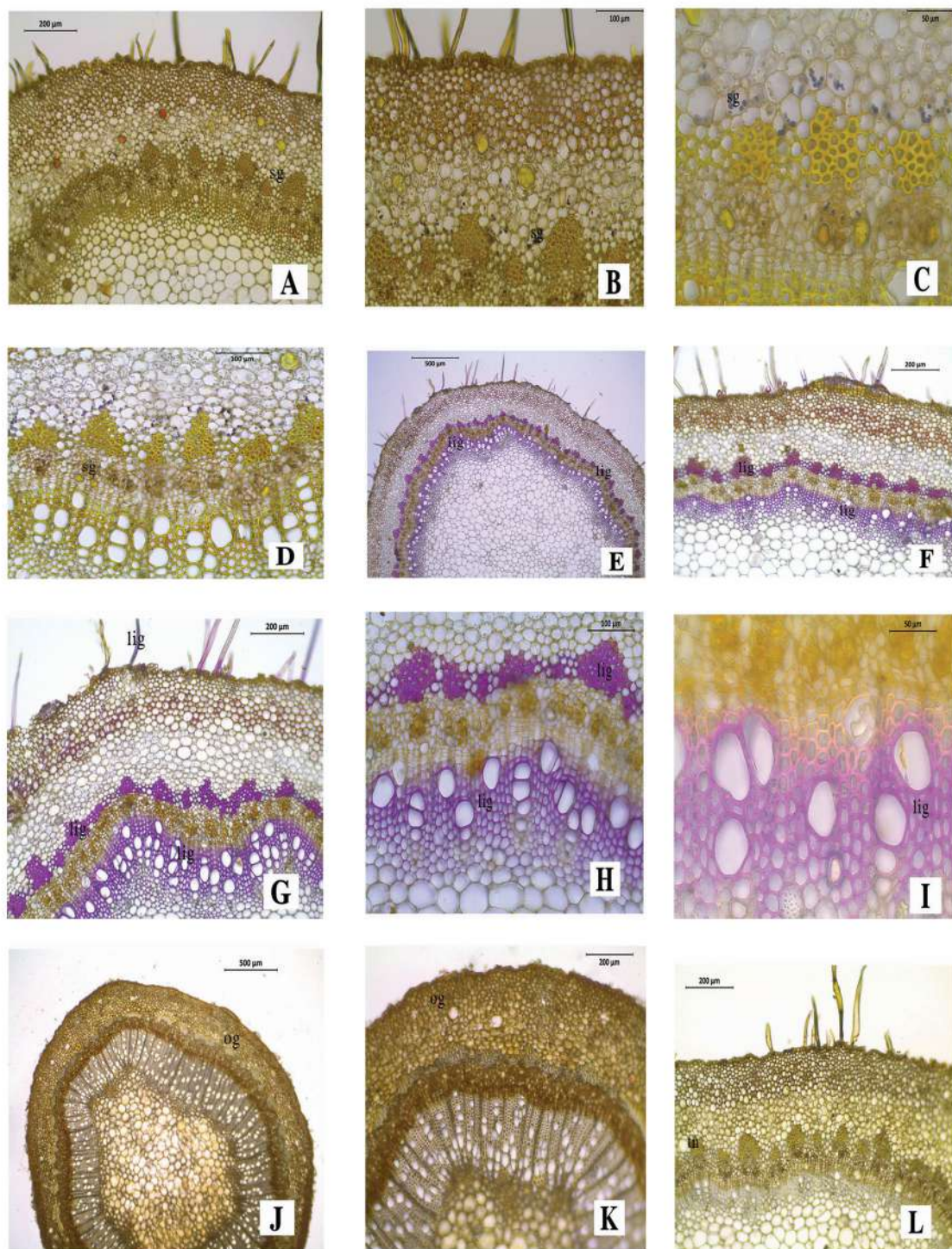


Fig 17 Histochemical localization of *L. bourdillonii* stem. A-D. Starch; E-I. Lignin; J&K. Fixed oil; L. Tannins

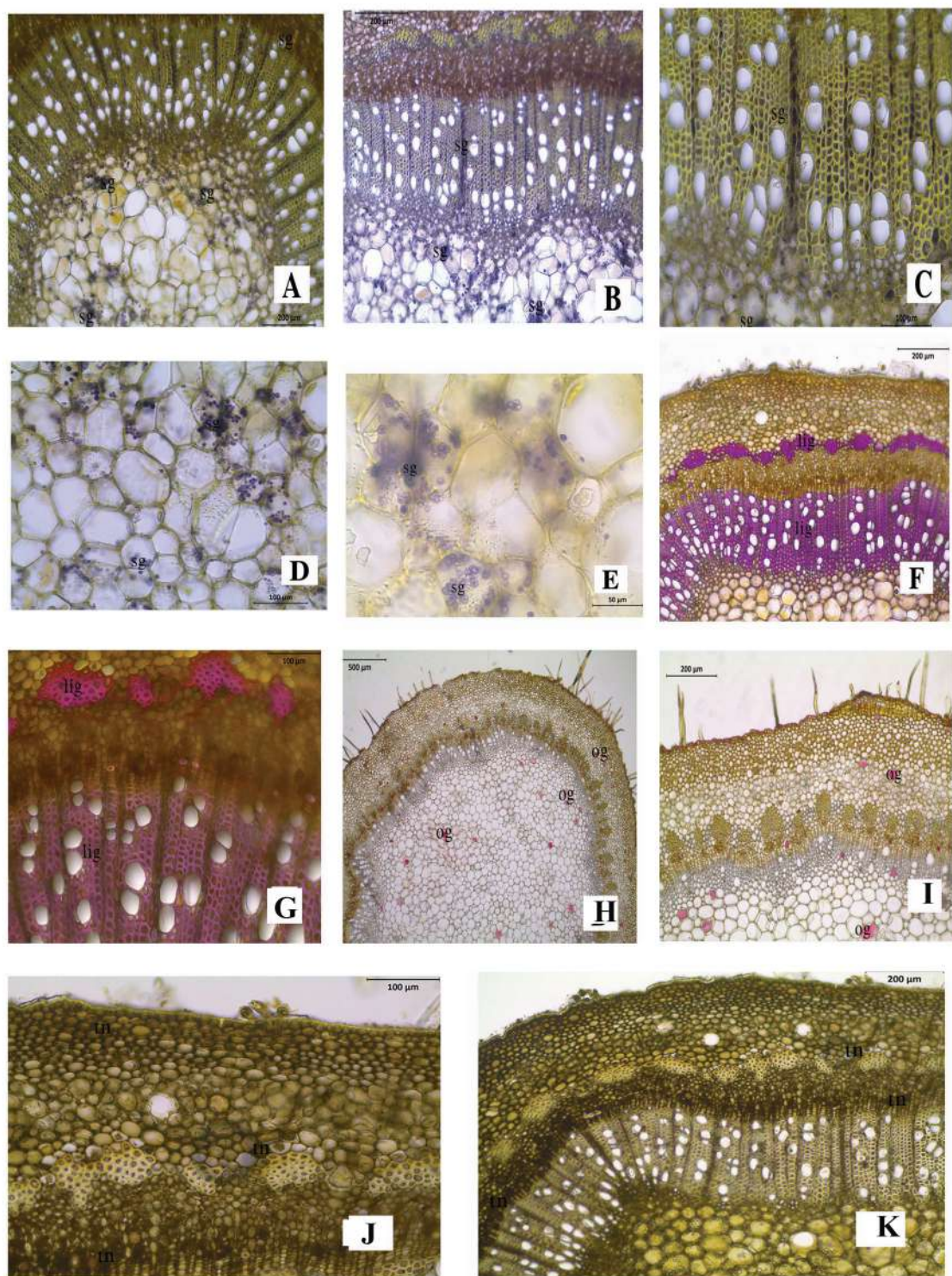


Fig 18 Histochemical localization of *L. coriacea* stem. A-E. Starch; F&G. Lignin; H&I. Fixed oil; J&K. Tannin

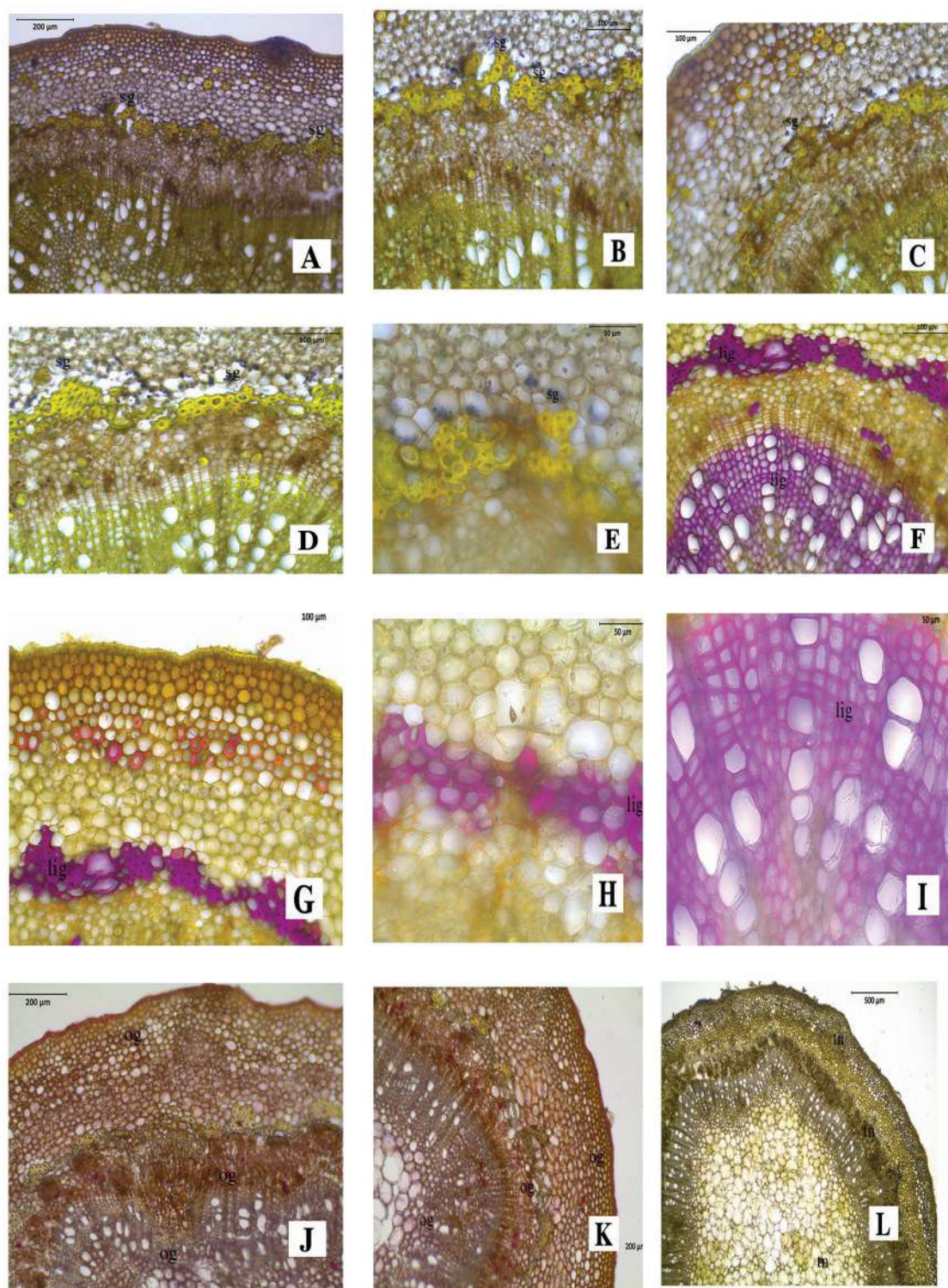


Fig 19 Histochemical localization of *L.wightiana* stem. A-E. Starch; F-I. Lignin; J&K Fixed oil; L. Tannin

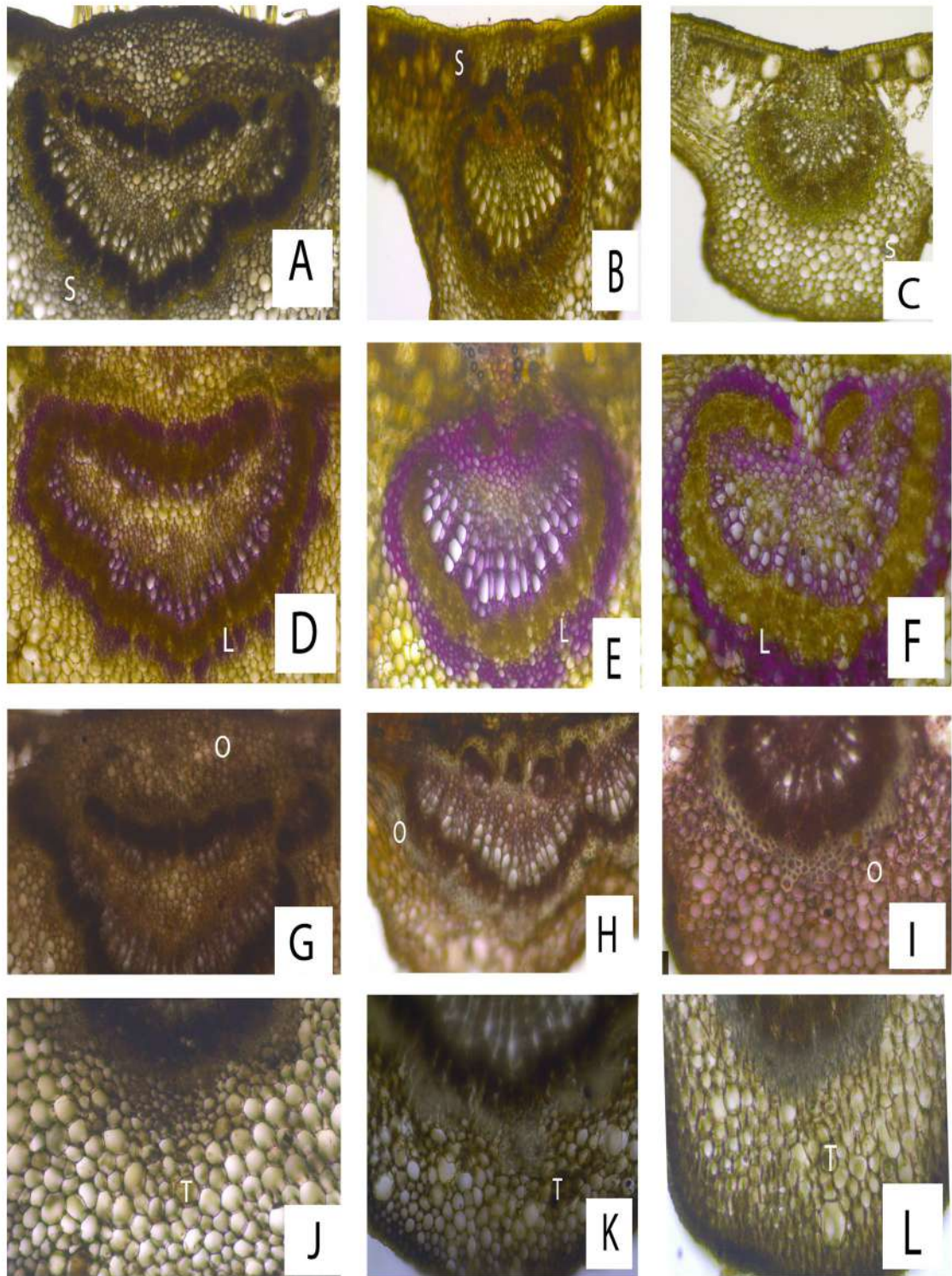


Fig 20 Histochemical localization of leaf of *L.bourdillonii*, *L.coriacea*, & *L.wightiana*. A-C. Starch; D-F. Lignin; G-I. Fixed oil; J-L. Tannin

Table 14 Comparative histochemical characterization of the leaves of three *Litsea* spp.

Test for histochemicals	<i>L. bourdillonii</i>	<i>L. coriacea</i>	<i>L. wightiana</i>
Starch	Absent	Present in cortical cells in the midrib region	A few deposits in cortical cells in the midrib region
Lignin	Present in the bundle sheath and xylem tracheids	Abundant in the bundle sheath and xylem, and a few in mesophyll cells	Present in the midrib, hypodermis, bundle sheath, and xylem wall
Tannins	Present in the midrib hypodermis and outer epidermal cells	Present in the midrib hypodermis. More than that in <i>L. bourdillonii</i>	Abundant in the midrib hypodermis. More than that in <i>L. coriacea</i> and <i>L. bourdillonii</i>
Fixed oils	Absent	High deposition of oil in mesophyll, hypodermis, and cortical cells in the midrib	Present in mesophyll and cortex but less than that in <i>L. coriacea</i>

d. Physicochemical study

Physicochemical analysis was performed on powder samples of *L. bourdillonii*, *L. coriacea* and *L. wightiana* leaves and bark samples. According to the study the percentage of water-soluble extractive, alcohol soluble extractive, crude fiber, total ash, acid-insoluble ash and sulphated ash in the leaves and bark samples varied noticeably according to the comparative physicochemical study. In leaves, the extractive values of both water and alcohol-soluble extracts were found to be higher in *L. wightiana* (10.36% and 10.76%) compared to those of *L. coriacea* (8.53% and 5.42%), and *L. bourdillonii* (6.71% and 5.97%), respectively. The total ash content was found to be high in *L. coriacea* (7.60%), followed by *L. bourdillonii* (6.60%), and *L. wightiana* (3.68%). The acid-insoluble ash value was 0.94% in *L. bourdillonii*, 0.54% in *L. coriacea* and 0.88% in *L. wightiana*. The sulphated ash and crude fiber content were observed to be 4.13% and 4.40% respectively in *L. wightiana*, 3.94% and 3.83% in *L. bourdillonii*, and comparatively lower in *L. coriacea* (1.96% and 3.17%) (Table 15).

In the bark samples, the water-soluble extractive values were found to be higher in *L. bourdillonii* (6.55%) compared to *L. wightiana* (4.63%) and *L. coriacea* (3.67%). At the same time, the alcohol-soluble extractives were higher in *L. wightiana* (6.07%) compared to *L. bourdillonii* (2.47%) and *L. coriacea* (1.59%). The total ash content was 24.64% in *L. coriacea* and 22.4% and 20.20% in *L. wightiana* and *L. bourdillonii*, respectively. The acid-insoluble ash was found higher in *L. wightiana* (0.59%) than in the other two species. In *L. bourdillonii*, the acid-insoluble ash was 0.54%, followed by 0.34% in *L. coriacea*. The total sulphated ash content was higher in *L. bourdillonii* (15.86%) followed by *L. coriacea* (9.62%) and *L. wightiana* (4.98%). Crude fibre content was also highest in *L. wightiana* (6.83%), followed by *L. coriacea* (5.2%) and *L. bourdillonii* (2.2%) (Table 16).

Table 15. Comparative physicochemical characterization of the leaves of three *Litsea* spp.

Parameters	<i>L. bourdillonii</i>	<i>L. coriacea</i>	<i>L. wightiana</i>
Water-soluble extractive	6.71%	8.53%	10.36%
Alcohol-soluble extractive	5.97%	5.42%	10.76%
Total ash	6.60%	7.60%	3.68%
Acid-insoluble ash	0.94%	0.54%	0.88%
Sulphated ash	3.94%	1.96%	4.13%
Crude fibre	3.83%	3.17%	4.40%

Table 16. Comparative physicochemical characterization of the bark of three *Litsea* spp.

Parameters tested	<i>L. bourdilonii</i>	<i>L. coriacea</i>	<i>L. wightiana</i>
Water-soluble extractive	6.55%	3.67%	4.63%
Alcohol-soluble extractive	2.47%	1.59%	6.07%
Total ash	20.20%	24.64%	22.41%
Acid-insoluble ash	0.54%	0.34%	0.59%
Sulphated ash	15.86%	9.62%	4.98%
Crude fibre	2.2%	5.2%	6.83%

e. Powder microscopic study

The stem bark of the three *Litsea* plants in powder form was subjected to powder characterization. The studies revealed the occurrence of cork cells, fibers, stone cells, starch grains and crystals in all three powdered stem barks and exhibited much similarity in structure. But the samples showed minor variations in powder characteristics like the shape of cells in sectional view, the nature of fibers, and the size of starch grains. It was observed that the fibers were long with a narrow lumen and pointed ends in *L. coriacea* and *L. wightiana*, but blunt ended in *L. bourdillonii* (Fig 21).

PHASE II. PHYTOCHEMICAL PROFILING

a. Determination of extractive value

Extracts of the leaves and bark samples of the three *Litsea* species selected for phytochemical studies were prepared by the Soxhlet extraction method. Methanol was used as the solvent. Before the phytochemical analysis, the extractive values of each sample were determined. The extractive value of *L. wightiana* bark was found to be higher (43.5%), followed by *L. bourdillonii* (35.65%) and *L. coriacea* (37%) leaf extracts. The least extractive value was found in *L. bourdillonii* bark (4.8%) (Table 17).

Table 17. Extractive values of the leaf and bark samples of three *Litsea* spp.

Sample	Weight of the sample (g)	Weight of dry extract (g)	Extractive value (%)
<i>L. bourdillonii</i> leaf	20	7.13	35.65
<i>L. coriacea</i> leaf	20	7.4	37
<i>L. wightiana</i> leaf	20	4.86	24.3
<i>L. bourdillonii</i> bark	20	0.96	4.8
<i>L. coriacea</i> brk	20	2.25	11.25
<i>L. wightiana</i> bark	20	8.7	43.5

Preliminary phytochemical screening of the methanolic leaf and bark extracts of the three *Litsea* plant samples were carried out both qualitatively and

quantitatively. The identification of non-volatile compounds was done by LC/MS-Q-TOF technique and GC/MS technique was adopted to identify the volatile compounds.

b. Qualitative analysis of phytochemicals

The results of qualitative phytochemical profiling of the leaves and bark showed the presence of phenols, flavonoids, tannins, and glycosides in *L. bourdillonii*, *L. coriacea* and *L. wightiana*. Alkaloids were absent in the bark of *L. wightiana*. Sterols and quinones were present in leaves of all the three *Litsea* spp. Terpenoids were found in leaves of all the three plants and the bark of *L. wightiana*. Fatty acid was observed in the leaves of *L. bourdillonii*, *L. coriacea* and *L. wightiana* and were absent in all other samples. The saponins were screened from the bark of *L. bourdillonii* and the leaves of *L. wightiana* (Table 18)

Table 18 Qualitative phytochemical screening of the methanolic extracts of three *Litsea* spp.

Sl. No	Class of phytochemicals	LBB	LBL	LCB	LCL	LWB	LWL
1	Phenols	+	+	+	+	+	+
2	Alkaloids	+	+	+	+	-	+
3	Flavonoids	+	+	+	+	+	+
4	Tannins	+	+	+	+	+	+
5	Glycosides	+	+	+	+	+	+
6	Phytosterols	-	+	-	+	-	+
7	Terpenoids	-	+	-	+	+	+
8	Saponins	+	-	-	-	-	+
9	Fatty acids	-	+	-	+	-	+
10	Quinones	-	+	-	+	-	+

(+) positive (Present), (-) negative (Absent)

LBB- *L. boudillonii* bark, LBL- *L. bourdillonii* leaf, LCB- *L. coriacea* bark, LCL- *L. coriacea* leaf, LWB- *L. wightiana* bark, LWL- *L. wightiana* leaf

c. Quantitative analysis of phytochemicals

The Aluminium chloride colourimetric method was used to assess the total flavonoid content and represented in terms of quercetin equivalent ($\mu\text{g QE/mg DW}$)

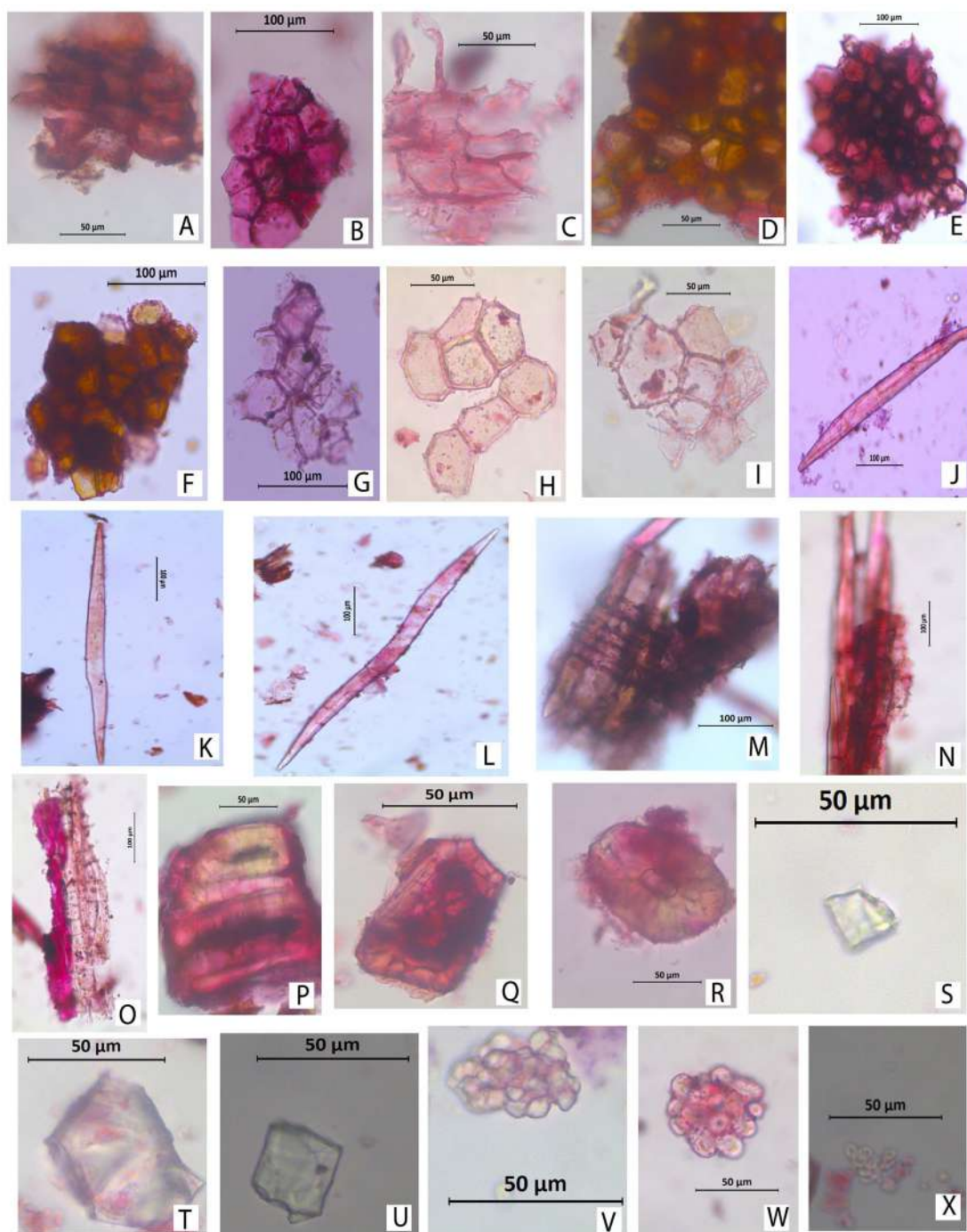


Fig 21 Powder microscopic characterization of stem bark of *L. bourdillonii*, *L. coriacea* & *L. wightiana*. A-C. Cork cell fragments in sectional view; D-F. Cork cell fragments in surface view; G-I. Cortical cells; J-L. Xylem fibres; M-O. Fibre crossing medullary rays; P-R. Stone cells; S-U. Prismatic crystals of calcium oxalate; V-X. Group of starch grains

as shown in Fig 22. The quantitative phytochemical estimation of methanolic extracts of leaves and bark extracts of selected *Litsea* samples showed that the total flavonoid content was high in *L. wightiana* leaf extracts ($37 \pm 0.04 \mu\text{g}/\text{mg}$) and lower in the bark of *L. bourdillonii* ($4 \pm 0.05 \mu\text{g}/\text{mg}$). Phenol content was quantified by the Folin-Ciocalteu method and expressed in terms of gallic acid equivalent (μg GAE/mg DW) as represented in Fig 23. The phenol content was observed to be higher in *L. wightiana* leaves ($119.14 \pm 0.26 \mu\text{g}/\text{mg}$) and lower in the bark of *L. coriacea* ($8.14 \pm 0.20 \mu\text{g}/\text{mg}$).

The tannin estimation was carried out by the Folin-Ciocalteu method and expressed in terms of tannic acid equivalent (μg TA/mg DW) as shown in Fig 24. The higher amount of tannin was noticed in the leaves of *L. wightiana* ($294.66 \pm 0.42 \mu\text{g}/\text{mg}$) and a lesser amount in the bark of *L. coriacea* ($21.5 \pm 0.04 \mu\text{g}/\text{mg}$) (Table 19 & Fig 25). The quantitative analysis revealed that the selected plant parts contained enough phytochemicals that may provide significant therapeutic value for the plants.

Table 19 Quantitative phytochemical analysis of the methanolic extracts of three *Litsea* spp.

Plant extracts	Flavonoids (μg QE/mg DW) $\pm$ SD	Phenols (μg GAE/mg DW) $\pm$ SD	Tannins (μg TAE/mg DW) $\pm$ SD
LBB	4 ± 0.05	79 ± 0.04	173 ± 0.03
LBL	25 ± 0.05	82.42 ± 0.30	163.5 ± 0.36
LCB	15 ± 0.52	8.14 ± 0.20	21.5 ± 0.04
LCL	29 ± 0.03	19.2 ± 0.15	30 ± 0.42
LWB	32 ± 0.04	21.4 ± 0.15	30.5 ± 0.30
LWL	37 ± 0.04	119.14 ± 0.26	294.66 ± 0.42

LBB- *L. boudillonii* bark, LBL- *L. bourdillonii* leaf, LCB- *L. coriacea* bark, LCL- *L. coriacea* leaf, LWB- *L. wightiana* bark, LWL- *L. wightiana* leaf. Values expressed as mean $\pm$ SD

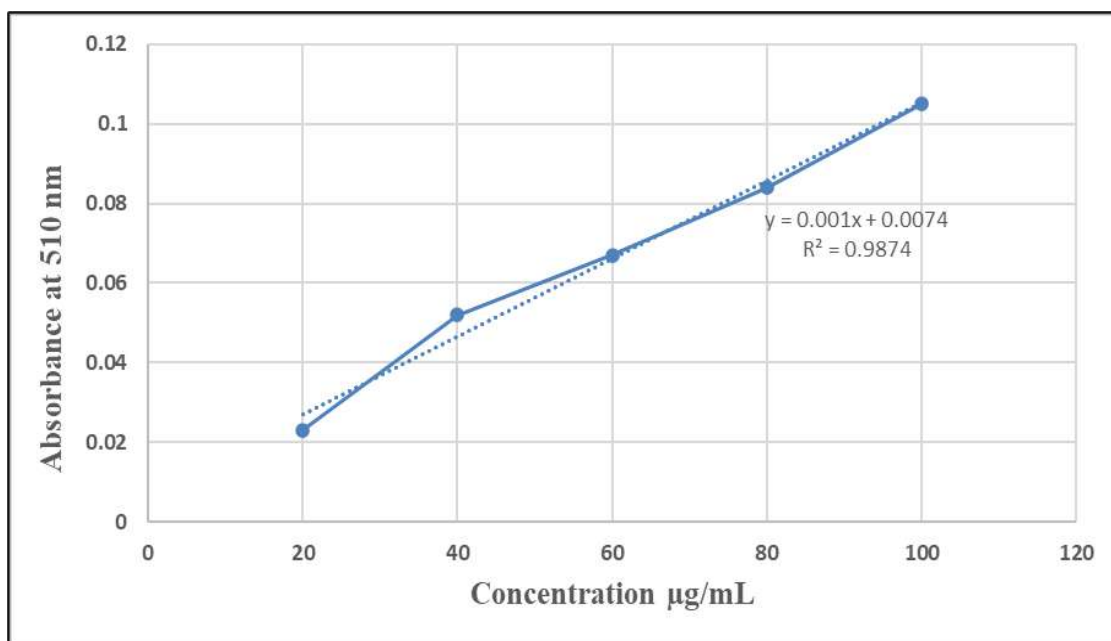


Fig 22 Standard graph of quercetin

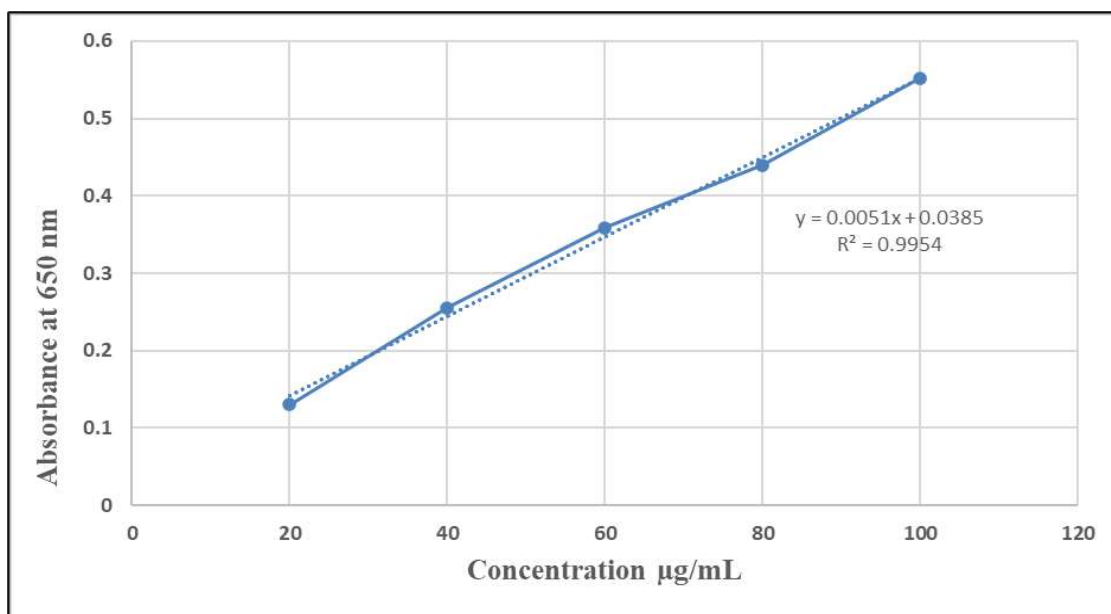


Fig 23 Standard graph of gallic acid

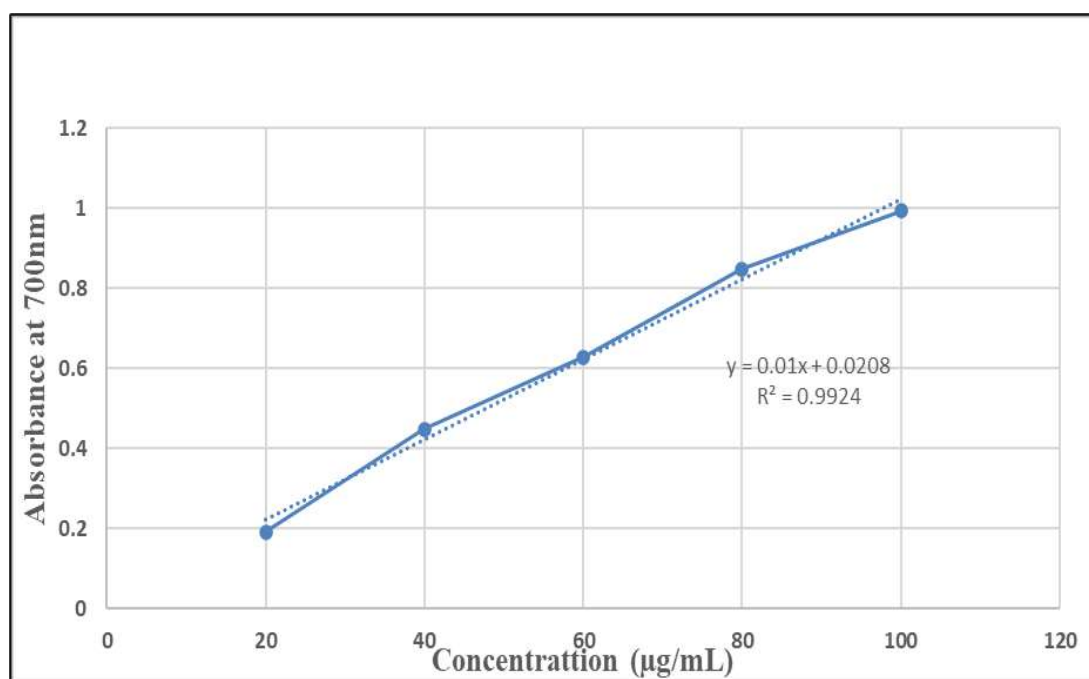


Fig 24 Standard graph of tannic acid

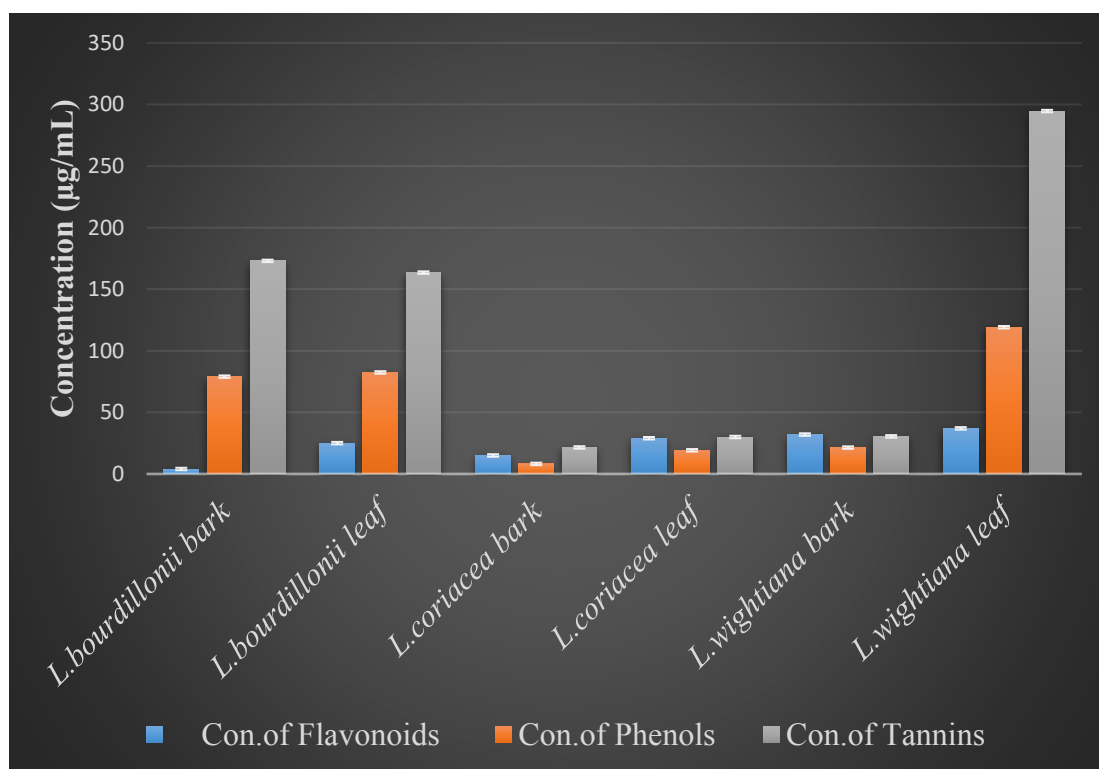
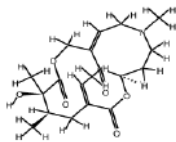
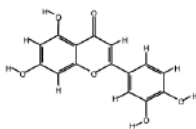
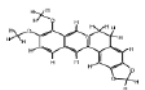


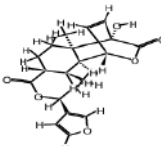
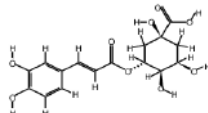
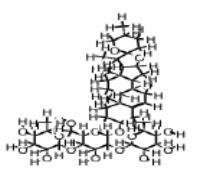
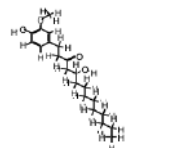
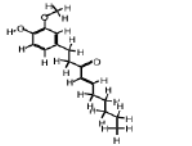
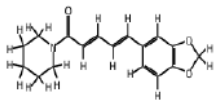
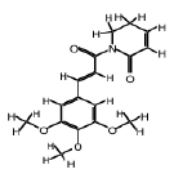
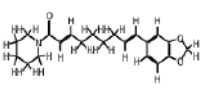
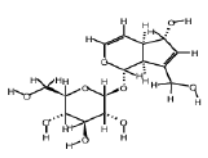
Fig 25 Quantitative analysis of phytochemicals

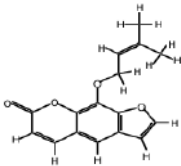
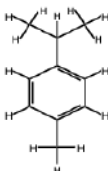
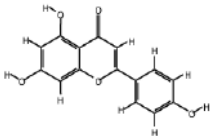
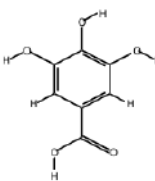
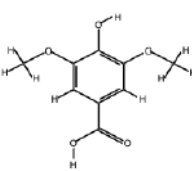
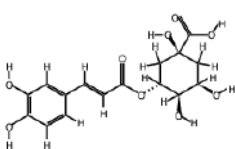
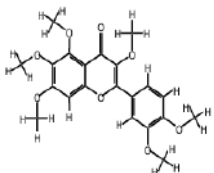
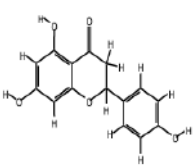
d. Liquid chromatography and mass spectrometric analysis

Liquid chromatography and mass spectrometric analysis was used for the identification of non-volatile compounds in the leaves of *L. bourdillonii*, *L. coriacea* and *L. wightiana*. LC/MS Quadrupole Time-of-Flight (Q-TOF) method was adopted. The results are given in Tables 20, 21 & 22 with the details of compounds like name, structure, molecular formula, retention time and mass. LC/MS chromatograms and mass spectra are depicted in Figures 26 & 27 (i-iv), 28 & 29 (i-v), 30 & 31 (i-iv). *L. bourdillonii* and *L. coriacea* showed the most similarity in the composition of compounds compared to *L. wightiana*. Compounds such as myristic acid, shogaol, columbin, p-cymene, chlorogenic acid, syringic acid, gingerol, luteolin, aucubin, apigenin and hexamethyl quercetagenin were found in *L. bourdillonii* and *L. coriacea* leaves and lauric acid were found in the leaves of three plants. The compounds such as senkirkine, berberine, dioscin, hesperidin are the phytochemicals present only in *L. bourdillonii* leaves. Caffeic acid, magnoflorine, diosgenin, and arbutin are the compounds present only in *L. coriacea*. Chalcone, corytuberine, dicentrinone, xanthoplanine, laurilitsine, isocorydine, reticuline, linalool are the specific compounds found in *L. wightiana* leaves.

Table 20 LC/MS analysis data of the leaves extract of *L. bourdillonii*

Sl. No	Name of compound	Structure	Molecular formula	RT (min)	Mass
1	Senkirkine		C ₁₉ H ₂₇ NO ₆	31.989	365.1845
2	Luteolin		C ₁₅ H ₁₀ O ₆	28.833	286.0453
3	Berberine		C ₂₀ H ₁₈ NO ₄	41.127	336.1229

4	Columbin		$C_{20}H_{22}O_6$	43.005	358.1367
5	Chlorogenic acid		$C_{16}H_{18}O_9$	41.775	354.0929
6	Dioscin		$C_{45}H_{72}O_{16}$	50.265	868.4798
7	(10)-Gingerol		$C_{21}H_{34}O_4$	35.861	350.2405
8	Shogaol		$C_{17}H_{24}O_3$	36.259	276.1716
9	Piperine		$C_{17}H_{19}NO_3$	20.675	285.1380
10	Piperlongumine		$C_{17}H_{19}NO_5$	3.812	317.1263
11	Pipernonaline		$C_{21}H_{27}NO_3$	41.592	341.1987
12	Aucubin		$C_{15}H_{22}O_9$	32.338	346.1267

13	Pentosalen		$C_{16}H_{14}O_4$	45.115	270.0863
14	p-Cymene		$C_{10}H_{14}$	29.514	134.1093
15	Apigenin		$C_{15}H_{10}O_5$	26.191	270.0516
16	Gallic acid		$C_7H_6O_5$	41.645	170.0224
17	Syringic acid		$C_9H_{10}O_5$	13.035	198.0501
18	Chlorogenic acid		$C_{16}H_{18}O_9$	37.691	354.0977
19	Hexamethyl quercetagenin		$C_{21}H_{22}O_8$	41.396	402.1265
20	5,7 – Dihydroxy-2-(4-hydroxyphenyl) Chroman-4-one		$C_{15}H_{12}O_5$	3.532	272.0699

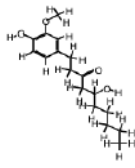
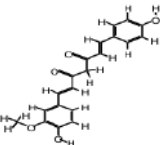
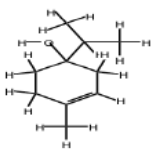
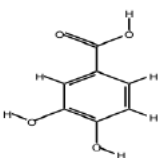
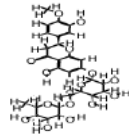
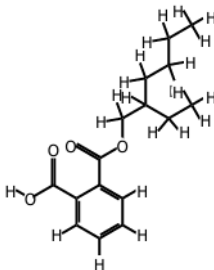
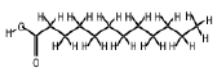
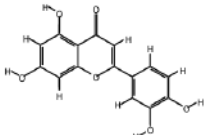
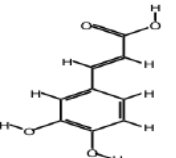
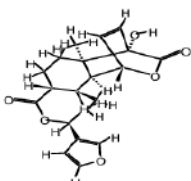
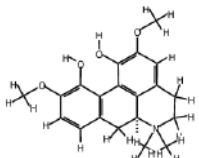
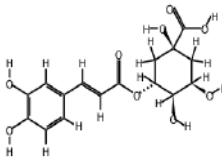
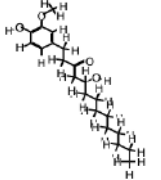
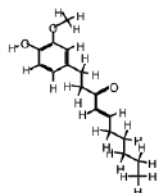
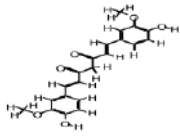
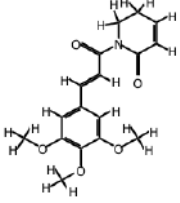
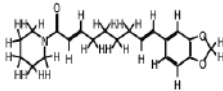
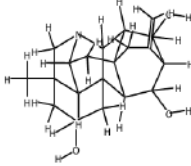
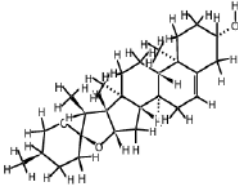
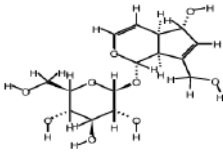
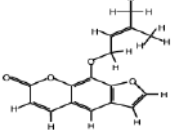
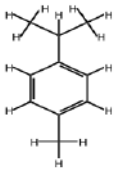
22	Gingerol		$C_{17}H_{26}O_4$	35.730	294.1786
23	Demethoxycurcumin		$C_{20}H_{18}O_5$	40.814	338.1139
24	4-Carvomenthenol		$C_{10}H_{18}O$	38.172	154.1351
25	3,4-Dihydroxybenzoic acid		$C_7H_6O_4$	9.812	154.0273
26	Hesperidin		$C_{28}H_{34}O_{15}$	24.084	610.1885
27	2-(((2-Ethylhexyl)oxy)carbonyl)benzoic acid		$C_{16}H_{22}O_4$	40.714	278.1484
28	Lauric acid		$C_{12}H_{24}O_2$	38.172	200.1770
29	Myristic acid		$C_{14}H_{28}O_2$	42.010	228.2090

Table 21 LC/MS analysis data of the leaves extract of *L. coriacea*

Sl. No	Name of compound	Structure	Molecular formula	RT (min)	Mass
1	Luteolin		C ₁₅ H ₁₀ O ₆	38.836	286.0477
2	Caffeic acid		C ₉ H ₈ O ₄	6.504	180.0466
3	Columbin		C ₂₀ H ₂₂ O ₆	42.840	358.1421
4	Magnoflorine		C ₂₀ H ₂₄ NO ₄	36.410	342.1705
5	Chlorogenic acid		C ₁₆ H ₁₈ O ₉	4.195	354.0960
6	(10)-Gingerol		C ₂₁ H ₃₄ O ₄	4.045	350.2426
7	Shogaol		C ₁₇ H ₂₄ O ₃	42.491	276.1726

8	Curcumin		$C_{21}H_{20}O_6$	42.009	368.1255
9	Piperlongumine		$C_{17}H_{19}NO_5$	3.813	317.1257
10	Pipernonaline		$C_{21}H_{27}NO_3$	40.082	341.1970
11	Hetisine		$C_{20}H_{27}NO_3$	21.241	329.1952
12	Diosgenin		$C_{27}H_{42}O_3$	21.241	329.1952
13	Aucubin		$C_{15}H_{22}O_9$	42.474	346.1264
14	Pentosalen		$C_{16}H_{14}O_4$	48.787	270.0862
15	p-Cymene		$C_{10}H_{14}$	24.614	1134.109 1

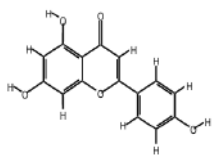
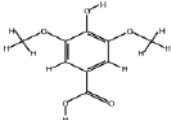
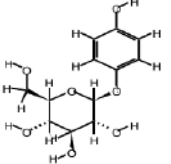
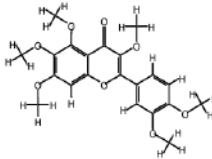
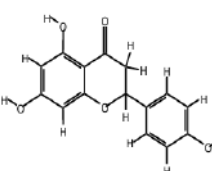
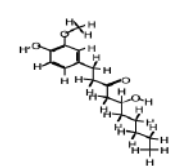
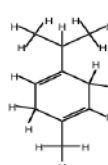
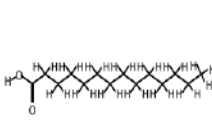
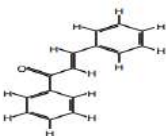
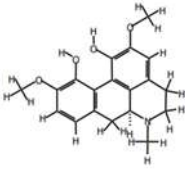
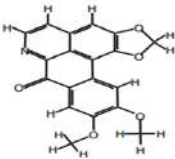
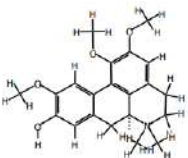
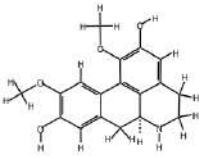
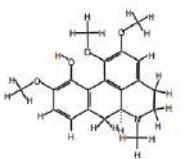
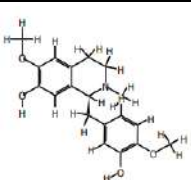
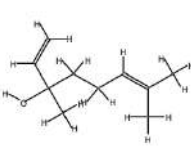
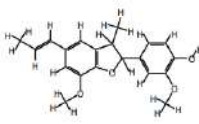
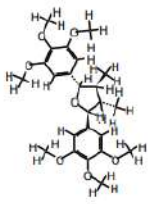
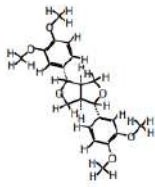
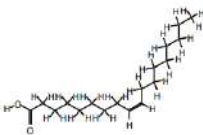
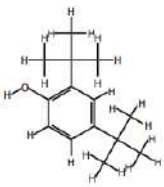
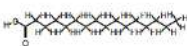
16	Apigenin		$C_{15}H_{10}O_5$	42.125	270.0528
17	Syringic acid		$C_9H_{10}O_5$	16.907	198.0528
18	Arbutin		$C_{12}H_{16}O_7$	24.251	272.0896
19	Hexamethylquercetin		$C_{21}H_{22}O_8$	30.514	402.1315
20	5,7-Dihydroxy-2(-4-hydroxyphenyl)chroman 4-one		$C_{15}H_{12}O_5$	24.001	272.0688
21	Gingerol		$C_{17}H_{26}O_4$	35.399	294.1837
22	Gamma-Terpinene		$C_{10}H_{16}$	41.064	136.1261
23	Lauric acid		$C_{12}H_{24}O_2$	37.260	200.1776
24	Myristic acid		$C_{14}H_{28}O_2$	37.077	228.2089

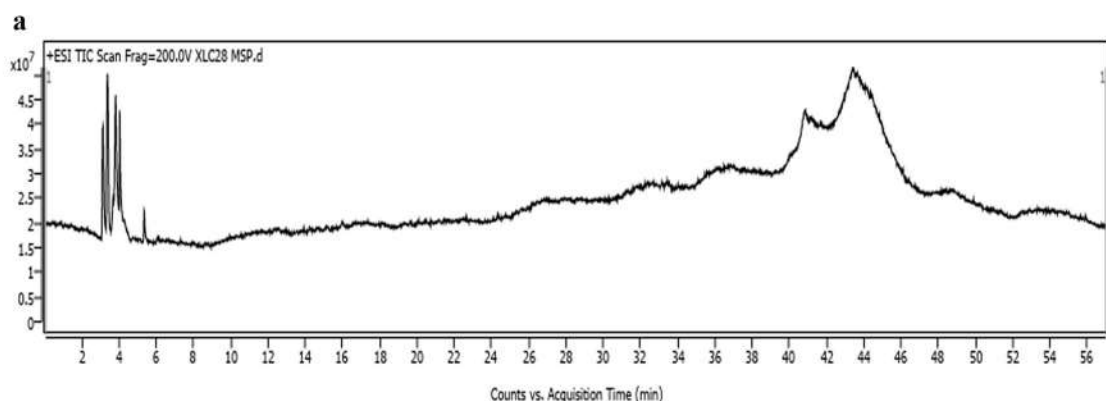
Table 22 LC/MS analysis data of the leaves extract of *L. wightiana*

Sl.No	Name of compound	Structure	Molecular formula	RT (min)	Mass
1	Chalcone		C ₁₅ H ₁₂ O	31.361	208.0875
2	Corytuberine		C ₁₉ H ₂₁ NO ₄	8.881	327.1467
3	Dicentrinone		C ₁₉ H ₁₃ NO ₅	19.215	335.0792
4	Xanthoplanine		C ₂₁ H ₂₆ NO ₄	33.936	356.1860
5	Laurolictsine		C ₁₈ H ₁₉ NO ₄	9.479	313.1320
6	Isocorydine		C ₂₀ H ₂₃ NO ₄	11.307	341.1621
7	Reticuline		C ₁₉ H ₁₉ NO ₄	9.263	329.1620
8	Linalool		C ₁₀ H ₁₈ O	1.936	154.1354
9	Dehydroisoeugenol		C ₂₀ H ₂₂ O ₄	12.437	326.1517

10	Grandisin		$C_{24}H_{32}O_7$	26.044	432.2124
11	(+)-Eudesmin		$C_{22}H_{26}O_6$	21.907	386.1731
12	Oleic acid		$C_{18}H_{34}O_2$	34.951	282.2579
13	Arachidic acid		$C_{20}H_{40}O_2$	34.835	312.303
14	2,4-Di- tert-butylphenol		$C_{14}H_{22}O$	33.838	206.1687
15	Palmitic acid		$C_{16}H_{32}O_2$	31.180	256.2416
16	Lauric acid		$C_{16}H_{32}O_2$	34.154	200.1788

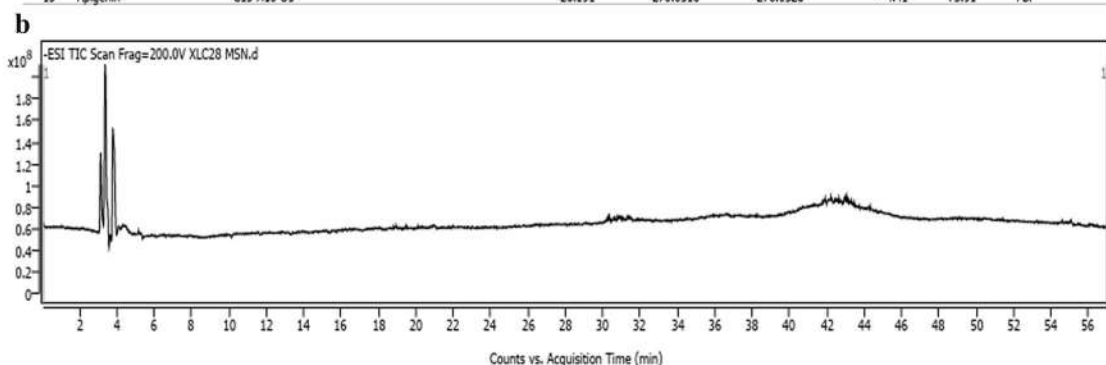
e. Gas chromatography and mass spectrometric analysis

The methanolic leaf extracts of all the three *Litsea* spp. were subjected to GC/MS analysis to find out the volatile compounds present in the samples. The GC/MS analysis of *L. wightiana* leaves revealed various compounds which include various classes of phytochemicals such as alkaloids, flavonoids and phenols (Table 23) Figures 32 & 33 (i-iv). The major compounds identified were Copaene (5.68%), 1H-cycloprop[e]azulene,decaydro-1,1,7-trimethyl-4-methylene(2.04%), naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1methylethyl)-(4.45%), naphthalene,1,2,4a,5,6,8a-hexahydro-4,7 dimethyl-1-(1-methylethyl)-



Compound Summary

Cpd	Name	Formula	CAS	RT	Mass	Mass (Tgt)	Diff (Tgt, ppm)	Score	Algorithm
1	Senkirkine	C19 H27 N O6		31.989	365.1845	365.1838	1.94	73.75	FBF
2	Luteolin	C15 H10 O6		28.833	286.0453	286.0477	-8.56	57.56	FBF
3	Berberine	C20 H18 N O4		41.127	336.1229	336.1236	-2.02	73.88	FBF
4	Columbin	C20 H22 O6		43.005	358.1367	358.1416	-13.72	57.98	FBF
5	Chlorogenic Acid	C16 H18 O9		41.775	354.0929	354.0951	-6.12	62.79	FBF
6	Dioscin	C45 H72 O16		50.265	868.4798	868.4820	-2.60	57.06	FBF
7	(10)-Gingerol	C21 H34 O4		35.861	350.2405	350.2457	-14.93	56.78	FBF
8	Shogaol	C17 H24 O3		36.259	276.1716	276.1725	-3.54	70.63	FBF
9	Piperine	C17 H19 N O3		20.675	285.1380	285.1365	5.42	65.42	FBF
10	Piperlongumine	C17 H19 N O5		3.812	317.1263	317.1263	-0.19	56.85	FBF
11	Pipernonaline	C21 H27 N O3		41.592	341.1987	341.1991	-1.25	73.75	FBF
12	Aucubin	C15 H22 O9		32.338	346.1267	346.1264	0.78	80.90	FBF
13	Pentosalen	C16 H14 O4		45.115	270.0863	270.0892	-10.76	60.50	FBF
14	p-CYMENE	C10 H14		29.514	134.1093	134.1096	-2.20	75.46	FBF
15	Apigenin	C15 H10 O5		26.191	270.0516	270.0528	-4.41	73.91	FBF



Compound Summary

Cpd	Name	Formula	CAS	RT	Mass	Mass (Tgt)	Diff (Tgt, ppm)	Score	Algorithm
1	Gallic acid	C7 H6 O5		41.645	170.0224	170.0215	5.10	58.11	FBF
2	Syringic acid	C9 H10 O5		13.035	198.0501	198.0528	-13.63	65.22	FBF
3	Chlorogenic acid	C16 H18 O9		37.691	354.0977	354.0951	7.34	61.75	FBF
4	Hexamethylquercetagenin	C21 H22 O8		41.396	402.1265	402.1315	-12.40	61.34	FBF
5	5,7-Dihydroxy-2-(4-hydroxyphenyl)chroman-4-one	C15 H12 O5		3.532	272.0699	272.0685	5.16	64.53	FBF
6	Gingerol	C17 H26 O4		35.730	294.1786	294.1831	-15.49	64.31	FBF
7	Demethoxycurcumin	C20 H18 O5		40.814	338.1139	338.1154	-4.44	80.03	FBF
8	4-Carvomenthenol	C10 H18 O		38.172	154.1351	154.1358	-4.23	74.20	FBF
9	4-Carvomenthenol	C10 H18 O		41.346	154.1367	154.1358	6.15	62.90	FBF
10	3,4-Dihydroxybenzoic acid	C7 H6 O4		9.812	154.0273	154.0266	4.28	70.16	FBF
11	Hesperidin	C28 H34 O15		24.084	610.1885	610.1898	-2.04	72.63	FBF
12	2-((2-Ethylhexyl)oxy)carbonyl)benzoic acid	C16 H22 O4		40.714	278.1484	278.1518	-12.18	64.36	FBF
13	Lauroic acid	C12 H24 O2		38.172	200.1770	200.1776	-3.06	86.83	FBF
14	Myristic acid	C14 H28 O2		42.010	228.2090	228.2089	0.09	77.89	FBF

Fig 26 LC/MS chromatogram of *L.bourdillonii* leaf methanolic extract. a. ESI+ spectrum and compounds; b. ESI- spectrum and compounds

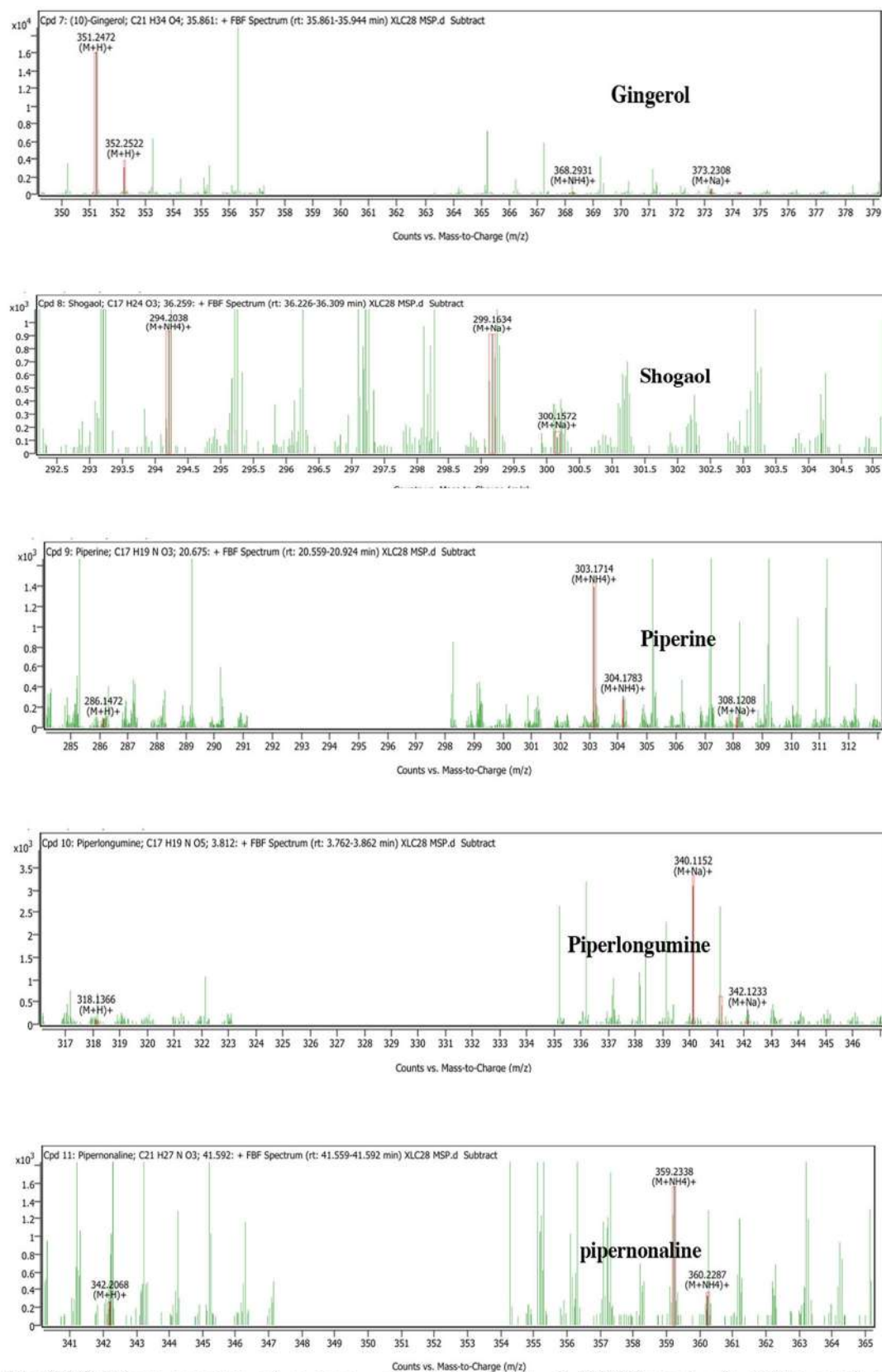


Fig 27(i) Mass spectra of compounds detected by LC/MS analysis of the methanolic extract of *L. bourdillonii* leaves

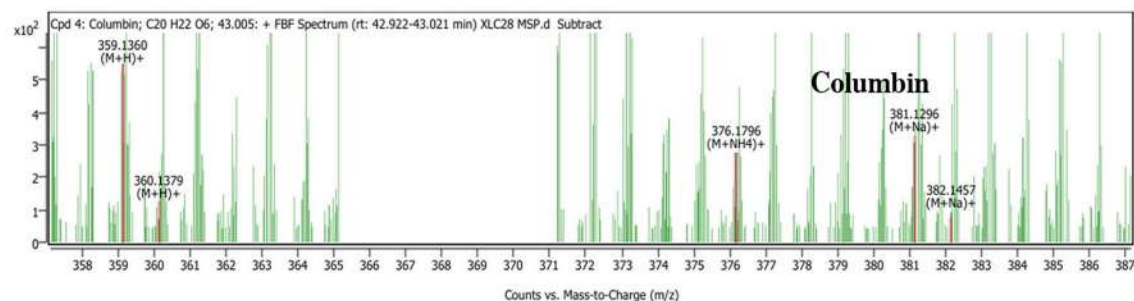
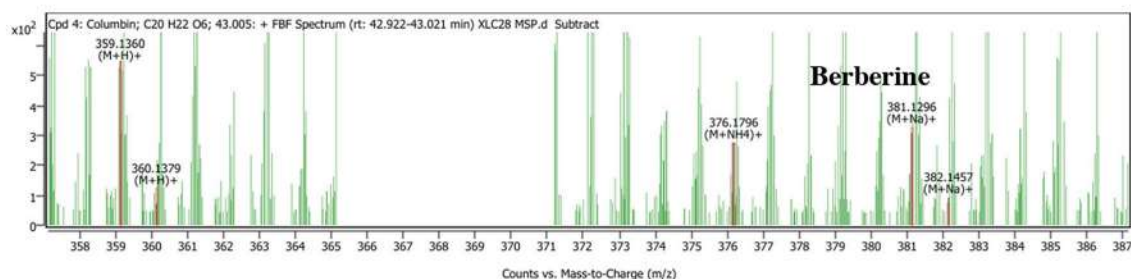
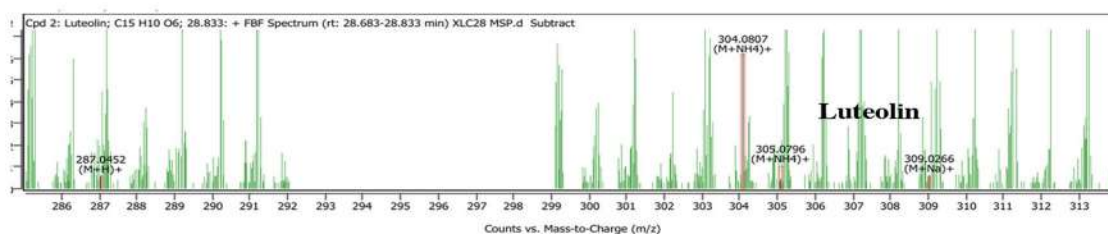
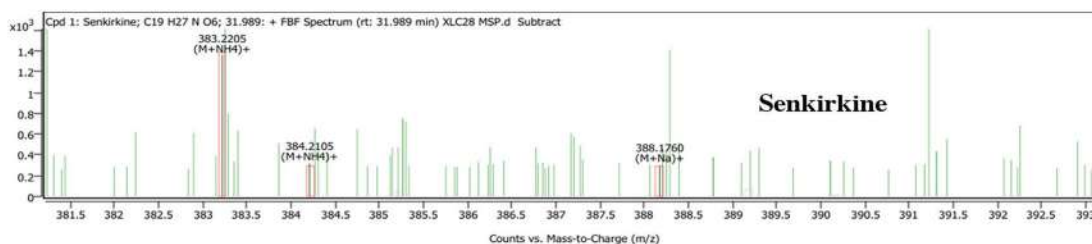
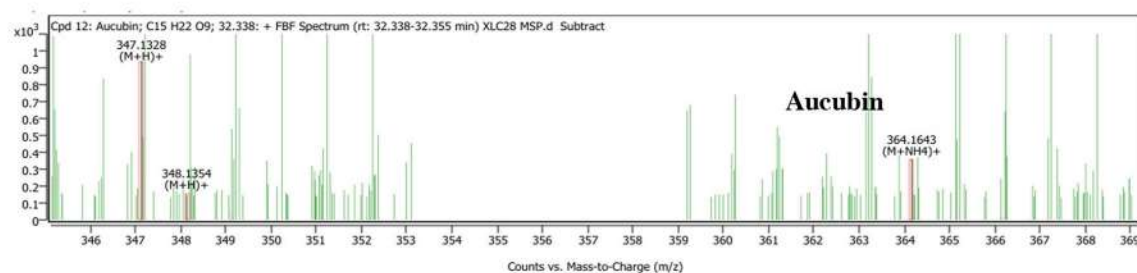


Fig 27(ii) Mass spectra of compounds detected by LC/MS analysis of the methanolic extract of *L.bourdillonii* leaves

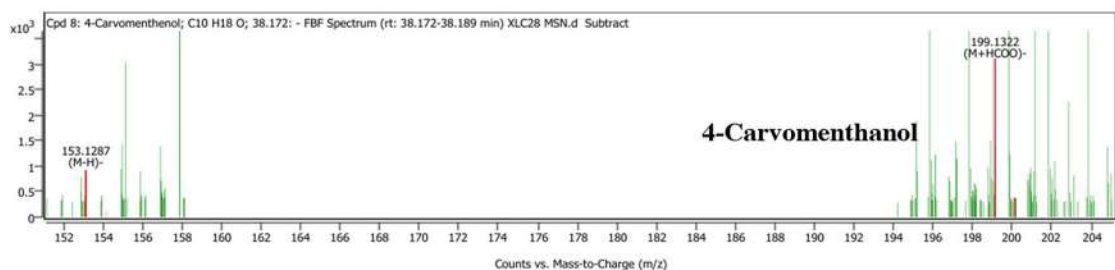
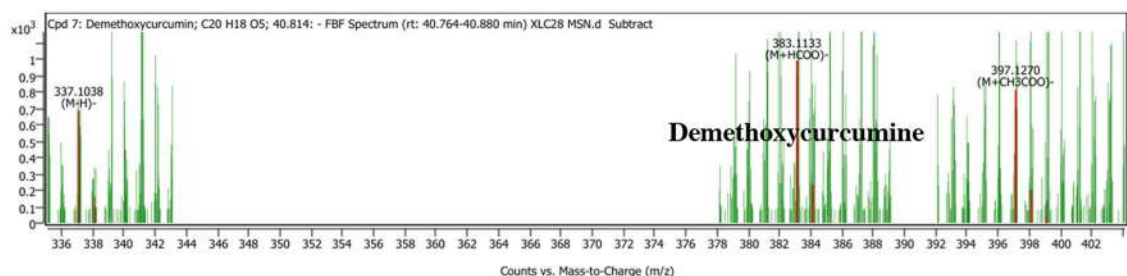
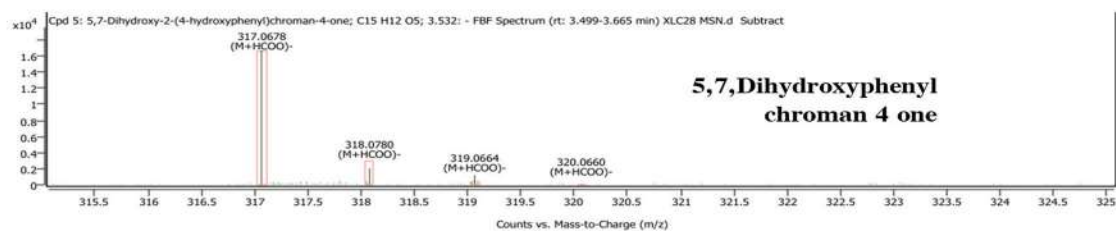
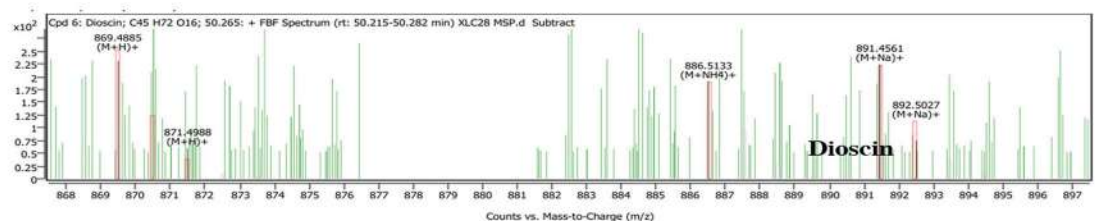
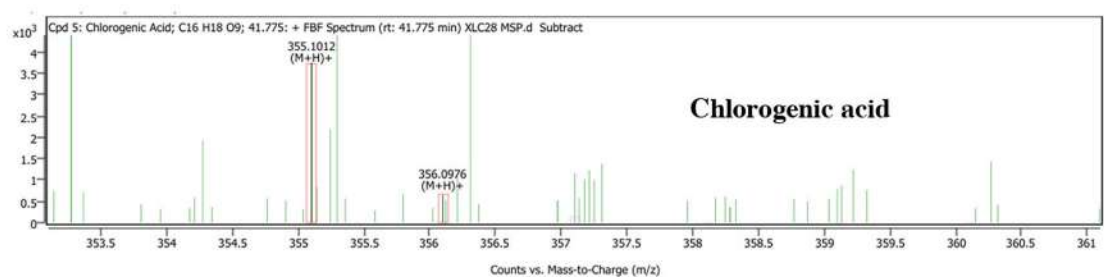


Fig 27(iii) Mass spectra of compounds detected by LC/MS analysis of the methanolic extract of *L.bourdillonii* leaves

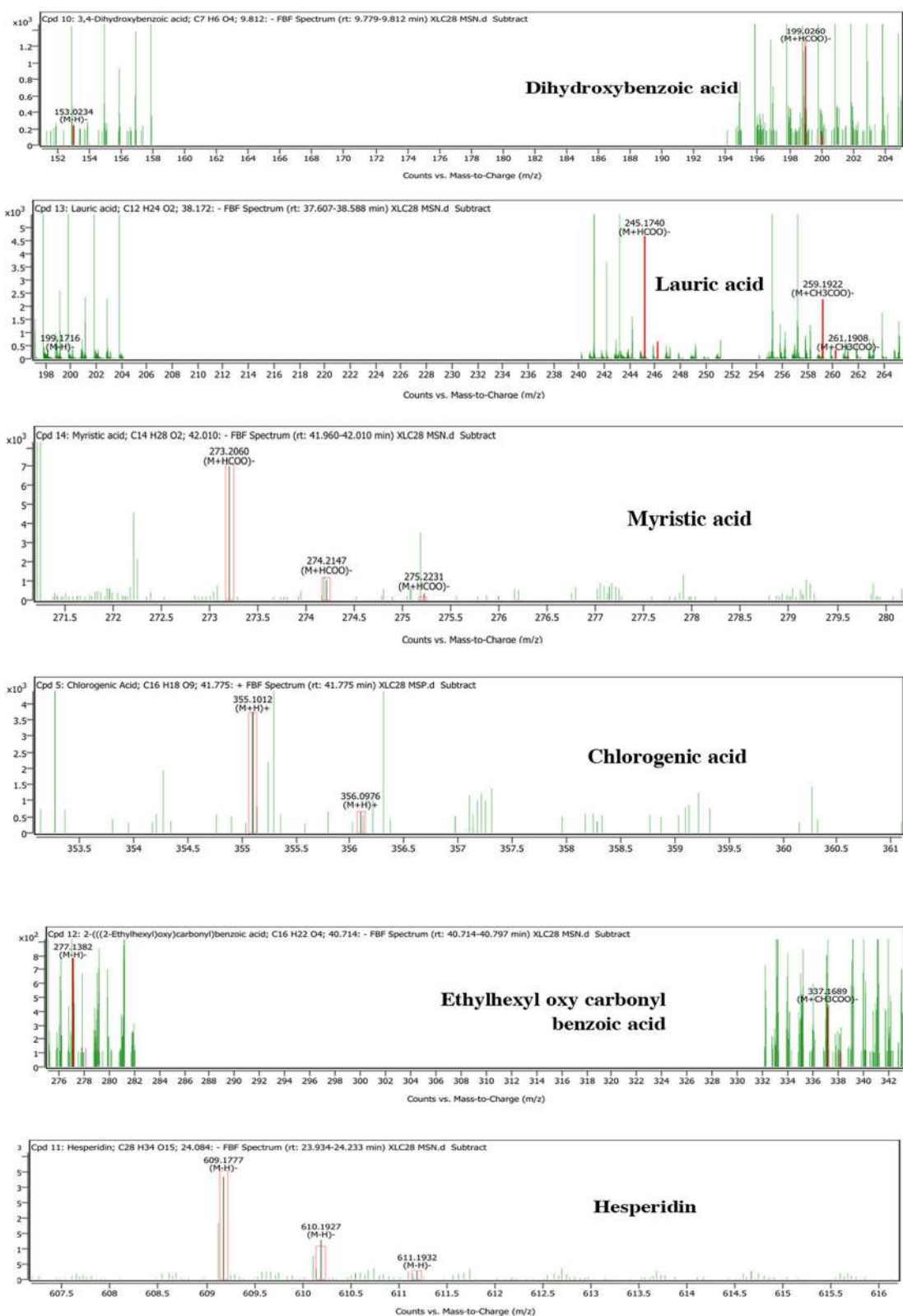
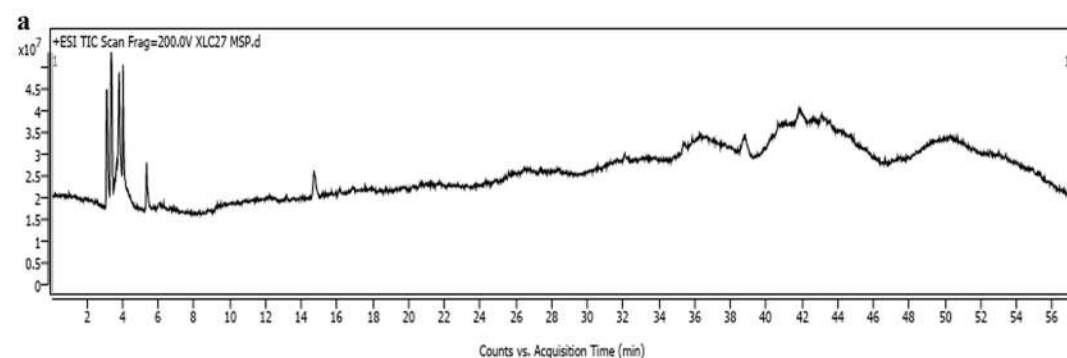
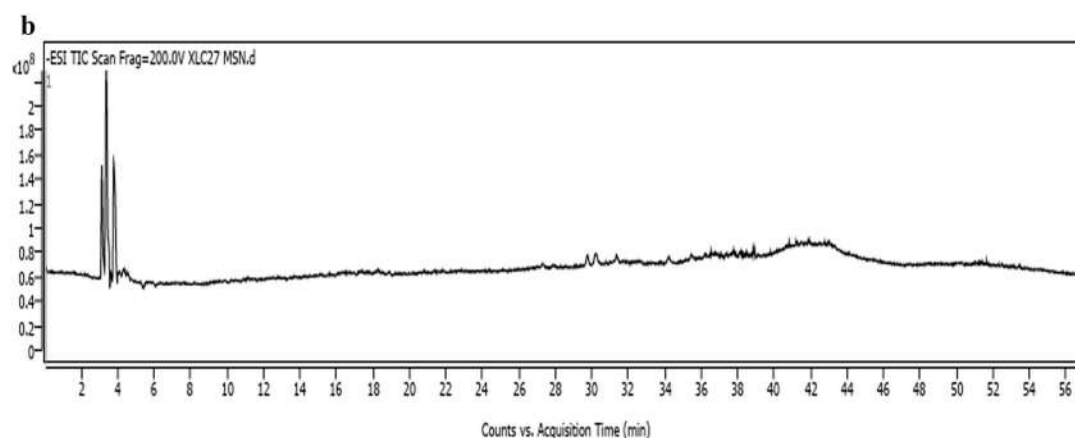


Fig 27(iv) Mass spectra of compounds detected by LC/MS analysis of the methanolic extract of *L.bourdillonii* leaves



Compound Summary

Cpd	Name	Formula	CAS	RT	Mass	Mass (Tgt)	Diff (Tgt, ppm)	Score	Algorithm
1	Luteolin	C15 H10 O6		38.836	286.0478	286.0477	0.34	98.12	FBF
2	Luteolin	C15 H10 O6		34.831	286.0466	286.0477	-3.99	88.97	FBF
3	Caffeic Acid	C9 H8 O4		6.504	180.0403	180.0423	-10.78	61.31	FBF
4	Columbin	C20 H22 O6		42.840	358.1421	358.1416	1.25	59.04	FBF
5	Magnoflorine	C20 H24 N O4		36.410	342.1722	342.1705	4.78	73.28	FBF
6	Chlorogenic Acid	C16 H18 O9		4.195	354.0960	354.0951	2.60	69.22	FBF
7	(10)-Gingerol	C21 H34 O4		4.045	350.2426	350.2457	-8.79	63.82	FBF
8	Shogaol	C17 H24 O3		42.491	276.1726	276.1725	0.24	65.53	FBF
9	(10)-Shogaol	C21 H32 O3		41.809	332.2272	332.2351	-23.77	65.39	FBF
10	Curcumin	C21 H20 O6		42.009	368.1255	368.1260	-1.35	56.29	FBF
11	Piperlongumine	C17 H19 N O5		3.813	317.1257	317.1263	-2.05	62.33	FBF
12	Piperonaline	C21 H27 N O3		40.082	341.1970	341.1991	-6.20	58.82	FBF
13	Hetsine	C20 H27 N O3		21.241	329.1952	329.1991	-11.68	55.69	FBF
14	Diosgenin	C27 H42 O3		35.911	414.3149	414.3134	3.57	55.13	FBF
15	Aucubin	C15 H22 O9		42.474	346.1292	346.1264	8.11	56.65	FBF
16	Pentosalen	C16 H14 O4		48.787	270.0862	270.0892	-11.31	83.93	FBF
17	p-CYMEENE	C10 H14		24.614	134.1091	134.1096	-3.51	74.21	FBF
18	Apigenin	C15 H10 O5		42.125	270.0523	270.0528	-1.90	84.10	FBF



Compound Summary

Cpd	Name	Formula	CAS	RT	Mass	Mass (Tgt)	Diff (Tgt, ppm)	Score	Algorithm
1	Syringic acid	C9 H10 O5		16.907	198.0510	198.0528	-9.37	67.85	FBF
2	Arbutin	C12 H16 O7		24.251	272.0916	272.0896	7.17	71.72	FBF
3	Hexamethylquercetagenin	C21 H22 O8		30.514	402.1303	402.1315	-2.95	67.50	FBF
4	5,7-Dihydroxy-2-(4-hydroxyphenyl)chroman-4-one	C15 H12 O5		24.001	272.0688	272.0685	1.05	61.94	FBF
5	Gingerol	C17 H26 O4		35.399	294.1837	294.1831	1.84	70.45	FBF
6	gamma-Terpinene	C10 H16		41.064	136.1261	136.1252	6.95	78.70	FBF
7	Lauric acid	C12 H24 O2		37.260	200.1757	200.1776	-9.84	80.94	FBF
8	Myristic acid	C14 H28 O2		37.077	228.2077	228.2089	-5.56	66.74	FBF

Fig 28 LC/MS chromatogram of *L. coriacea* leaf methanolic extract. a. ESI+ spectrum and compounds; b. ESI- spectrum and compounds

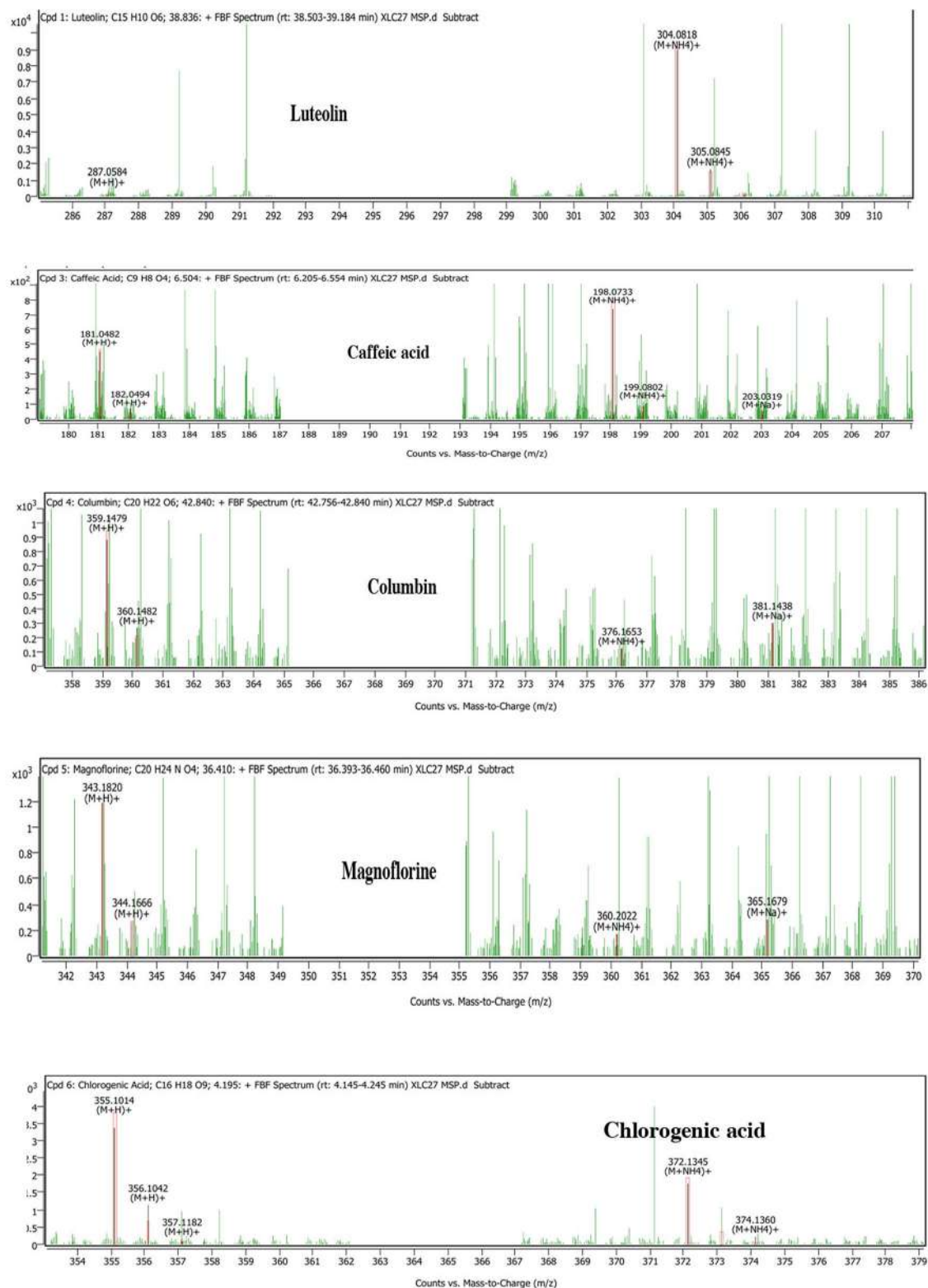


Fig 29(i) Mass spectra of compounds detected by LC/MS analysis of the methanolic extract of *L.coriacea* leaves

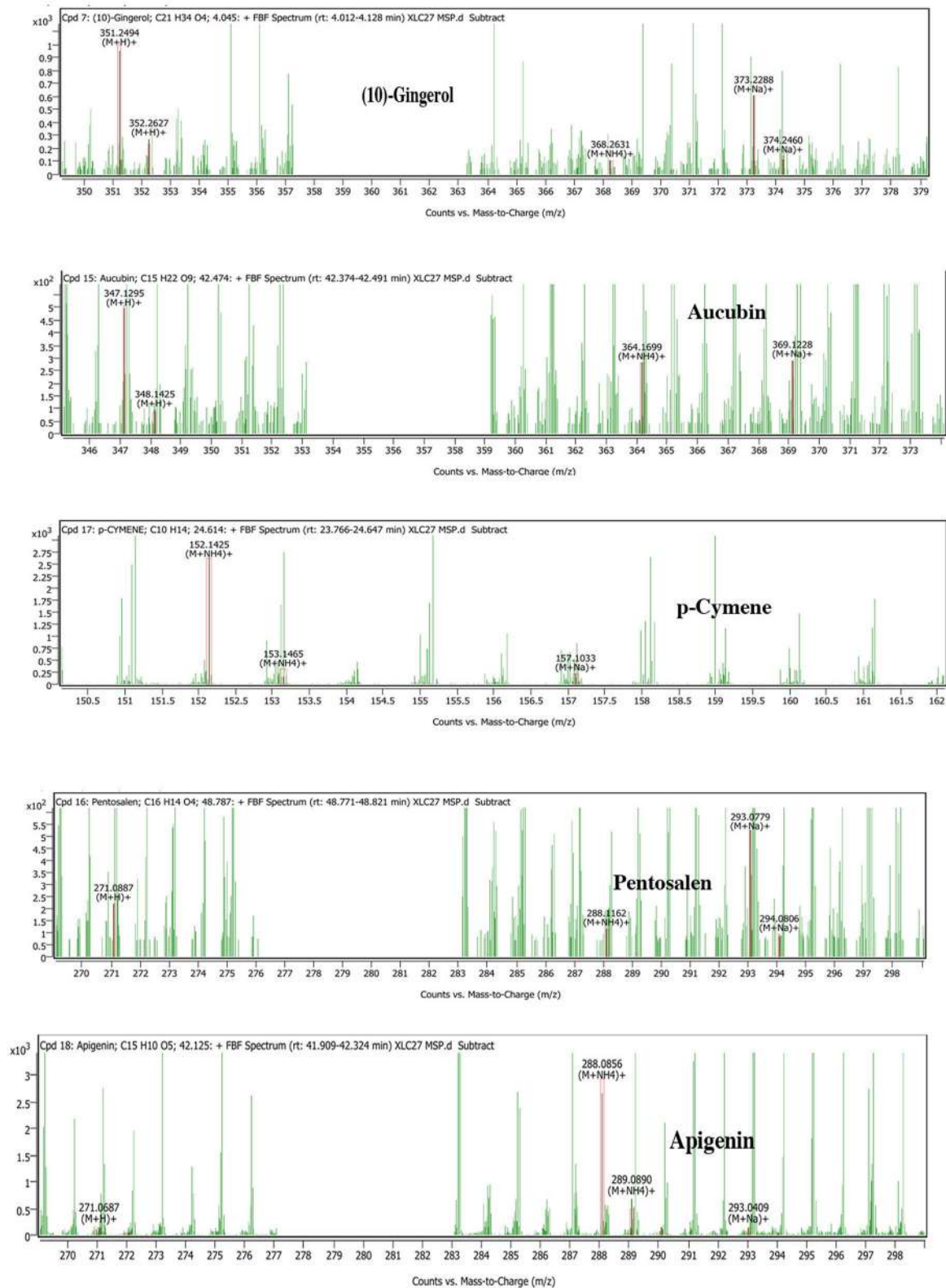


Fig 29(ii) Mass spectra of compounds detected by LC/MS analysis of the methanolic extract of *L.coriacea* leaves

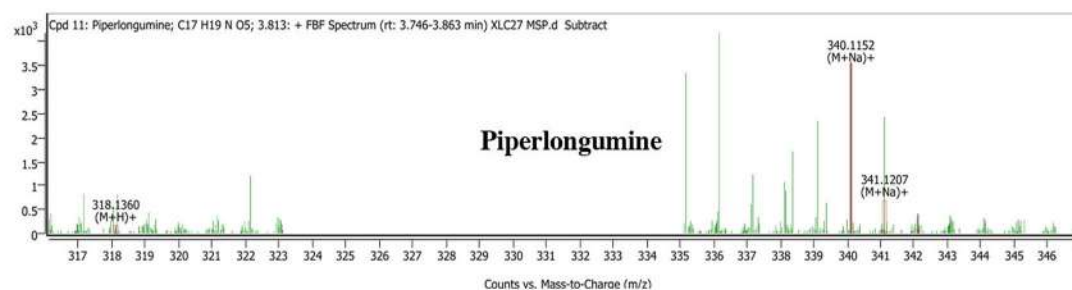
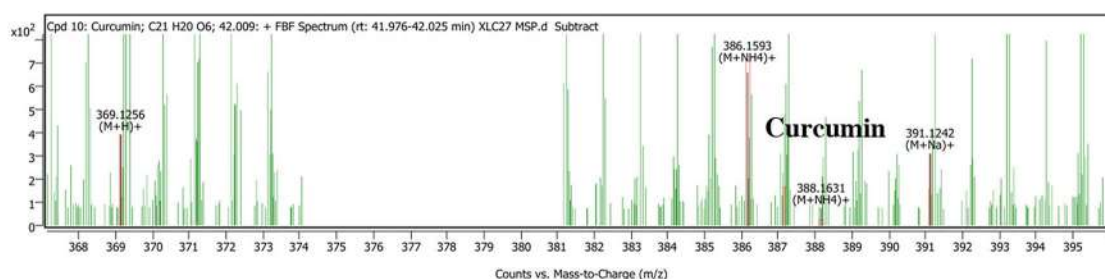
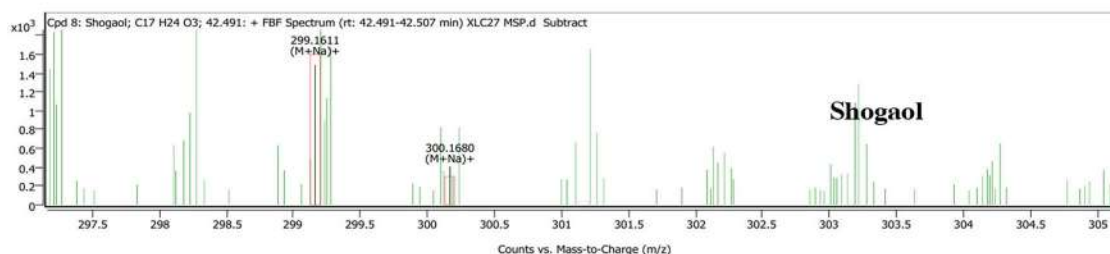
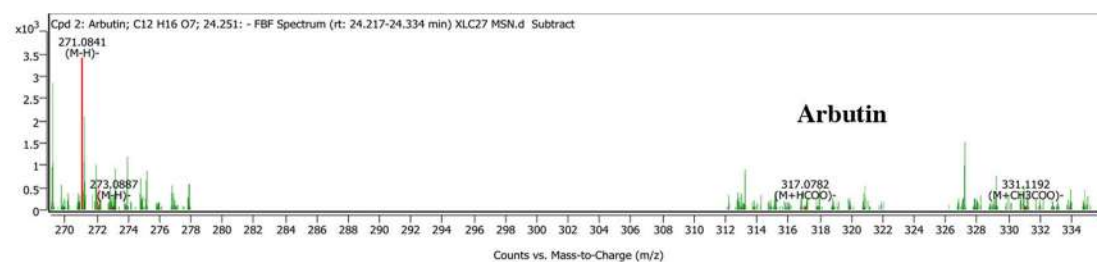
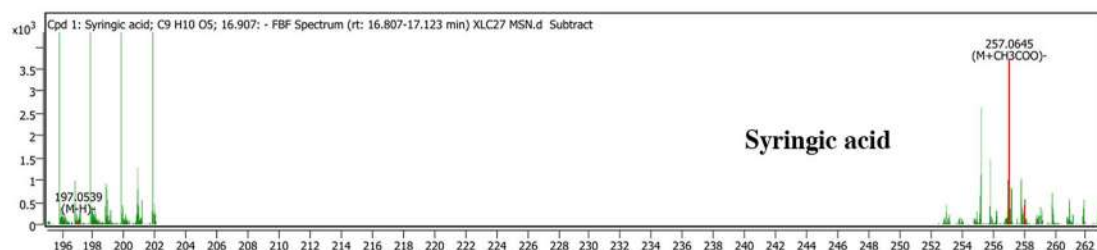


Fig 29(iii) Mass spectra of compounds detected by LC/MS analysis of the methanolic extract of *L.coriacea* leaves

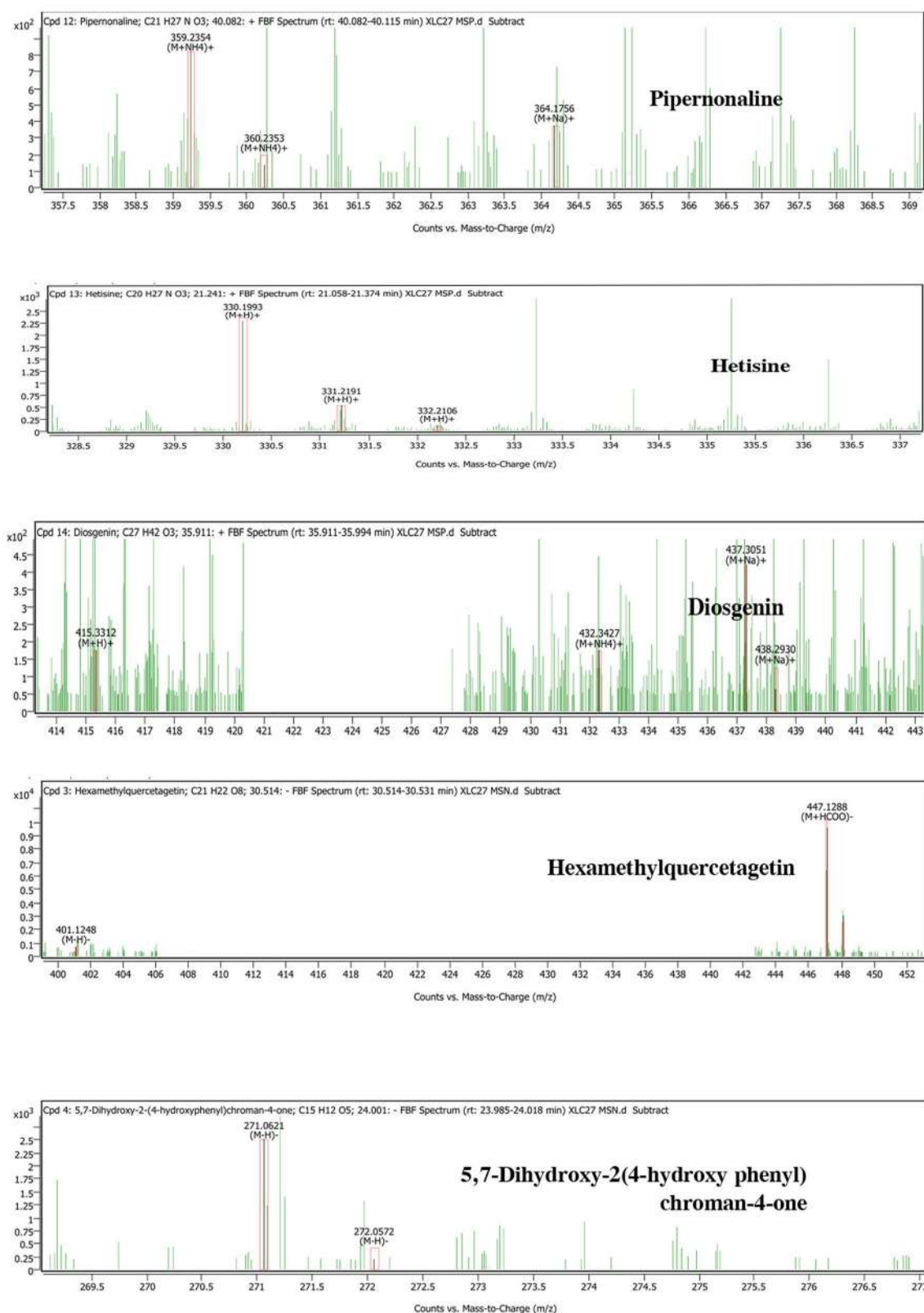


Fig 29(iv) Mass spectra of compounds detected by LC/MS analysis of the methanolic extract of *L. coriacea* leaves

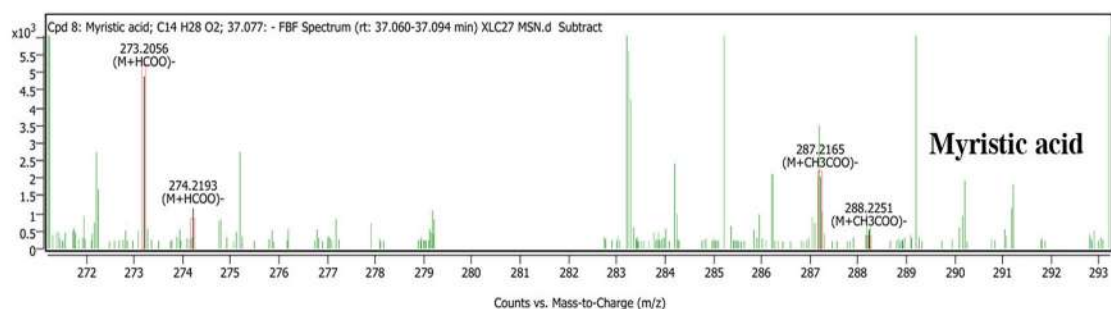
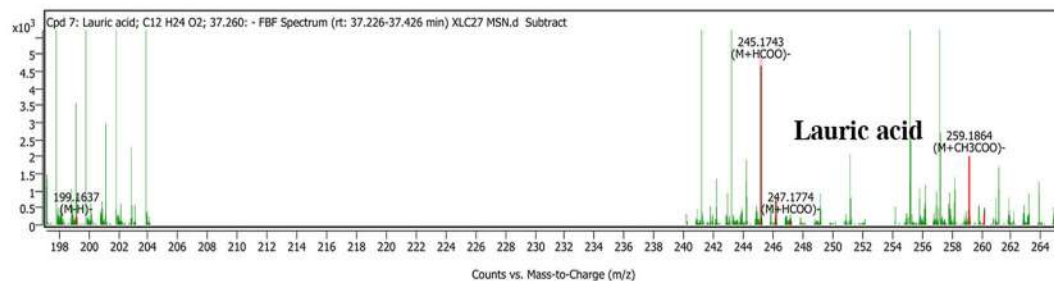
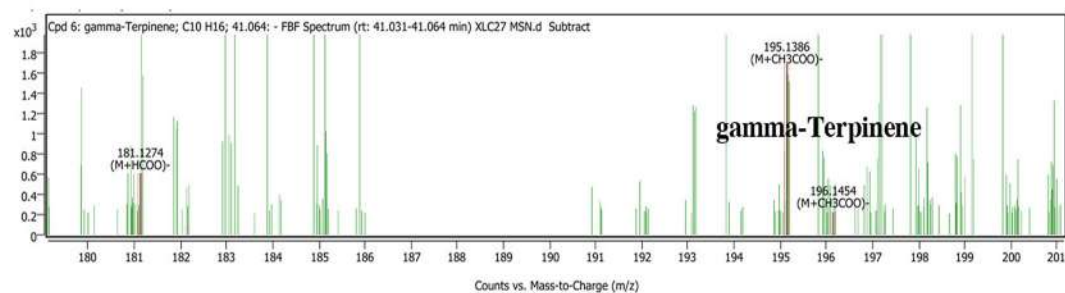
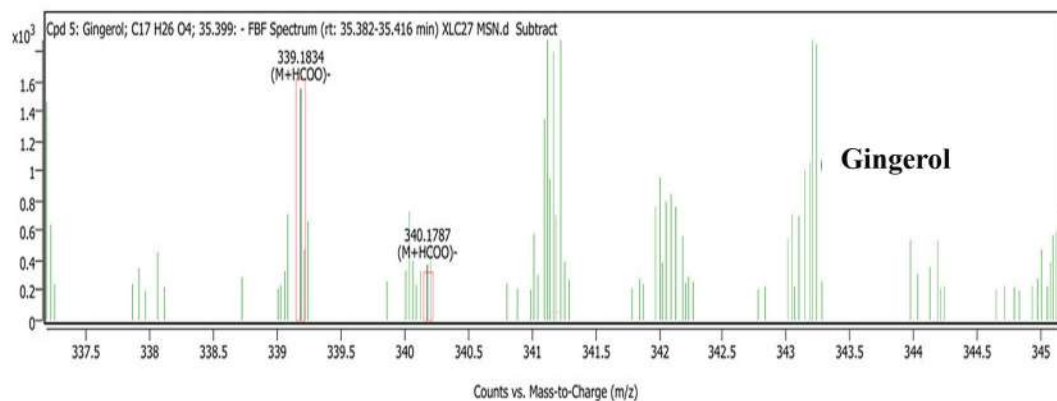
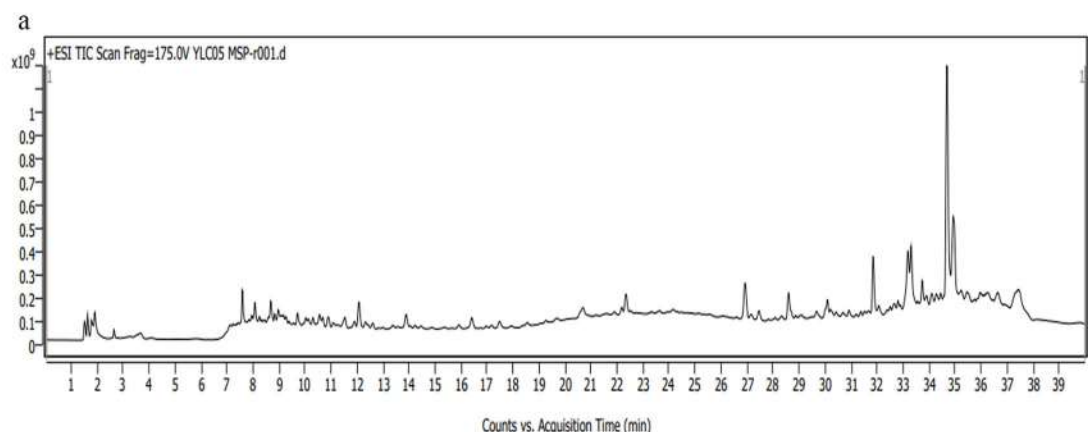
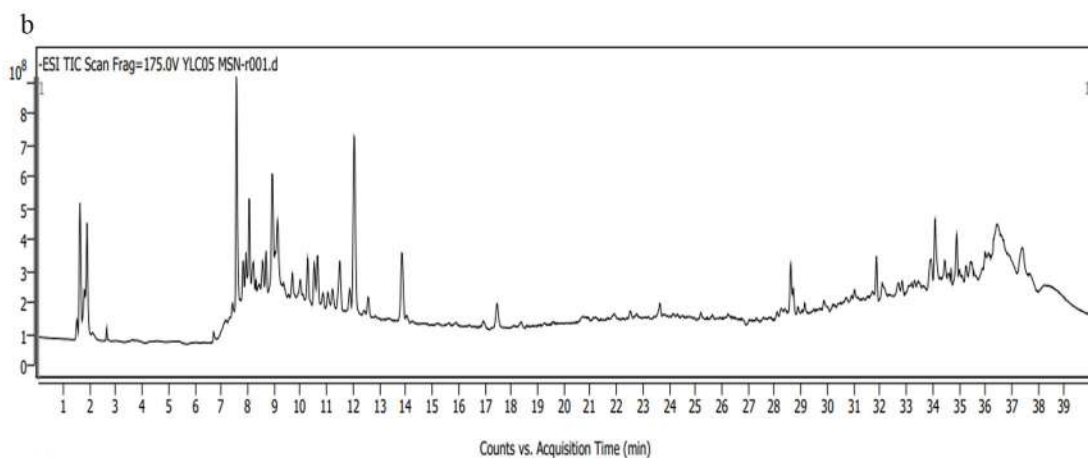


Fig 29(v) Mass spectra of compounds detected by LC/MS analysis of the methanolic extract of *L.coriaacea* leaves



Compound Summary

Cpd	Name	Formula	CAS	RT	Mass	Mass (Tgt)	Diff (Tgt, ppm)	Score	Algorithm
1	Chalcone	C15 H12 O		31.361	208.0875	208.0888	-6.35	90.02	FBF
4	Coryuberine	C19 H21 N O4		8.881	327.1467	327.1471	-0.96	98.85	FBF
5	Dicentrinone	C19 H13 N O5		19.215	335.0792	335.0794	-0.48	95.90	FBF
6	Xanthoplanine	C21 H26 N O4		33.936	356.1860	356.1862	-0.51	80.05	FBF
7	Lauroilsine	C18 H19 N O4		9.479	313.1320	313.1314	1.99	98.55	FBF
8	Isocorydine	C20 H23 N O4		11.307	341.1621	341.1627	-1.73	97.63	FBF
10	Reticuline	C19 H23 N O4		9.263	329.1620	329.1627	-2.18	94.55	FBF
11	Linalool	C10 H18 O		1.936	154.1364	154.1358	4.14	77.35	FBF
12	Dehydrolisoeugenol	C20 H22 O4		12.437	326.1517	326.1518	-0.19	95.82	FBF
13	Grandisin	C24 H32 O7		26.044	432.2124	432.2148	-5.49	84.99	FBF
14	(+)-Eudesmin	C22 H26 O6		21.907	386.1731	386.1729	0.43	83.10	FBF



Cpd	Name	Formula	RT	Mass	Mass (Tgt)	Diff (Tgt, ppm)	Score	Algorithm
1	Oleic acid	C18 H34 O2	34.951	282.2579	282.2559	7.08	76.07	FBF
2	Arachidic acid	C20 H40 O2	34.835	312.3031	312.3028	0.92	93.53	FBF
3	2,4-Di-tert-butylphenol	C14 H22 O	33.838	206.1687	206.1671	7.94	87.67	FBF
4	Palmitic acid	C16 H32 O2	31.180	256.2416	256.2402	5.19	92.19	FBF
5	Lauric acid	C12 H24 O2	34.154	200.1788	200.1776	5.66	75.25	FBF

Fig 30 LC/MS chromatogram of *L.wightiana* leaf methanolic extract. a. ESI+ spectrum and compounds; b. ESI- spectrum and compounds

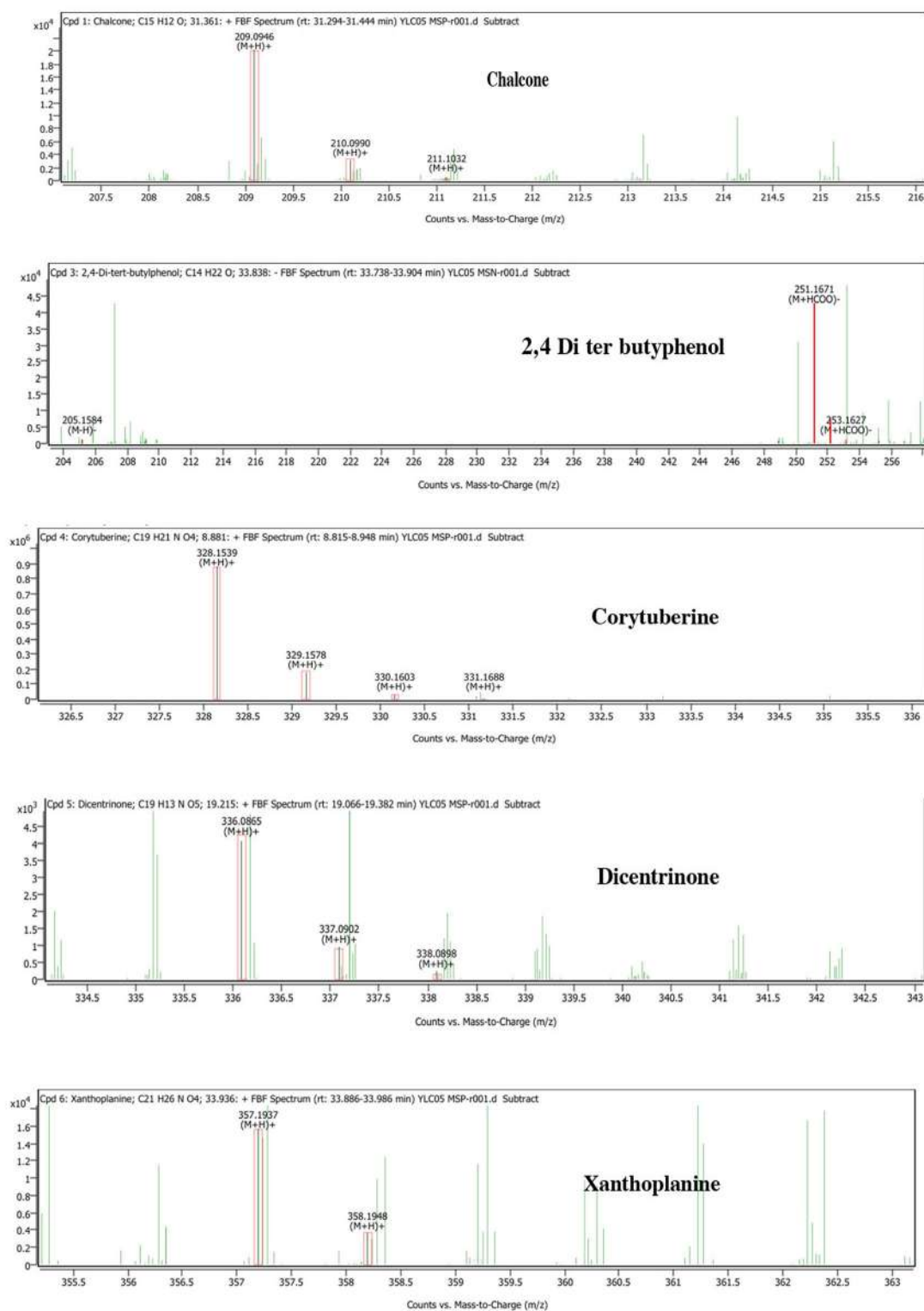


Fig 31(i) Mass spectra of compounds detected by LC/MS analysis of the methanolic extract of *L.wightiana* leaves

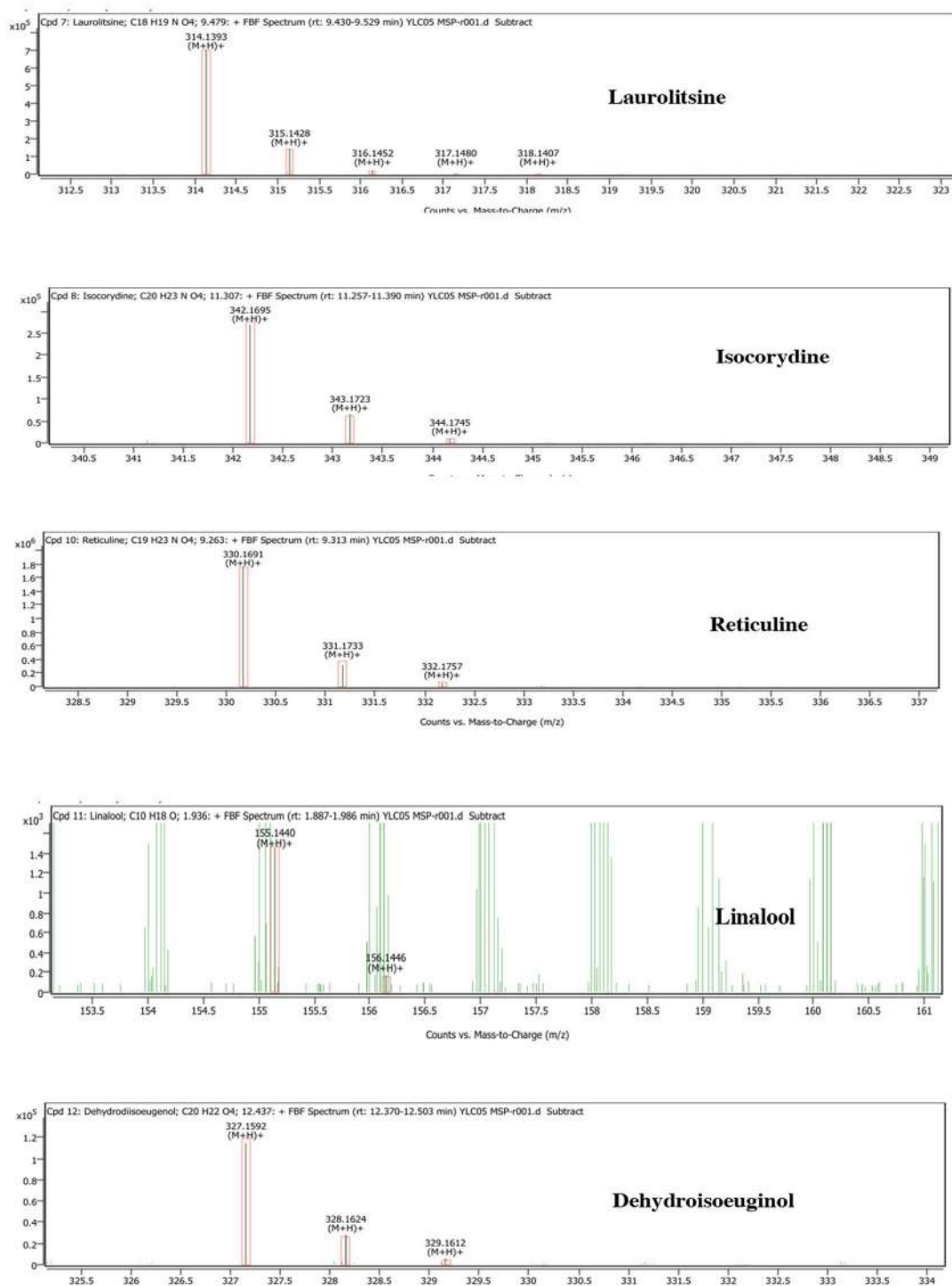


Fig 31(ii) Mass spectra of compounds detected by LC/MS analysis of the methanolic extract of *L.wightiana* leaves

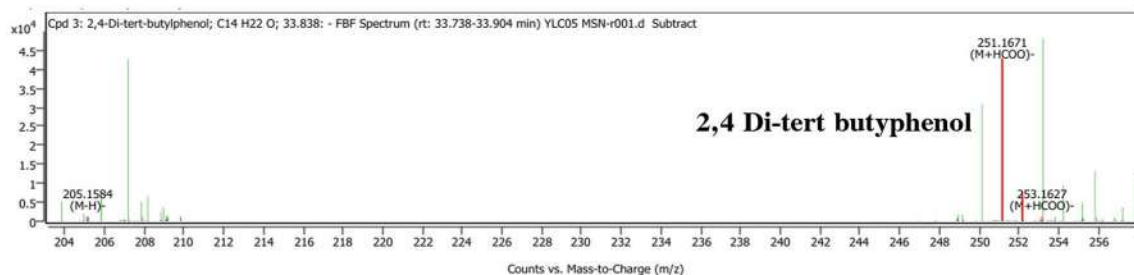
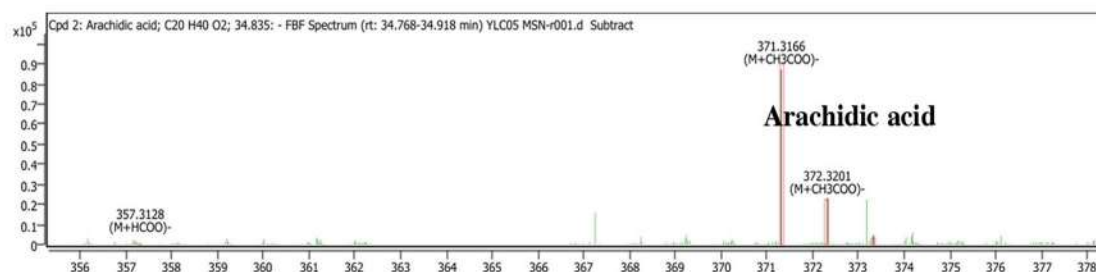
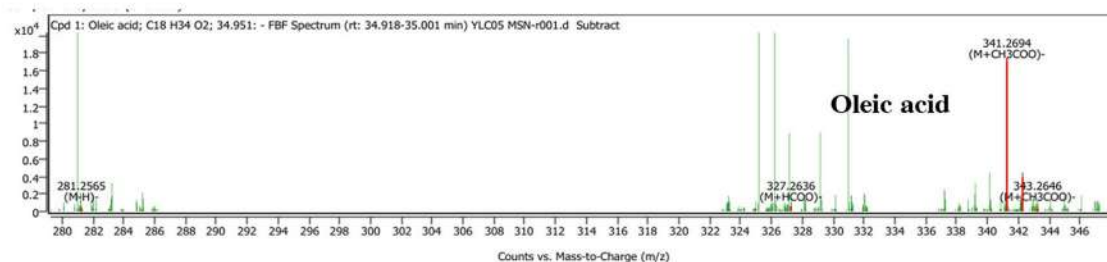
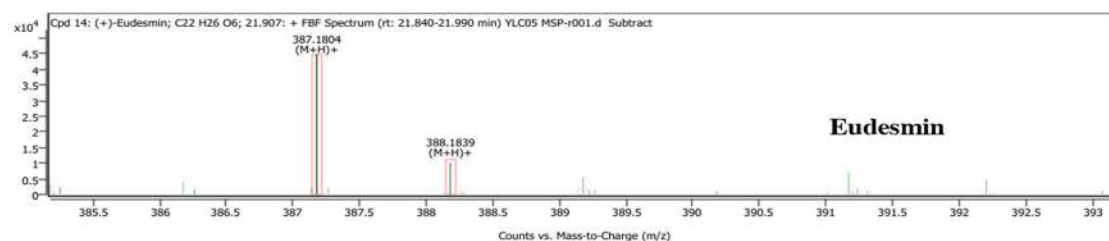
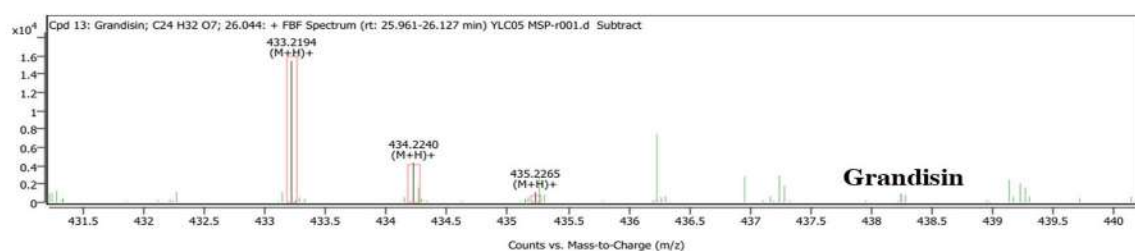


Fig 31(iii) Mass spectra of compounds detected by LC/MS analysis of the methanolic extract of *L.wightiana* leaves

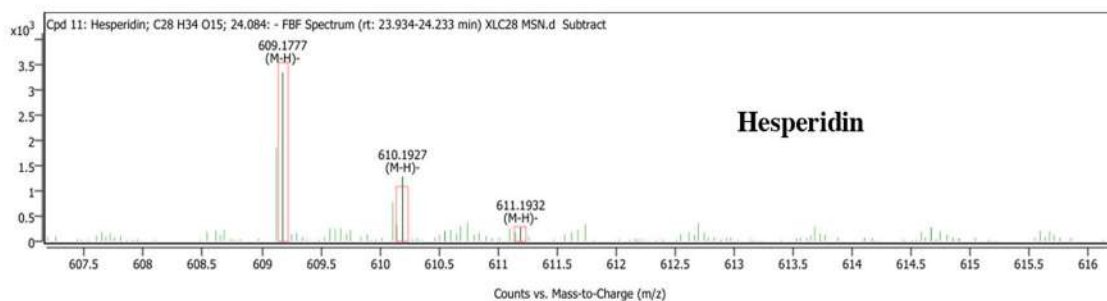
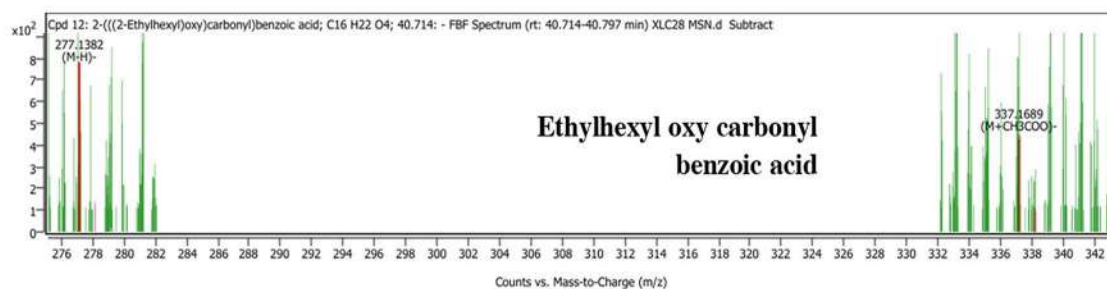
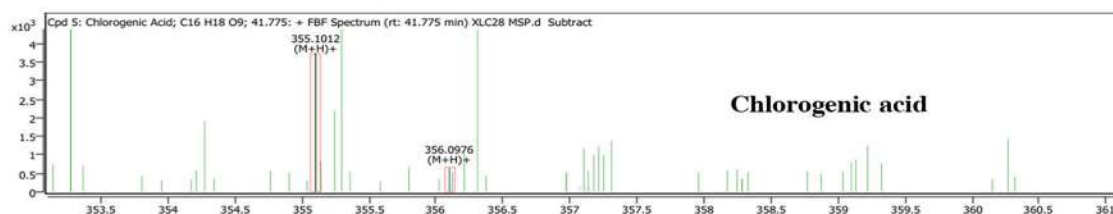
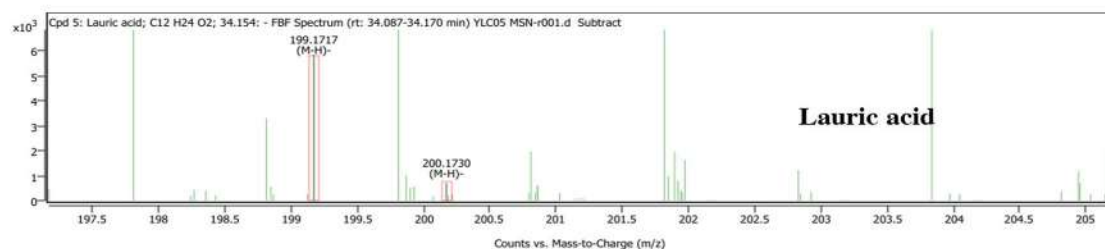
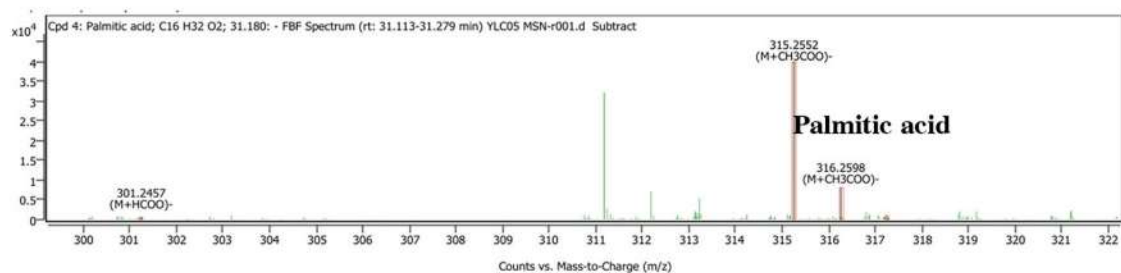


Fig 31(iv) Mass spectra of compounds detected by LC/MS analysis of the methanolic extract of *L.wightiana* leaves

(1 α ,4 $\alpha\alpha$,8 $\alpha\alpha$)(5.99%), spathulenol (2.71%), globulol (5.52%), cubenol (2.70%), tau-cadenol (3.94%), α -Cadinol (2.81%), hexadecanoic acid, methylester (10.49%), n-hexadecanoic acid (100%), 9,12-octadecadienoic acid (Z,Z)-methylester (5.68%), 11-octadecanoic acid, methylester (13.22%), oleic acid (65.16%), decanedioic acid, bis(2-ethylhexyl)ester (5.50%). n-hexadecanoic acid and oleic acid are the compounds found in higher amounts. The GC/MS analysis of *L. bourdillonii* and *L. coriacea* yielded no compounds.

Table 23 GC/MS analysis data of methanolic extract of leaves of *L. wightiana*

Peak	Name of compound	Molecular formula	Retention time (min)	Peak area (%)
1	Copaene	C ₁₅ H ₂₄	31.841	5.68
2	I-H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene	C ₁₅ H ₂₄	33.890	2.04
3	Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-	C ₁₅ H ₂₄	34.991	4.45
4	Naphthalene,1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-(1 α ,4 $\alpha\alpha$,8 $\alpha\alpha$)-	C ₁₅ H ₂₄	35.753	5.99
5	Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-1-(methylethyl)1-(1 α ,4 $\alpha\alpha$,8 $\alpha\alpha$)	C ₁₅ H ₂₄	36.238	3.90
6	Naphthalene,1,2,2,4-tetrahydro-1,6-dimethyl-1-4(1-methylethyl)-(1S-cis)-	C ₁₅ H ₂₄	36.544	7.46
7	Spathulenol	C ₁₅ H ₂₄ O	38.716	2.71
8	9-Isopropyl-1-methyl-2-methylene-5-oxatricyclo[5.4.0.0(3,8)] undecane	C ₁₅ H ₂₄ O	38.901	2.84
9	Globulol	C ₁₅ H ₂₆ O	39.101	5.52
10	2-Naphthalenemethanol, decahydro- α - α ,4a-trimethyl-8-methylene- β -eudesmol	C ₁₅ H ₂₆ O	40.075	1.57
11	Cubenol	C ₁₅ H ₂₆ O	40.900	2.70
12	Carotol	C ₁₅ H ₂₆ O	41.045	2.29
13	tau-Cadinol	C ₁₅ H ₂₆ O	41.879	3.94
14	α -Cadinol	C ₁₅ H ₂₆ O	41.986	2.81
15	Naphthalene,1,6-dimethyl-4-(1-methylethyl)-(Cadalene)	C ₁₅ H ₁₈	43.637	5.57

16	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	53.695	10.49
17	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	55.043	100
18	9,12-Octadecadienoic acid (Z, Z)-methylester	C ₁₉ H ₃₄ O ₂	57.633	5.68
19	11-Octadecanoic acid, methyl ester	C ₁₉ H ₃₆ O ₂	57.790	13.22
20	Octadecanoic acid, methyl ester	C ₁₉ H ₃₈ O ₂	58.371	3.70
21	Oleic acid	C ₁₈ H ₃₄ O ₂	58.837	65.16
22	Decanedioic acid,bis(2-ethylhexyl) ester	C ₂₆ H ₅₀ O ₄	65.559	5.50

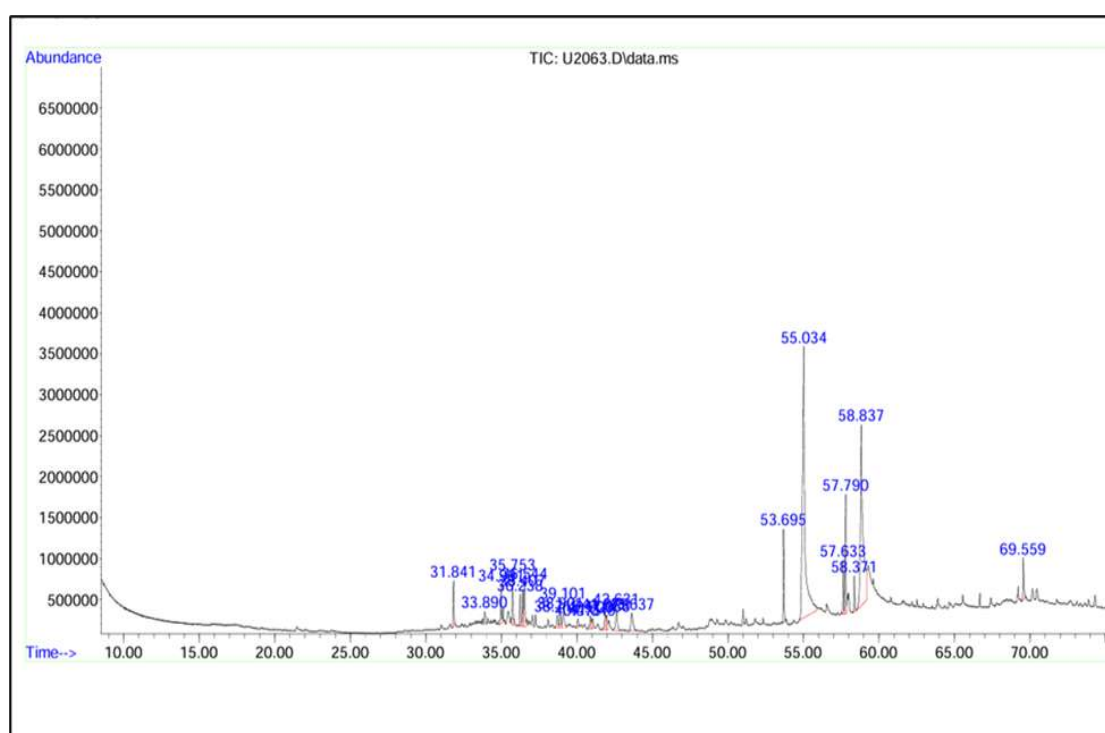
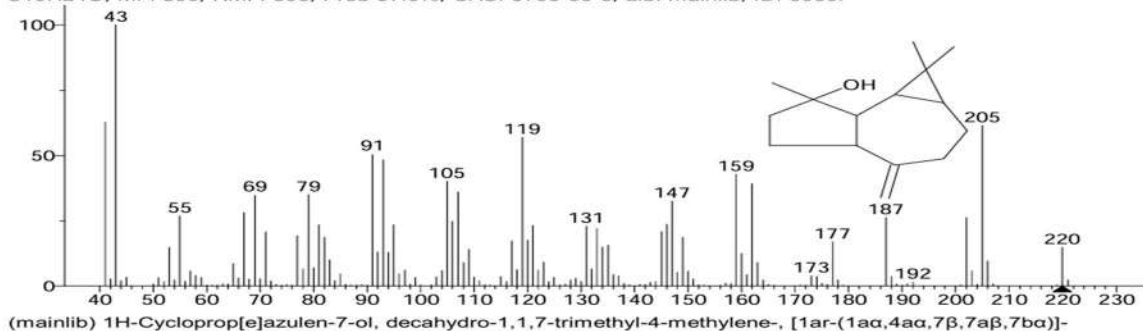


Fig 32 GC/MS chromatogram of *L. wightiana* leaves methanolic extract

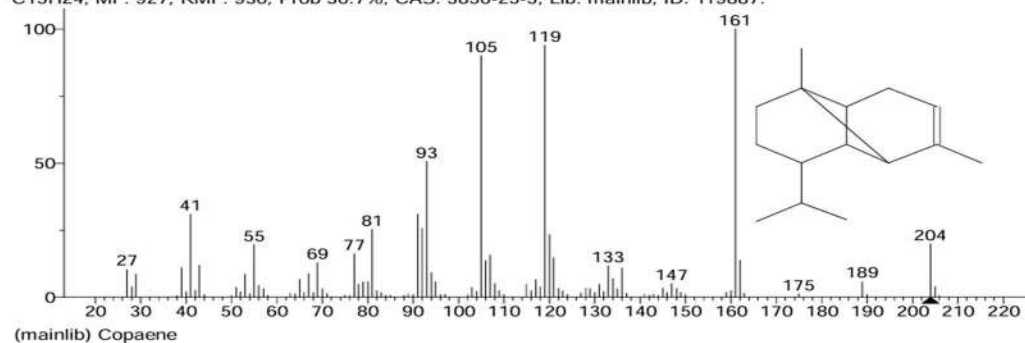
f. Analysis of the essential oil

The essential oil from *L. coriacea* leaves was extracted by the hydro distillation method using the Clevenger apparatus and GC/MS-GC-FID analysis was carried out and the oil components were screened. The descriptions of the compounds are given in Table 24 and chromatogram and mass spectra of compounds depicted in Figures 34 & 35. Various components of essential oil include, α -terpineol (75.81%), linalool (41.06%), bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-(1S)-(6.26%), endo borneol (4.98%), cyclohexanol,5-methyl-2-(1-

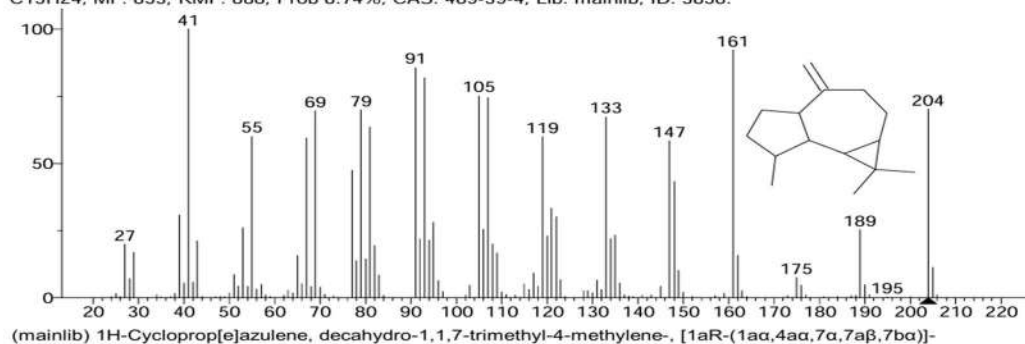
C₁₅H₂₄O; MF: 853; RMF: 858; Prob 37.9%; CAS: 6750-60-3; Lib: mainlib; ID: 6035.



C₁₅H₂₄; MF: 927; RMF: 930; Prob 30.7%; CAS: 3856-25-5; Lib: mainlib; ID: 119887.



C₁₅H₂₄; MF: 853; RMF: 888; Prob 8.74%; CAS: 489-39-4; Lib: mainlib; ID: 3858.



C₁₅H₂₄; MF: 910; RMF: 916; Prob 31.9%; CAS: 39029-41-9; Lib: replib; ID: 21213.

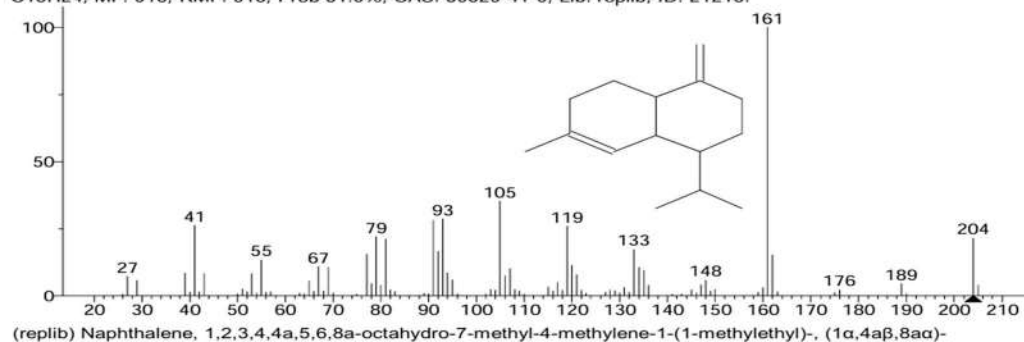
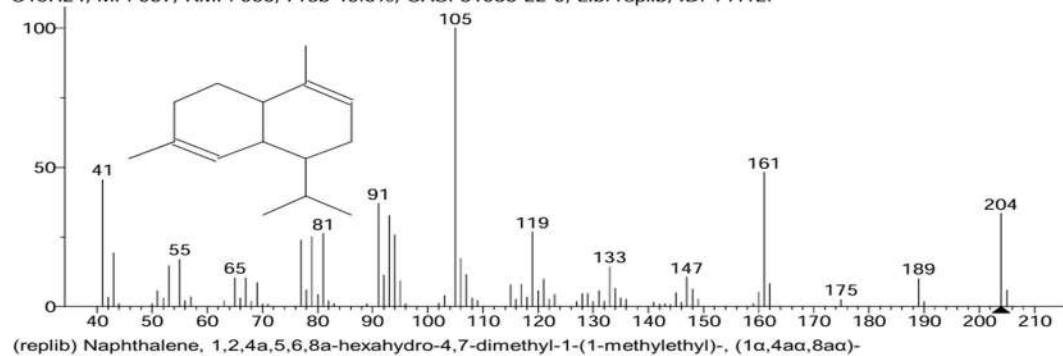
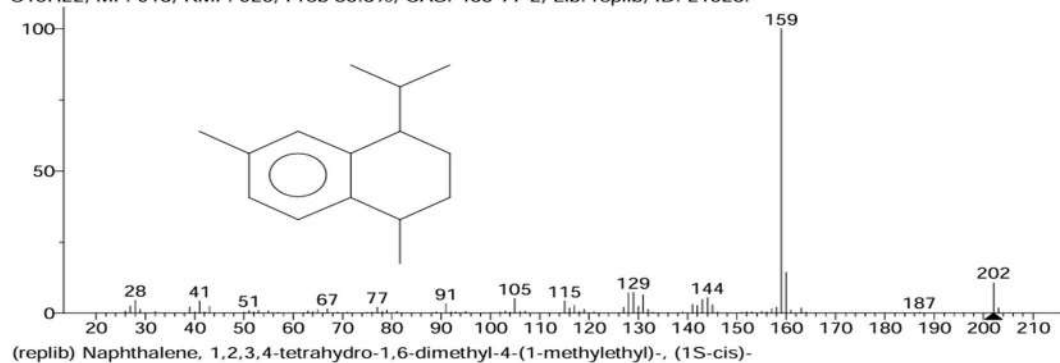


Fig 33(i) Mass spectra of compounds detected by GC/MS analysis of methanolic extract of *L.wightiana* leaves

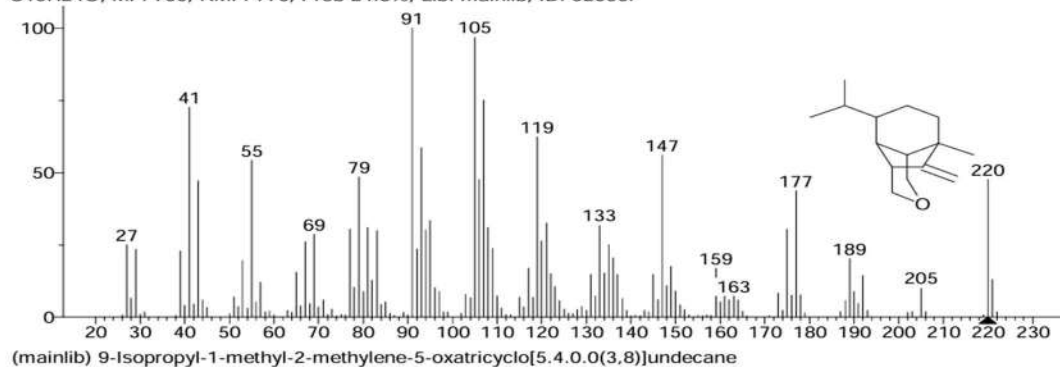
C₁₅H₂₄; MF: 937; RMF: 950; Prob 40.5%; CAS: 31983-22-9; Lib: replib; ID: 14112.



C₁₅H₂₂; MF: 918; RMF: 920; Prob 85.8%; CAS: 483-77-2; Lib: replib; ID: 21028.



C₁₅H₂₄O; MF: 760; RMF: 776; Prob 24.8%; Lib: mainlib; ID: 52066.



C₁₅H₂₆O; MF: 881; RMF: 892; Prob 29.9%; CAS: 51371-47-2; Lib: mainlib; ID: 5786.

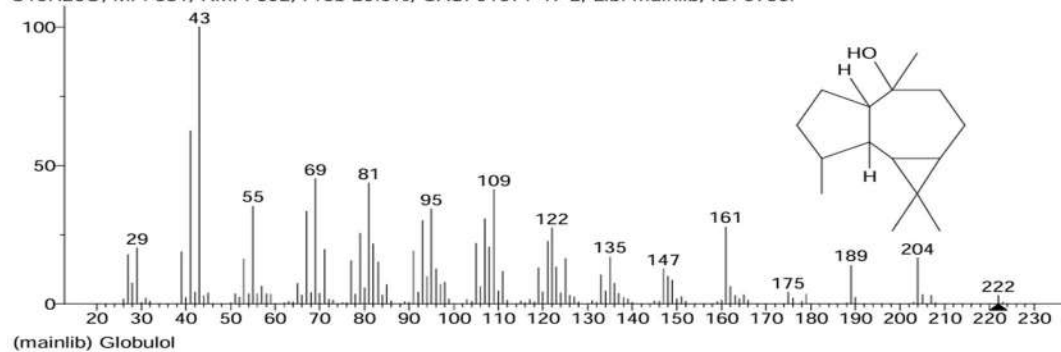
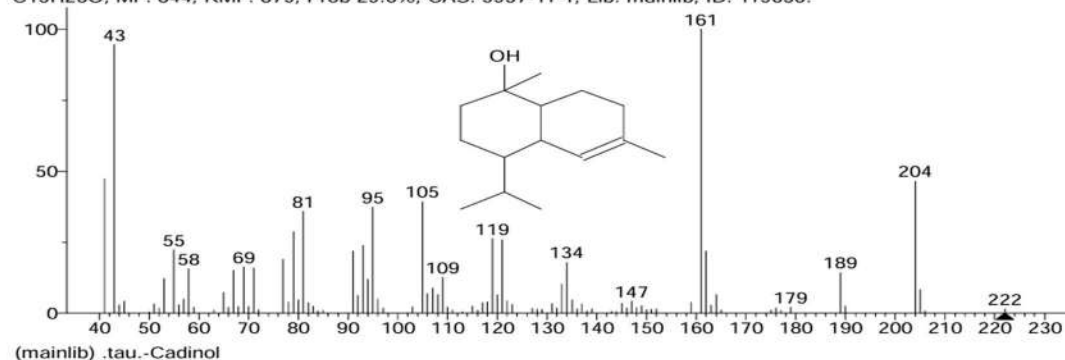
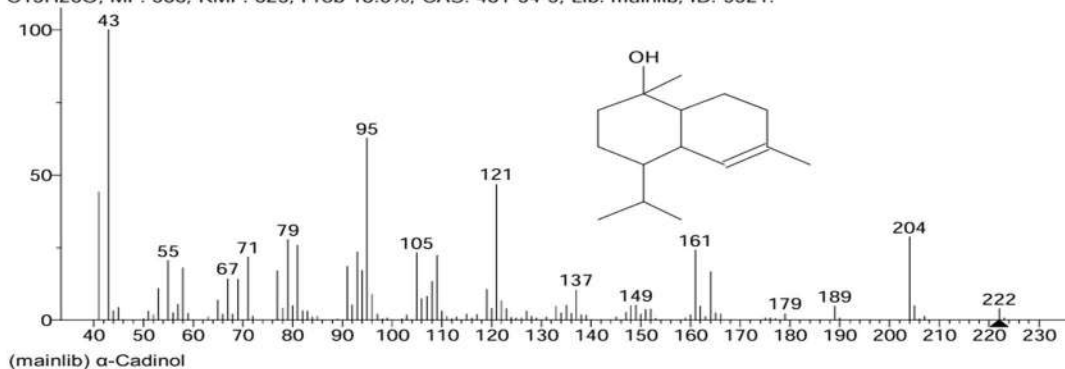


Fig 33(ii) Mass spectra of compounds detected by GC/MS analysis of methanolic extract of *L.wightiana* leaves

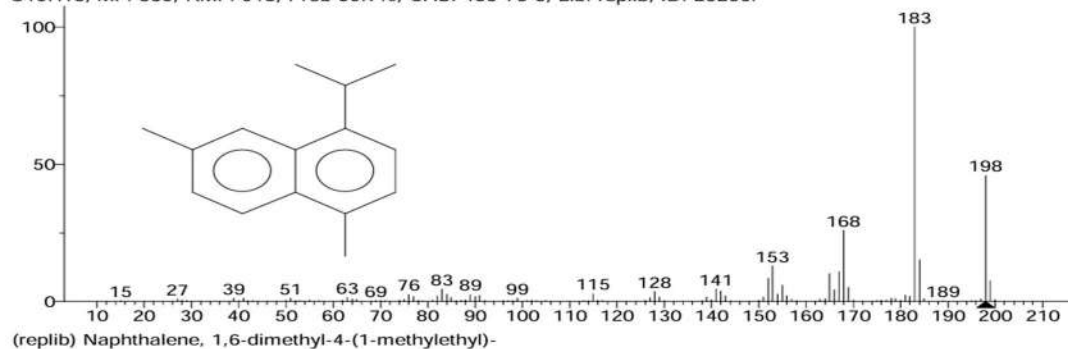
C₁₅H₂₆O; MF: 844; RMF: 879; Prob 29.6%; CAS: 5937-11-1; Lib: mainlib; ID: 119630.



C₁₅H₂₆O; MF: 566; RMF: 623; Prob 18.0%; CAS: 481-34-5; Lib: mainlib; ID: 9521.



C₁₅H₁₈; MF: 889; RMF: 918; Prob 65.7%; CAS: 483-78-3; Lib: replib; ID: 23236.



C₁₇H₃₄O₂; MF: 910; RMF: 942; Prob 73.0%; CAS: 112-39-0; Lib: replib; ID: 9050.

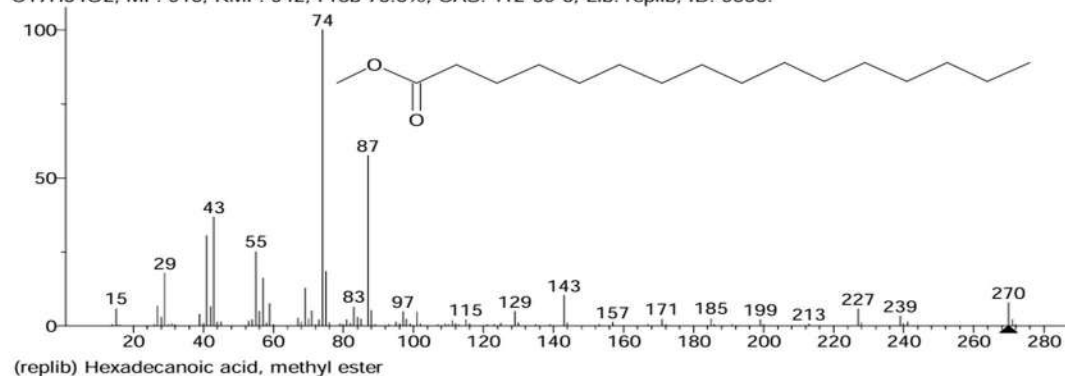
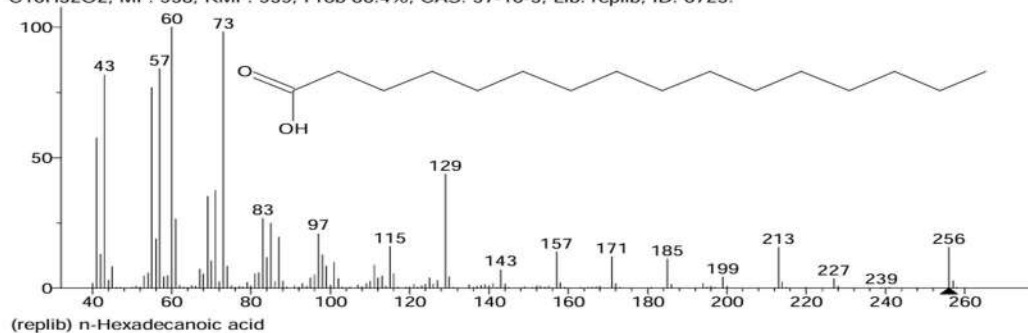
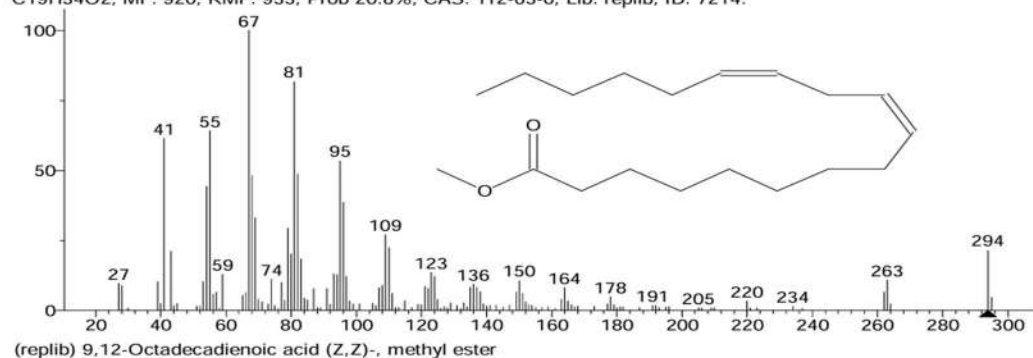


Fig 33(iii) Mass spectra of compounds detected by GC/MS analysis of methanolic extract of *L. wightiana* leaves

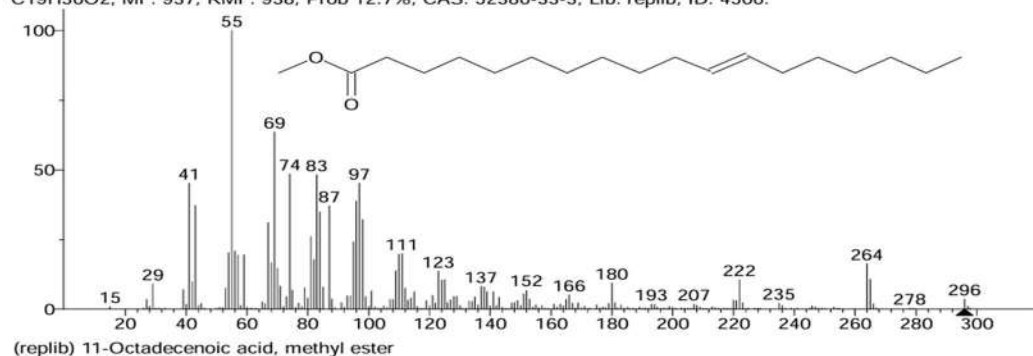
C16H32O2; MF: 938; RMF: 939; Prob 86.4%; CAS: 57-10-3; Lib: replib; ID: 6723.



C19H34O2; MF: 920; RMF: 933; Prob 20.8%; CAS: 112-63-0; Lib: replib; ID: 7214.



C19H36O2; MF: 937; RMF: 938; Prob 12.7%; CAS: 52380-33-3; Lib: replib; ID: 4506.



C19H38O2; MF: 903; RMF: 931; Prob 69.1%; CAS: 112-61-8; Lib: replib; ID: 9086.

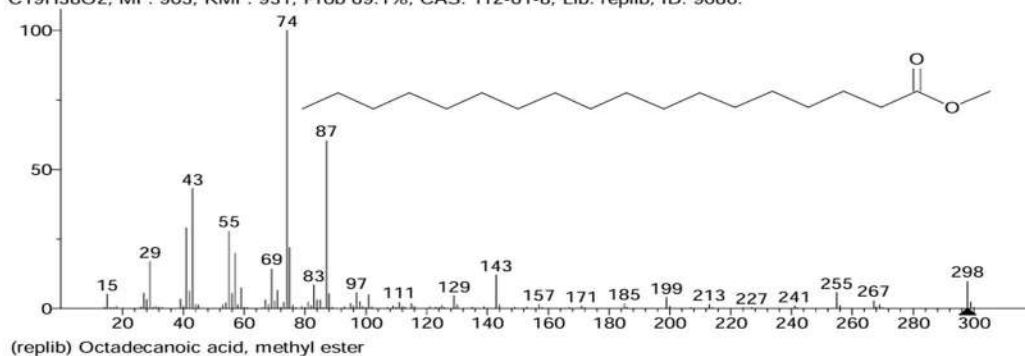


Fig 33(iv) Mass spectra of compounds detected by GC/MS analysis of methanolic extract of *L.wightiana* leaves

methylethyl)-, (1 α ,2 β ,5 β)-(2.58%), eucalyptol (2.12%), linalyl acetate (2.485%), and bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-(1S-endo)-(1.84%).

Table 24 GC/MS with GC-FID analysis data of *L. coriacea* leaves essential oil

Sl.No.	Name of compound	Molecular formula	Retention time (min)	Area %
1	Eucalyptol	C ₁₀ H ₁₈ O	13.819	2.12
2	Linalool	C ₁₀ H ₁₈ O	16.316	41.06
3	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-(1S)-	C ₁₀ H ₁₆ O	17.701	6.26
4	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-(1S-endo)-	C ₁₀ H ₁₈ O	18.364	1.84
5	endo-Borneol	C ₁₀ H ₁₈ O	18.729	4.98
6	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1 α ,2 β ,5 β)-	C ₁₀ H ₂₀ O	19.077	2.58
7	α -Terpineol	C ₁₀ H ₁₈ O	19.782	75.81
8	Cyclohexanol, 2-methyl-5-(1-methylethenyl)-, (1 α ,2 α ,5 β)-	C ₁₀ H ₁₈ O	19.910	41.56
9	Linalyl acetate	C ₁₂ H ₂₀ O ₂	22.408	2.485

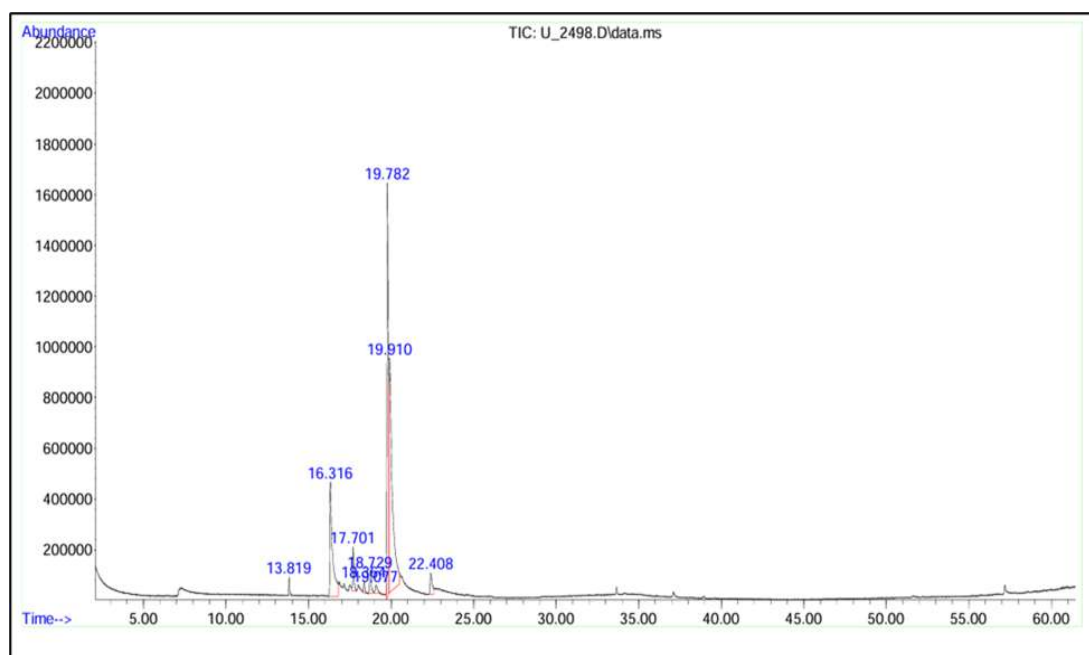


Fig 34 GC/MS chromatogram of *L. coriacea* leaves essential oil

PHASE III- PHARMACOLOGICAL STUDY

Pharmacological evaluation of leaves methanolic extracts and bark extracts of the selected species of *Litsea* were carried out. Bioactivities like antimicrobial, antioxidant, antidiabetic and cytotoxicity studies of three species of *Litsea* namely *L. bourdillonii*, *L. coriacea* and *L. wightiana* were evaluated. Additionally, the anti-inflammatory and wound healing studies of *L. wightiana* and the essential oil analysis of *L. coriacea* were carried out.

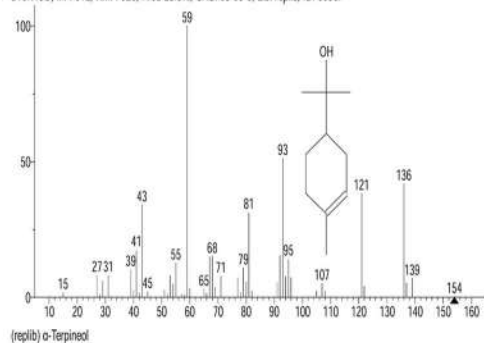
A. ANTIMICROBIAL ACTIVITY

a. Antibacterial study

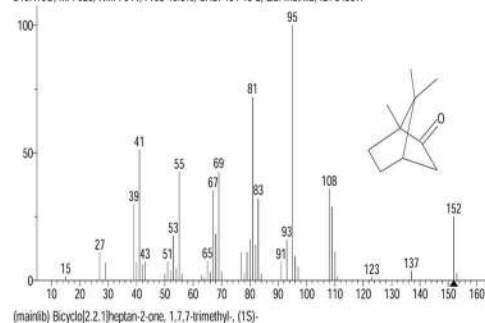
The leaves and bark of the three species of *Litsea* were subjected to antibacterial screening against pathogens such as *S. aureus*, *E. coli*, and *S. aeruginosa* using the agar well diffusion method. The results implicated that the extracts have significant inhibitory activity against gram-negative and less effect towards gram-positive bacteria (Table 25 and Figures 36, 37 & 38). The antibacterial results were observed by measuring the zone of inhibition in mm. The inhibition zone of *E. coli* by the 1000 µg/mL leaf extracts of *L. bourdillonii*, *L. coriacea*, and *L. wightiana* was 14±0.01 mm, 15±0.02 mm, and 14±0.20 mm, respectively, and that of *P. aeruginosa* was 18±0.13 mm, 17±0.43 mm, and 15±0.02 mm each. The bark extracts showed antibacterial activity with inhibition zones such as 13±0.13 mm, 14±0.25 mm, and 16±0.06 mm against *E. coli*, and 17±0.05 mm, 16±0.30 mm, and 17±0.32 mm against *P. aeruginosa*. The bark extract of *L. wightiana* showed maximum inhibition (13±0.80mm) against *S. aureus* at 1000µg/mL when compared to other extracts. The standard antibiotic streptomycin showed antibacterial activity against all selected strains of bacteria with inhibition zones ranging from 27-32mm. The minimum inhibitory concentration (MIC) of the extracts against bacteria was also determined.

The minimum inhibitory concentration of antibacterial activity of leaves and bark methanolic extracts were analyzed and found that the *L. wightiana* leaf extracts showed good activity at minimal concentration against selected strains of bacteria as

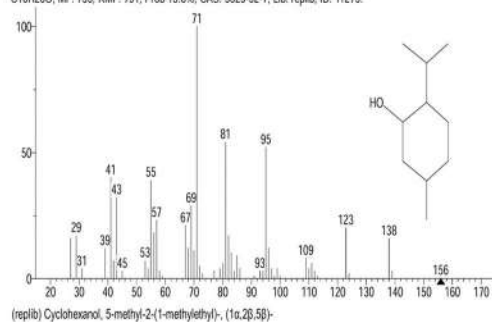
C₁₀H₁₈O; MF: 912; RMF: 929; Prob 28.3%; CAS: 98-55-5; Lib: replib; ID: 9095.



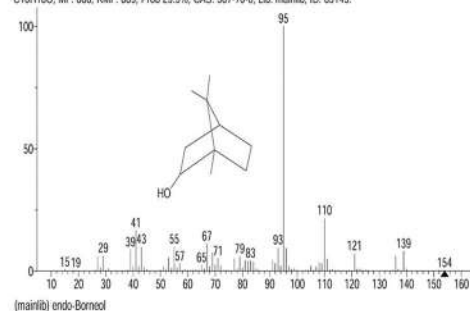
C₁₀H₁₆O; MF: 925; RMF: 944; Prob 46.3%; CAS: 464-48-2; Lib: mainlib; ID: 84807.



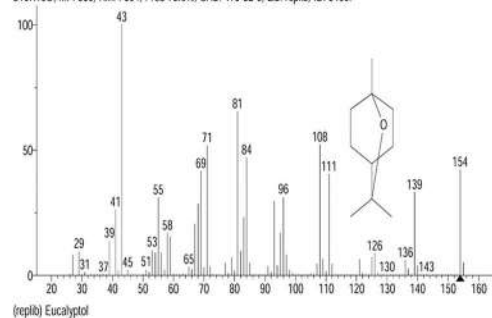
C₁₀H₂₀O; MF: 735; RMF: 791; Prob 13.8%; CAS: 3623-52-7; Lib: replib; ID: 11275.



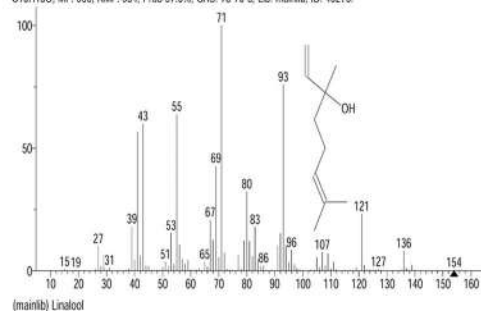
C₁₀H₁₈O; MF: 888; RMF: 889; Prob 29.9%; CAS: 507-70-0; Lib: mainlib; ID: 85143.



C₁₀H₁₈O; MF: 886; RMF: 891; Prob 73.6%; CAS: 470-82-6; Lib: replib; ID: 3400.



C₁₀H₁₈O; MF: 933; RMF: 934; Prob 67.3%; CAS: 78-70-6; Lib: mainlib; ID: 45273.



C₁₂H₂₀O₂; MF: 897; RMF: 899; Prob 39.7%; CAS: 115-95-7; Lib: mainlib; ID: 82075.

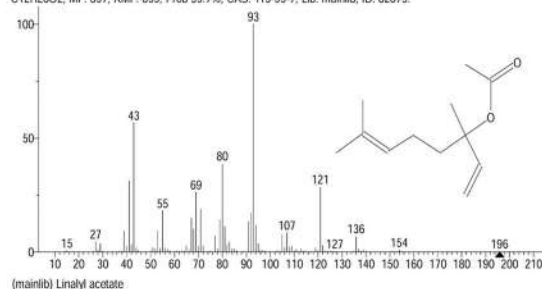


Fig 35 Mass spectra of compounds detected by GC/MS analysis of essential oil of *L.coriacea* leaves

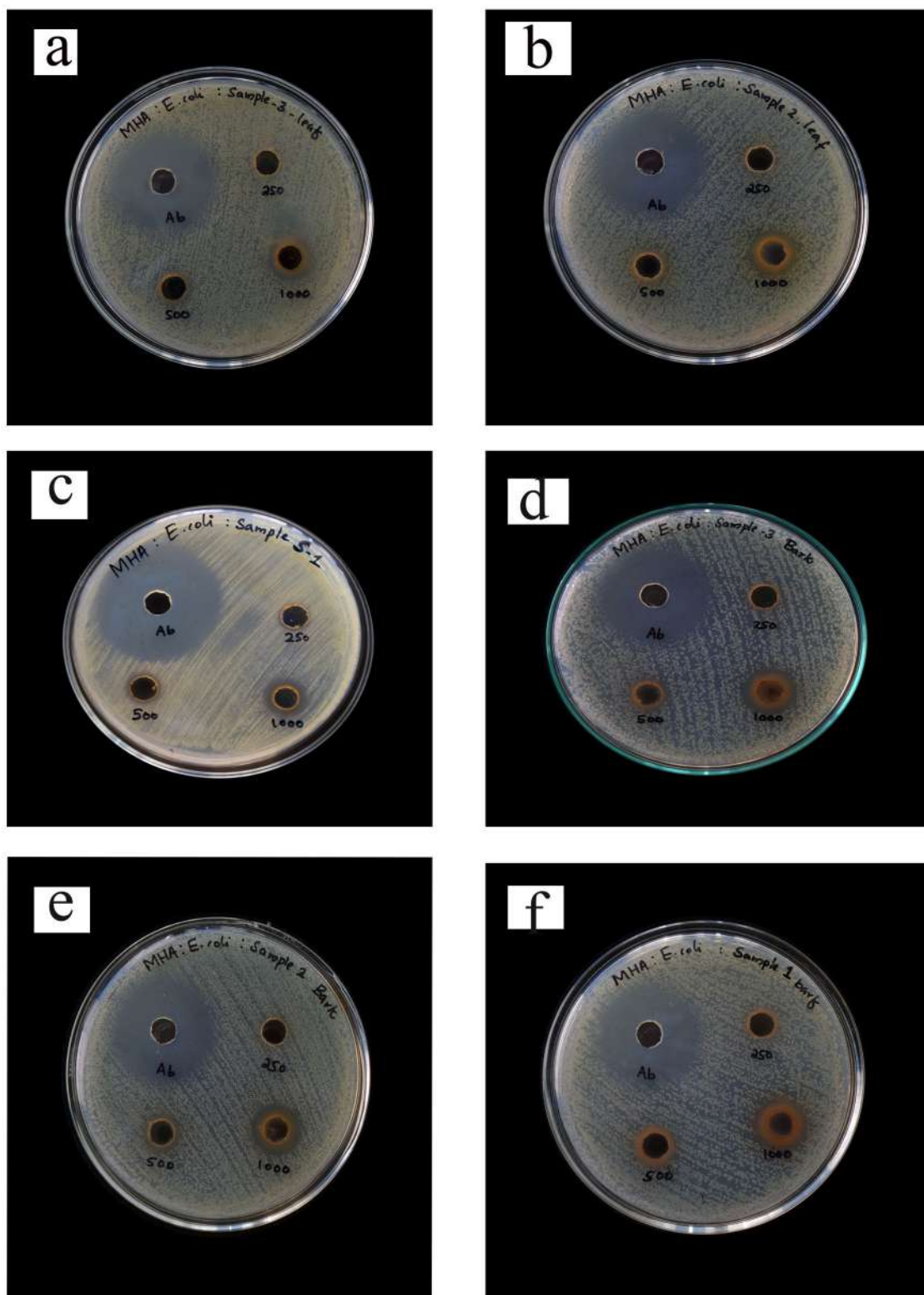


Fig 36 Antibacterial activity of Methanolic leaf & bark extracts. a. Inhibition of *E.coli* by leaf extract of *L.bourdillonii*; b. By leaf extract of *L.coriacea*; c. By leaf extract of *L.wightiana*; d. Inhibition of *E.coli* by bark extract of *L.bourdillonii*; e. By bark extract of *L.coriacea*; f. By bark extract of *L.wightiana*

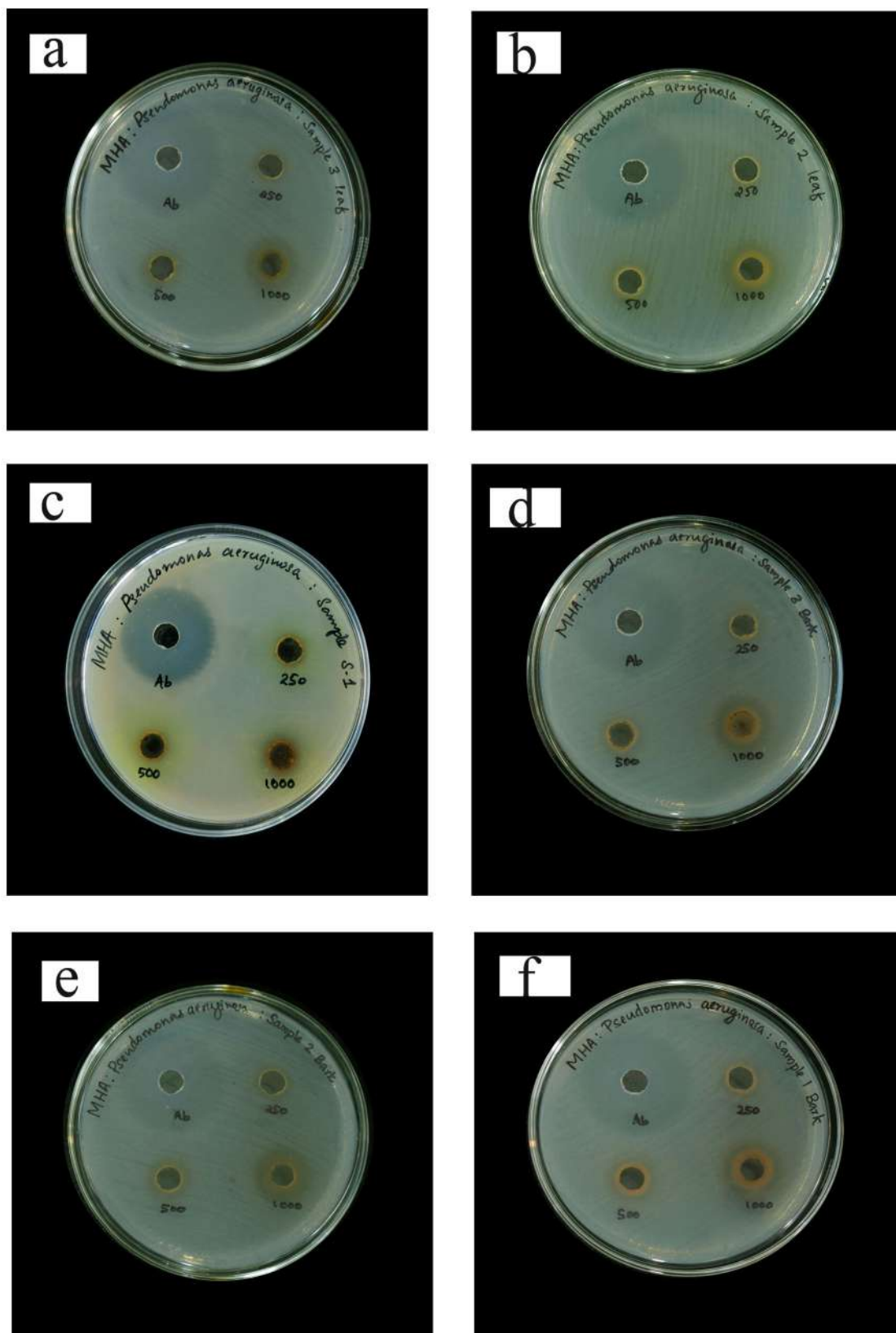


Fig 37 Antibacterial activity of Methanolic leaf & bark extracts. a. Inhibition of *P.aeruginosa* by leaf extract of *L.bourdillonii*; b. By leaf extract of *L.coriacea*; c. By leaf extract of *L.wightiana*; d. Inhibition of *P.aeruginosa* by bark extract of *L.bourdillonii*; e. By bark extract of *L.coriacea*; f. By bark extract of *L.wightiana*

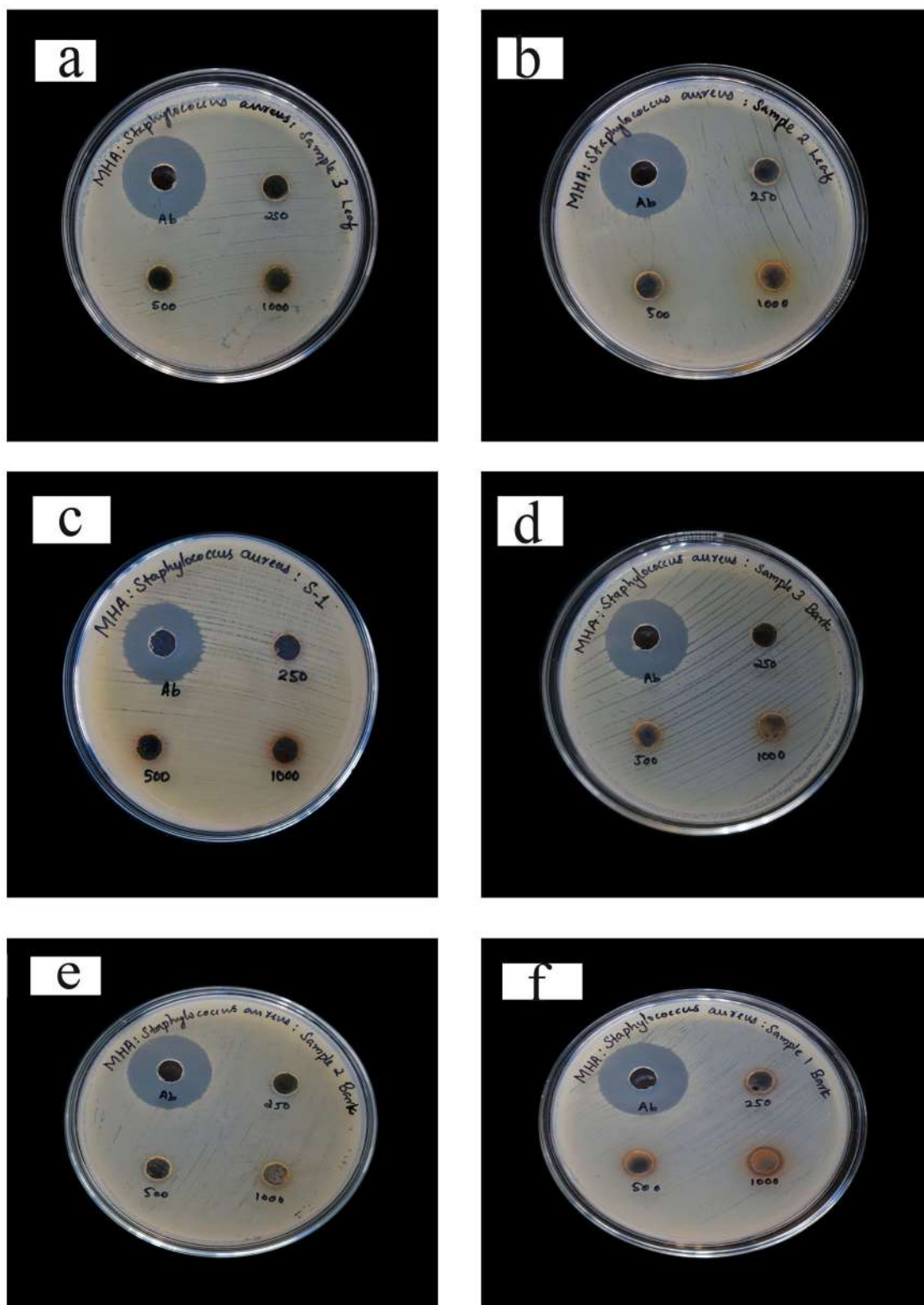


Fig 38 Antibacterial activity of Methanolic leaf & bark extracts. a. Inhibition of *S. aureus* by leaf extract of *L. bourdillonii*; b. By leaf extract of *L. coriacea*; c. By leaf extract of *L. wightiana*; d. Inhibition of *S. aureus* by bark extract of *L. bourdillonii*; e. By bark extract of *L. coriacea*; f. By bark extract of *L. wightiana*

compared to other extracts. The lowest inhibition concentration was 191.401 ± 0.44 $\mu\text{g/mL}$ against *E. coli*, followed by *P. aeruginosa* (488.124 ± 0.23 $\mu\text{g/mL}$) and *S. aureus* (622.666 ± 0.22 $\mu\text{g/mL}$) as shown in Table 26.

Table 25 Antibacterial activity of the leaves and bark extracts of three *Litsea* spp.

Extract	Concentration ($\mu\text{g/mL}$)	Zone of inhibition (mm)& $\pm$ SD		
		<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>
Streptomycin LBL	100	30 ± 0.13	32 ± 0.09	26 ± 0.65
	250	ND	11 ± 0.12	ND
	500	11 ± 0.07	13 ± 0.04	ND
	1000	14 ± 0.01	18 ± 0.13	11 ± 0.07
Streptomycin LCL	100	30 ± 0.05	32 ± 0.05	27 ± 0.43
	250	ND	ND	ND
	500	12 ± 0.12	12 ± 0.09	ND
	1000	15 ± 0.02	17 ± 0.43	11 ± 0.07
Streptomycin LWL	100	30 ± 0.17	30 ± 0.20	27 ± 0.02
	250	11 ± 0.17	11 ± 0.12	ND
	500	12 ± 0.21	12 ± 0.03	ND
	1000	14 ± 0.20	15 ± 0.02	11 ± 0.012
Streptomycin LBB	100	30 ± 0.12	32 ± 0.72	27 ± 0.12
	250	ND	12 ± 0.12	ND
	500	11 ± 0.01	13 ± 0.70	ND
	1000	13 ± 0.13	17 ± 0.05	ND
Streptomycin LCB	100	30 ± 0.15	32 ± 0.06	27 ± 0.02
	250	ND	12 ± 0.11	ND
	500	11 ± 0.30	14 ± 0.18	ND
	1000	14 ± 0.25	16 ± 0.30	11 ± 0.06
Streptomycin LWB	100	30 ± 0.10	32 ± 0.04	27 ± 0.12
	250	ND	11 ± 0.30	11 ± 0.10
	500	12 ± 0.12	13 ± 0.12	12 ± 0.91
	1000	16 ± 0.06	17 ± 0.32	13 ± 0.80

LBL- *L. bourdillonii* leaf, LCL- *L. coriacea* leaf, LWL- *L. wightiana* leaf, LBB- *L. bourdillonii* bark, LCB- *L. coriacea* bark, LWB- *L. wightiana* bark ND-Not Detected

Table 26 Antibacterial activity expressed by minimum inhibitory concentration (MIC) of the leaf and bark extracts of three *Litsea* spp.

Plant sample	Minimum Inhibitory Concentration (MIC) ($\mu\text{g/mL}$)		
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>
LBL	777.012 $\pm$ 0.12	702.40 $\pm$ 0.15	777.012 $\pm$ 0.54
LCL	661.026 $\pm$ 0.22	756.007 $\pm$ 0.15	759.941 $\pm$ 0.23
LWL	191.401 $\pm$ 0.44	488.124 $\pm$ 0.23	622.666 $\pm$ 0.22
L.BB	625.572 $\pm$ 0.23	546.601 $\pm$ 0.20	625.572 $\pm$ 0.25
LCB	643.811 $\pm$ 0.41	533.069 $\pm$ 0.22	643.811 $\pm$ 0.34
LWL	720.355 $\pm$ 1.23	682.387 $\pm$ 0.14	905.62 $\pm$ 0.30

LBL- *L. bourdillonii* leaf, LCL- *L. coriacea* leaf, LWL- *L. wightiana* leaf, LBB- *L. bourdillonii* bark, LCB- *L. coriacea* bark, LWL- *L. wightiana* leaf

b. Antifungal Study

Antifungal activity of studies of leaves and bark samples of *L. bourdillonii*, *L. coriacea*, and *L. wightiana* were carried out. The results (Figures 39 & 40) showed no significant fungal activity by the extracts. The leaves and bark extracts of *L. coriacea* and the leaf extract of *L. wightiana* showed moderate activity with inhibition zones (12 $\pm$ 0.012mm, 11 $\pm$ 0.15mm and 11 $\pm$ 0.01mm) against *C. albicans*. No extracts showed antifungal effect against *A. niger*.

B. ANTIOXIDANT ASSAY

The methanolic leaves extract of selected *Litsea* spp. were subjected to *in vitro* antioxidant activity assays. Four different methods, like DPPH radical scavenging, hydroxyl radical scavenging, superoxide radical scavenging assays and ferrous reducing power assay, were adopted. Each assay results were compared with known antioxidants.

i. DPPH (1,1-Diphenyl-1-Picrylhydrazyl) radical scavenging activity

In phytochemistry the DPPH radical scavenging assay is a commonly used technique to evaluate the antioxidant qualities of different compounds including natural products, pharmaceuticals, *etc.* This test evaluates a compound's ability to donate an electron and a hydrogen atom to neutralize the DPPH radical. For the current study, the methanolic extracts of *L. bourdillonii*, *L. coriacea*, and *L.*

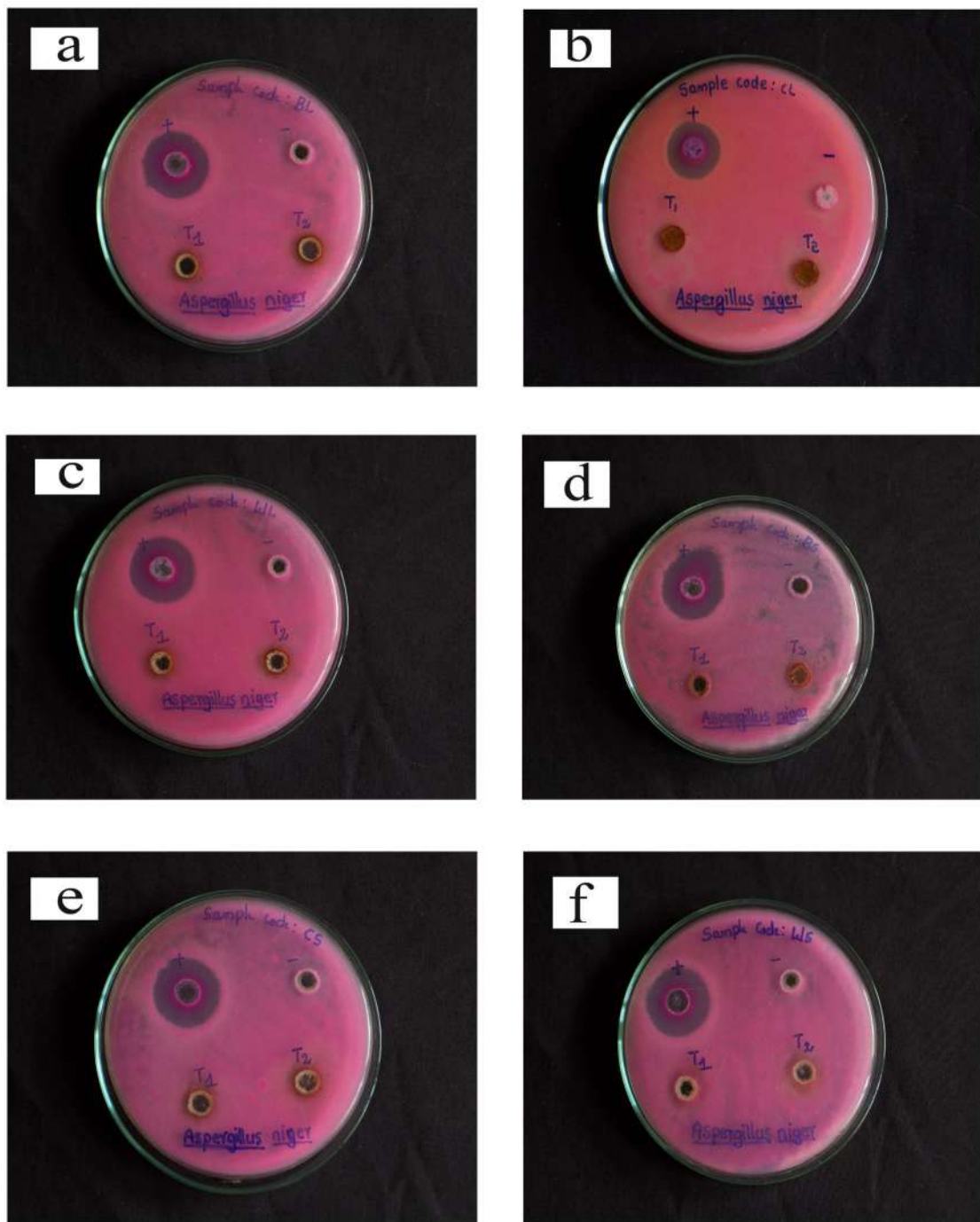


Fig 39 Antifungal activity of Methanolic leaf & bark extracts. a. Inhibition of *A.niger* by leaf extract of *L.bourdillonii*; b. By leaf extract of *L.coriacea*; c. By leaf extract of *L.wightiana*; d. Inhibition of *A.niger* by bark extract of *L.bourdillonii*; e. By bark extract of *L.coriacea*; f. By bark extract of *L.wightiana*

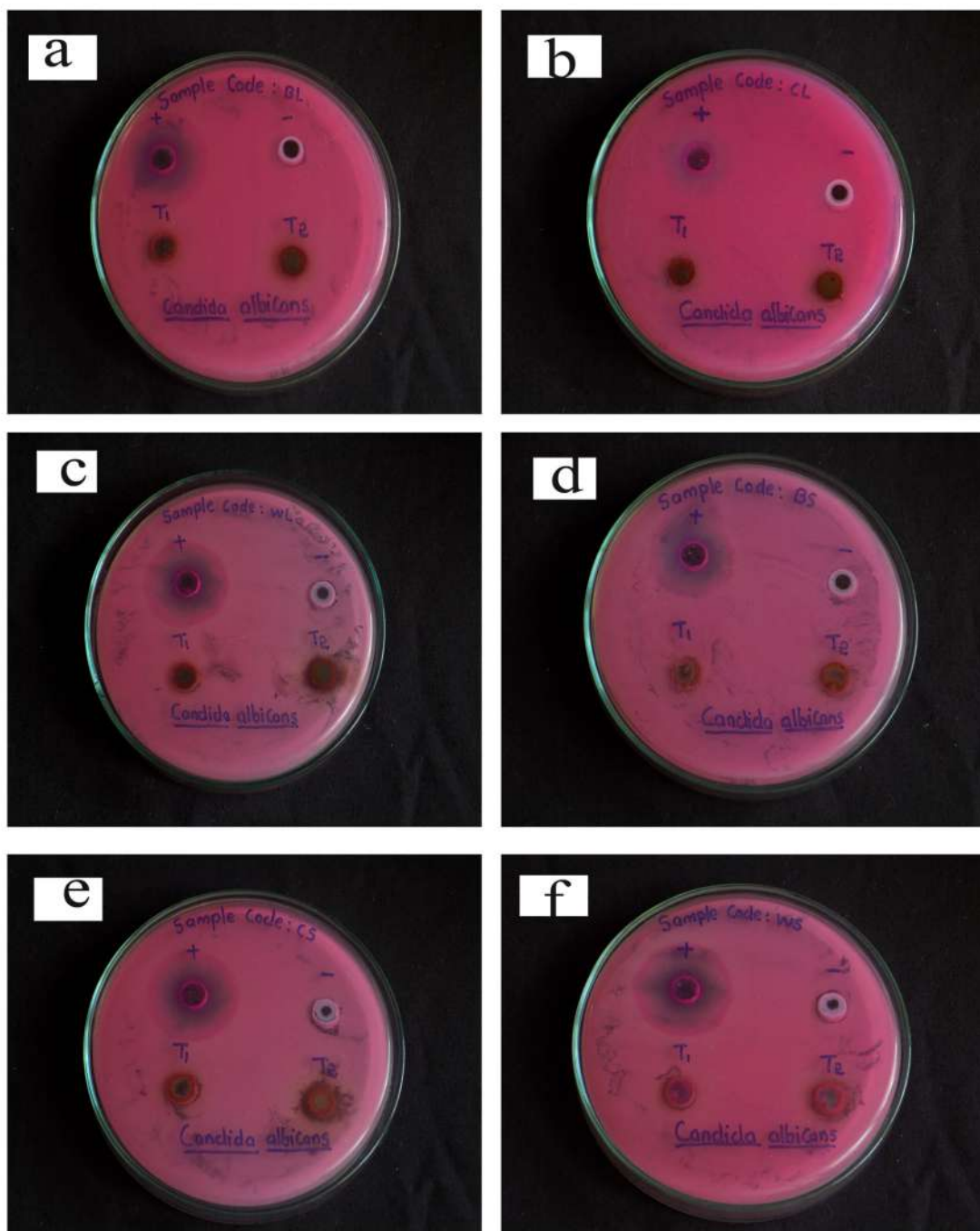


Fig 40 Antifungal activity of Methanolic leaf & bark extracts. a. Inhibition of *C.albicans* by leaf extract of *L.bourdillonii*; b. By leaf extract of *L.coriacea*; c. By leaf extract of *L.wightiana*; d. Inhibition of *C.albicans* by bark extract of *L.bourdillonii*; e. By bark extract of *L.coriacea*; f. By bark extract of *L.wightiana*

wightiana leaves were taken at different concentrations (6.5, 12.5, 25, 50, 100 µg/mL). The results are represented in Table 27 and Fig. 41. From the comparative assessment, it is comprehended that the *L. wightiana* leaves extract showed better antioxidant activity with an IC₅₀ value of 30.32 µg/mL among the remaining extracts. This indicates the high antioxidant potential of *L. wightiana* as compared to the standard antioxidant, ascorbic acid, with an IC₅₀ value of 54.53 µg/mL. Hence, the other methods of antioxidant activity, such as hydroxyl radical scavenging activity, superoxide radical scavenging activity, and ferrous reducing power antioxidant assay studies, were attempted by *L. wightiana* leaf methanolic extracts.

Table 27 DPPH free radical scavenging activity of the leaves of three *Litsea* spp.

Sample	Concentration (µg/mL)	DPPH scavenging activity (%) ±SD	IC ₅₀ (µg/mL)
<i>L.bourdillonii</i>	6.25	5.218±0.01	89.79
	12.5	7.160±0.57	
	25	15.6±0.06	
	50	34.83±0.15	
	100	53.03±0.15	
<i>L.coriacea</i>	6.25	14.05±0.31	67.5
	12.5	17.41±0.20	
	25	25.99±0.52	
	50	44.52±0.11	
	100	66.54±0.73	
<i>L.wightiana</i>	6.25	10.6±0.01	30.32
	12.5	25.15±0.07	
	25	46.63±0.51	
	50	89.45±0.65	
	100	97.58±0.20	
Ascorbic acid	6.25	25.18±0.95	54.53
	12.5	39.80±0.01	
	25	44.93±0.25	
	50	72.67±0.55	
	100	94.98±0.68	

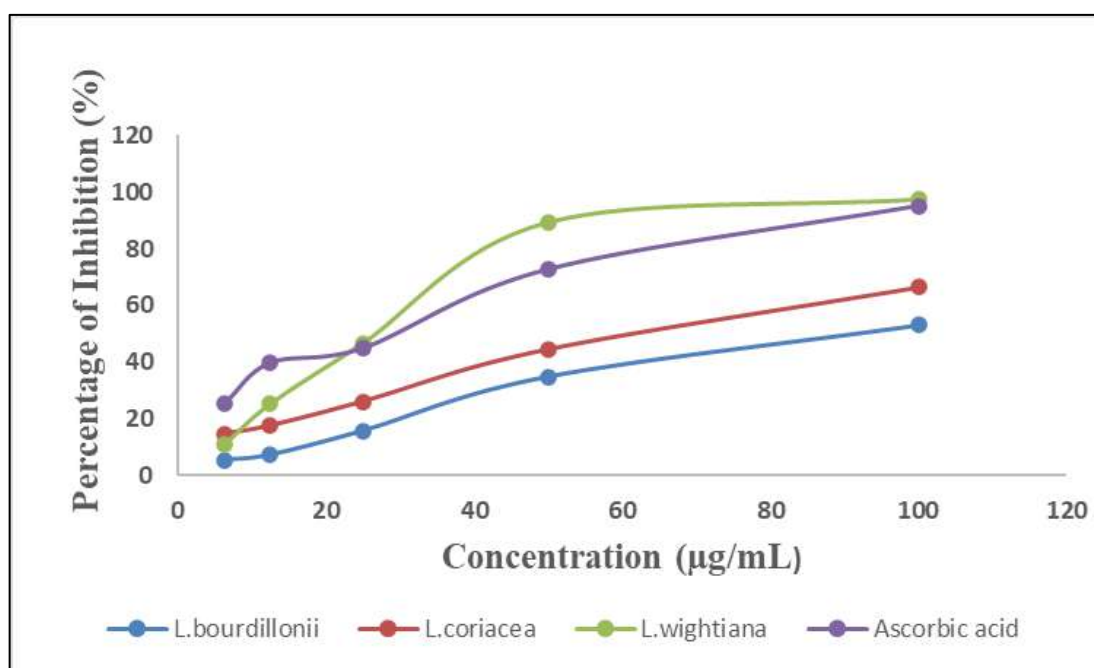


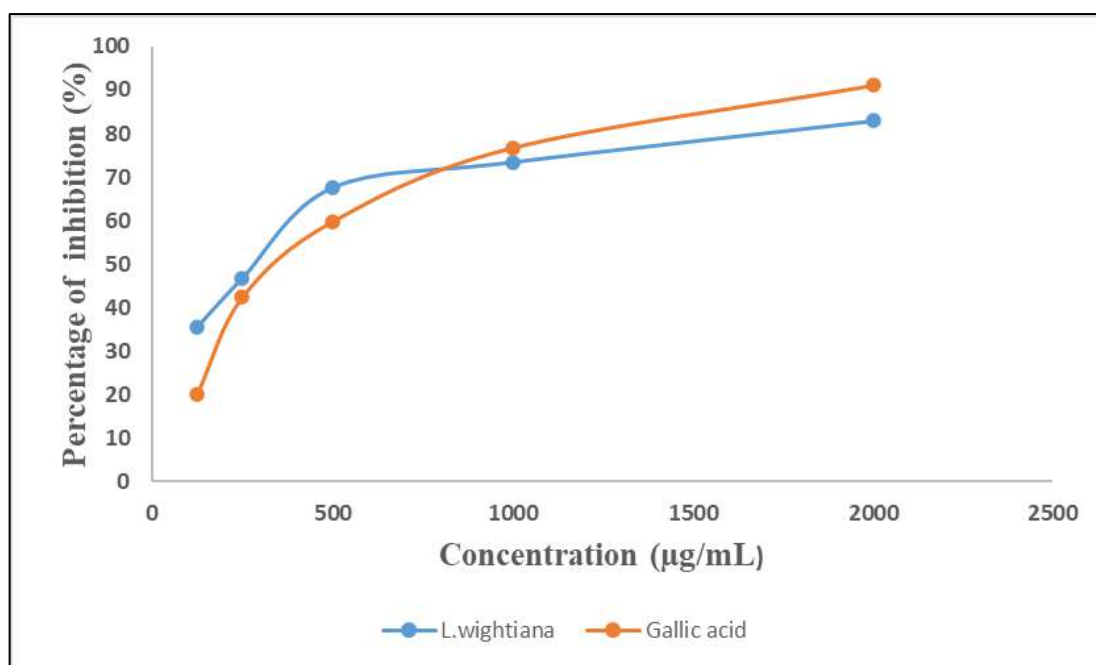
Fig 41 Antioxidant activity of leaves extract of the three *Litsea* spp. using DPPH method

ii. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity is the capability of a substance to nullify hydroxyl radicals, which are highly reactive and damaging free radicals. Hydroxyl radicals are generated within biological systems and can cause oxidative stress, leading to various health issues and cellular damage. Hence, the assessment of hydroxyl radical scavenging activity of compounds is crucial in evaluating their antioxidant properties and potential health benefits. In the present study, the leaf extracts of *L. wightiana* in various concentrations, like (125, 250, 500, 1000, 2000 µg/mL), were used. The antioxidant activity of *L. wightiana* leaves extract was determined with an IC_{50} value of 271.255 µg/mL, and the standard gallic acid, with an IC_{50} value of 533.504 µg/mL. The results are shown in Table 28 & Fig 42.

Table 28 Hydroxyl radical scavenging activity of the leaves extract of *L. wightiana*

Extract	Concentration $\mu\text{g/mL}$	% inhibition	IC ₅₀ Value ($\mu\text{g/mL}$)
<i>L. wightiana</i> leaf	125	35.54 $\pm$ 0.02	271.255
	250	46.76 $\pm$ 0.32	
	500	67.55 $\pm$ 0.15	
	1000	73.43 $\pm$ 0.05	
	2000	82.91 $\pm$ 0.17	
Standard (Gallic Acid)	125	20.17 $\pm$ 0.15	533.504
	250	42.36 $\pm$ 0.72	
	500	59.74 $\pm$ 0.95	
	1000	76.5 $\pm$ 0.26	
	2000	91.11 $\pm$ 0.20	

**Fig 42** Antioxidant activity of *L. wightiana* leaves extract using hydroxyl radical scavenging assay**iii. Superoxide radical scavenging activity**

Superoxide radical scavenging activity refers to the potential of compounds to neutralize and remove superoxide radicals from biological systems. Superoxide radicals are highly reactive and are considered as one of the most significant reactive

oxygen species (ROS) generated in living organisms during various physiological and pathological processes. Their overproduction can lead to oxidative stress, which is associated with numerous health issues, including inflammation, aging, and various diseases. In the present investigation, the methanolic extracts of leaves of *L. wightiana* were subjected to a superoxide radical scavenging activity study. The extract in different concentrations (125, 250, 500, 1000, 2000 µg/mL) were used and encouraging results were obtained. The methanolic extracts of *L. wightiana* leaves showed maximum radical scavenging with an IC₅₀ value of 1052.57 µg/mL compared to ascorbic acid, which was used as the standard with an IC₅₀ value of 567.07 µg/mL (Table 29 & Fig 43).

Table 29 Superoxide radical scavenging activity of the leaves extract of *L. wightiana*

Extract	Concentration(µg/mL)	% inhibition	IC ₅₀ Value (µg/mL)
<i>L. wightiana</i> leaf	125	13.30±0.02	1052.27
	250	25.10±0.16	
	500	34.74±0.01	
	1000	59.71±0.65	
	2000	73.82±0.045	
Standard (Ascorbic Acid)	125	23.13±0.45	567.07
	250	39.80±0.82	
	500	59.21±0.47	
	1000	70.52±0.01	
	2000	91.26±0.05	

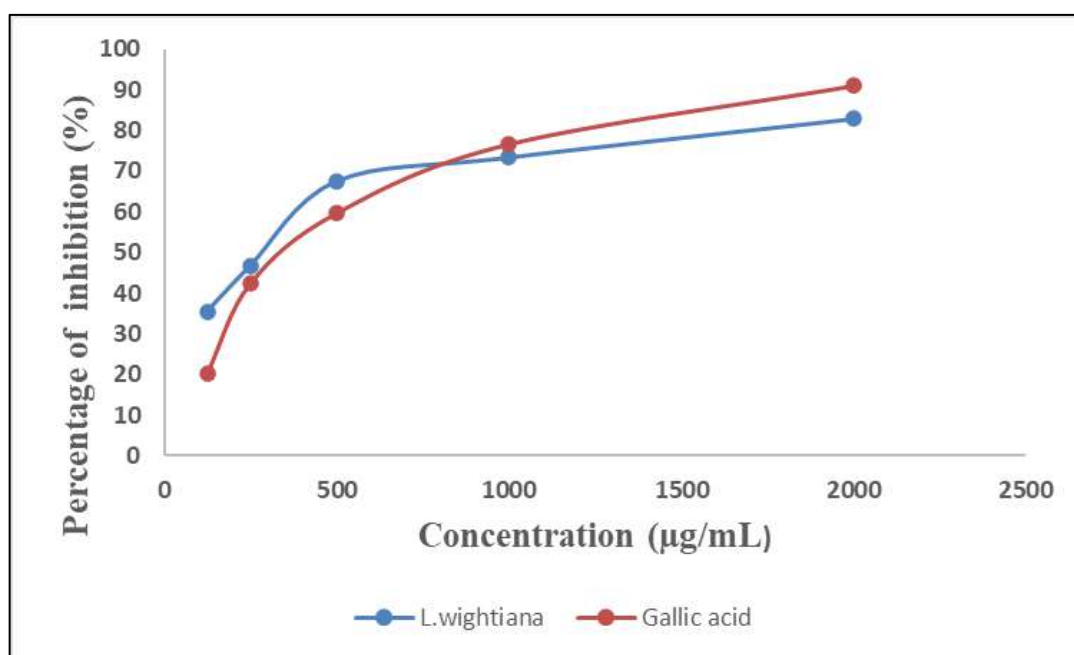


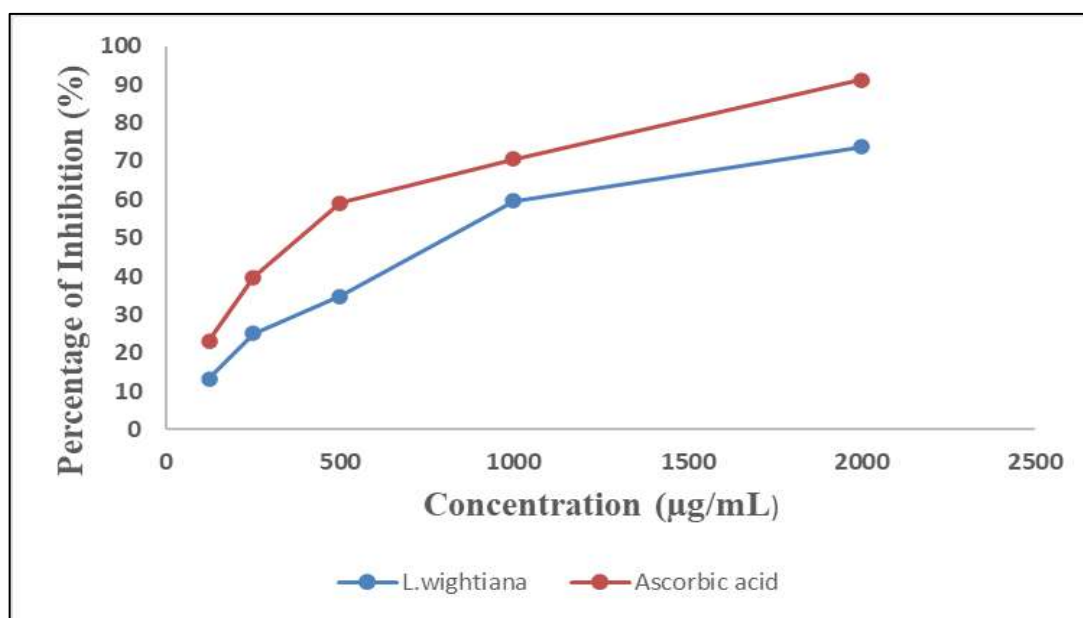
Fig 43 Antioxidant activity of *L. wightiana* leaves extract by Superoxide radical scavenging activity

iv. Ferrous reducing antioxidant power assay (FRAP)

FRAP is a common method used to assess the antioxidant activity of plant extracts. It assesses the ability of a substance to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). This assay is based on the principle that the antioxidants can donate electrons to reduce Fe^{3+} to Fe^{2+} ions, resulting in a color change that can be measured spectrophotometrically. The results of the FRAP assay in *L. wightiana* methanolic leaf extracts indicated an increase in absorbance from 0.135 ± 0.017 to 0.488 ± 0.011 in a dose-dependent manner, and this increase in absorbance was indicated due to increased reducing power of the extracts. The results were compared with the absorbance of standard quercetin in which the absorbance ranged from 0.347 ± 0.05 to 0.878 ± 0.005 (Table 30 and Fig 44).

Table 30. Ferrous reducing antioxidant power assay of the leaves extract of *L. wightiana*

Extract	Concentration($\mu\text{g/mL}$)	Absorbance $\pm$ SD at 700 nm
<i>L. wightiana</i> leaf	125	0.135 $\pm$ 0.17
	250	0.207 $\pm$ 0.26
	500	0.237 $\pm$ 0.20
	1000	0.308 $\pm$ 0.01
	2000	0.488 $\pm$ 0.11
Standard (Quercetin)	125	0.347 $\pm$ 0.05
	250	0.569 $\pm$ 0.01
	500	0.645 $\pm$ 0.02
	1000	0.700 $\pm$ 0.02
	2000	0.878 $\pm$ 0.05

**Fig 44 Antioxidant activity of *L. wightiana* leaves extract by FRAP method****C. ANTIDIABETIC STUDY**

Antidiabetic activity of the leaves and bark extracts of the three *Litsea* species were studied using the alpha amylase and alpha glucosidase enzyme inhibition assays, and the extracts showed remarkable antidiabetic activity. In the alpha amylase assay, the leaves and bark extracts of *L. wightiana* showed significant activity with IC_{50} values, 134.67 $\mu\text{g/mL}$ and 148.83 $\mu\text{g/mL}$. *L. coriacea* leaves and bark showed lower inhibition with IC_{50} 228.95 $\mu\text{g/mL}$ and 412.90 $\mu\text{g/mL}$

respectively. In the alpha glucosidase assay, *L. wightiana* leaf and *L. coriacea* bark exhibited higher inhibition of the enzyme among other extracts with IC₅₀ of 50.30 µg/mL and 88.771 µg/mL. In both assays acarbose, an antidiabetic agent used as standard which showed antidiabetic activity with IC₅₀ values 45.41µg/mL in alpha amylase inhibitory assay and 20.23 µg/mL in alpha glucosidase inhibitory assay. The details of the results are depicted in Table 31& 32 and Figures 45 & 46 below.

Table 31. Antidiabetic activity of the leaves and bark extracts of three *Litsea* spp. by α -amylase inhibition method

Sample	Concentration(µg/mL)	Absorbance at 540 nm	% of inhibition	IC ₅₀ Value µg/mL
LBB	50	0.400± 0.12	34.70	176.20
	100	0.210±0.02	46.37	
	200	0.178±0.23	52.68	
	400	0.135±0.12	70.03	
LBL	50	0.164±0.31	41.28	219.06
	100	0.155±0.01	49.56	
	200	0.141±0.30	58.77	
	400	0.128±0.22	70.97	
LCB	50	0.258±0.54	49.77	412.90
	100	0.249±0.03	58.1	
	200	0.204±0.51	66.91	
	400	0.150±0.43	89.17	
LCL	50	0.235±0.01	27.44	228.95
	100	0.198±0.02	35.45	
	200	0.178±0.76	49.52	
	400	0.123±0.34	68.47	
LWB	50	0.140±0.03	36.85	148.83
	100	0.099±0.12	45.21	
	200	0.086±0.24	59.04	
	400	0.080±0.91	75.69	
LWL	50	0.087±0.02	34.56	134.67
	100	0.081±0.56	48.65	
	200	0.079±0.66	62.21	
	400	0.075±0.30	80.9	
Acarbose (Standard)	50	0.219±0.40	45.32	45.41
	100	0.181±0.38	59.21	
	200	0.156±0.35	74	
	400	0.121±0.36	90.34	
Blank	-	0.515±0.013	-	
Control	-	0.032±0.001	-	

LBB- *L. bourdillonii* bark, LBL- *L. bourdillonii* leaf, LCB- *L. coriacea* bark, LCL- *L. coriacea* leaf, LWB- *L. wightiana* bark, LWL- *L. wightiana* leaf. Absorbance value expressed as ± SEM (n=3)

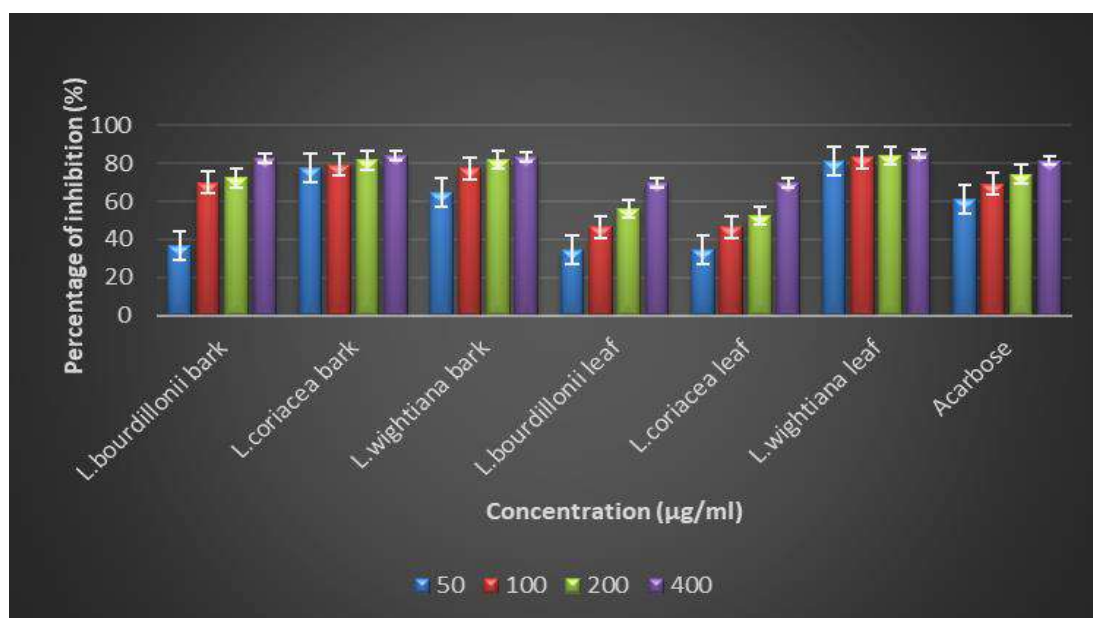


Fig 45 Antidiabetic assay of leaf and bark extracts of *Litsea* spp. using alpha amylase inhibition method

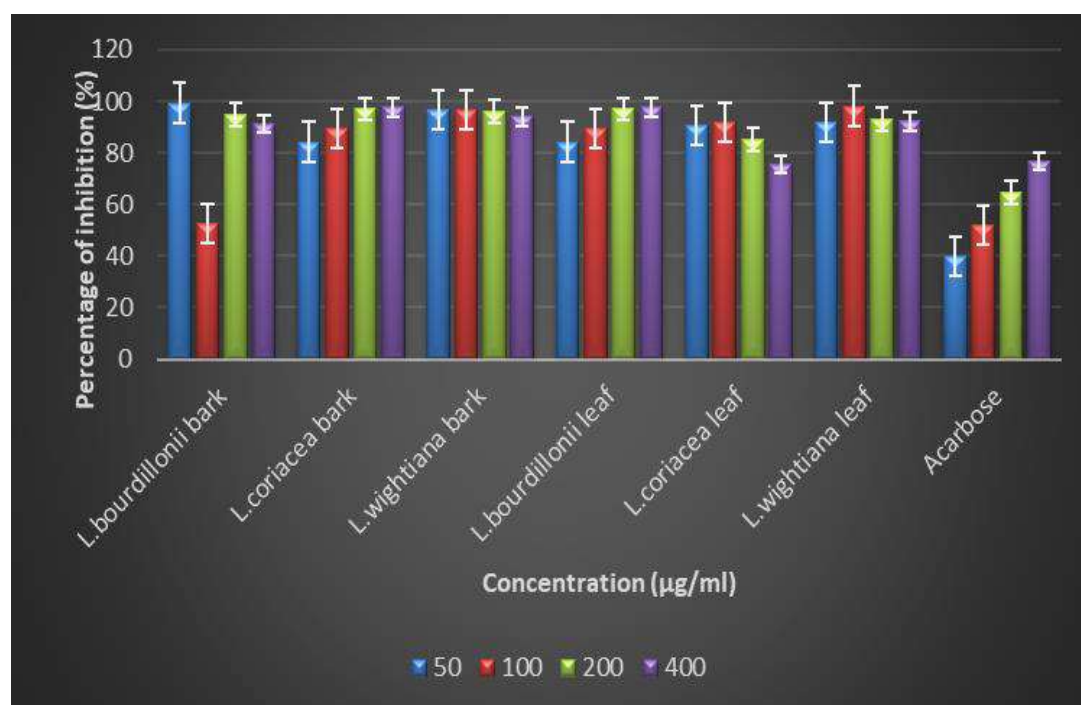


Fig 46 Antidiabetic assay of leaf and bark extracts of *Litsea* spp. by alpha glucosidase inhibition method

Table 32 Antidiabetic activity of leaf and bark extracts of the three *Litsea* spp. by α -glucosidase inhibition method

Extract	Concentration($\mu\text{g/mL}$)	Absorbance at 400 nm	% of inhibition	IC ₅₀ value($\mu\text{g/mL}$)
LBB	50	0.020 $\pm$ 0.16	28.28	217.779
	100	0.276 $\pm$ 0.32	39.43	
	200	0.044 $\pm$ 0.11	48.52	
	400	0.064 $\pm$ 0.65	65.33	
LBL	50	0.040 $\pm$ 0.32	38.23	164.09
	100	0.50 $\pm$ 1.23	44.32	
	200	0.084 $\pm$ 0.93	54.48	
	400	0.116 $\pm$ 0.75	74.82	
LCB	50	0.102 $\pm$ 0.12	44.20	88.771
	100	0.075 $\pm$ 0.54	53.38	
	200	0.033 $\pm$ 0.34	60.08	
	400	0.029 $\pm$ 0.41	75.12	
LCL	50	0.067 $\pm$ 0.11	30.23	176.763
	100	0.062 $\pm$ 0.98	39.28	
	200	0.097 $\pm$ 0.76	58.03	
	400	0.165 $\pm$ 0.09	78.28	
LWB	50	0.036 $\pm$ 0.87	36.40	99.32
	100	0.035 $\pm$ 0.35	54.38	
	200	0.038 $\pm$ 0.65	69.20	
	400	0.049 $\pm$ 0.76	85.43	
LWL	50	0.061 $\pm$ 0.08	40.43	50.30
	100	0.027 $\pm$ 0.023	61.28	
	200	0.055 $\pm$ 0.37	72.30	
	400	0.59 $\pm$ 0.23	81.11	
Standard (Acarbose)	50	0.575 $\pm$ 0.49	48.29	20.23
	100	0.464 $\pm$ 0.65	63.28	
	200	0.348 $\pm$ 0.63	75.82	
	400	0.236 $\pm$ 0.21	86.43	
Blank	-	0.943	-	-
Control	-	0.021		

LBB- *L. bourdillonii* bark, LBL- *L. bourdillonii* leaf, LCB- *L. coriacea* bark, LCL- *L. coriacea* leaf, LWB- *L. wightiana* bark, LWL- *L. wightiana* leaf. Values expressed as $\pm$ SEM (n=3).

D. IN VITRO CYTOTOXICITY

The cytotoxicity of leaf and bark extracts of *L. bourdillonii*, *L. coriacea*, and *L. wightiana* were studied by MTT assay by applying various concentrations such as 6.25 $\mu\text{g/mL}$, 12.5 $\mu\text{g/mL}$, 25 $\mu\text{g/mL}$, 50 $\mu\text{g/mL}$, and 100 $\mu\text{g/mL}$, and the results

indicated that no extracts showed significant cytotoxicity in SK-MEL cell lines (Table 33 and Figures 47&48). Almost all the treated cell lines showed cell viability greater than or equal to 50% indicating less or no cytotoxicity effect. All experiments were conducted three times as replicates. The *L. coriacea* leaves extract showed moderate cytotoxicity with 40.67% of inhibition at 100 µg/mL concentration.

Table 33 Cytotoxicity effect of leaf and bark extracts of the three *Litsea* spp. in SK-MEL cell lines

Extract	Concentration(µg/mL)	Absorbance at 570 nm	Cell viability (%)
LBB	6.25	0.411±0.12	93.55
	12.5	0.381±0.98	86.87
	25	0.346±0.07	78.90
	50	0.307±0.42	69.87
	100	0.259±0.51	60.92
LBL	6.25	0.381±0.01	86.87
	12.5	0.352±0.025	80.12
	25	0.311±0.65	70.86
	50	0.281±0.32	64.03
	100	0.237±0.33	54.09
LCB	6.25	0.390±0.12	88.92
	12.5	0.353±0.04	80.42
	25	0.305±0.75	69.57
	50	0.283±0.43	64.49
	100	0.239±0.32	54.47
LCL	6.25	0.352±0.04	80.19
	12.5	0.312±0.32	71.01
	25	0.275±0.03	62.59
	50	0.228±0.34	51.97
	100	0.200±0.12	45.67
LWB	6.25	0.369±0.24	84.14
	12.5	0.332±0.07	75.56
	25	0.306±0.23	69.72
	50	0.271±0.46	61.83
	100	0.223±0.11	50.75
LWL	6.25	0.316±0.03	97.21
	12.5	0.293±0.12	92.19
	25	0.271±0.13	87.72
	50	0.227±0.81	83.48
	100	0.165±0.04	77.21
Control	-	0.439±0.12	-

LBB- *L. bourdillonii* bark, LBL- *L. bourdillonii* leaf, LCB- *L. coriacea* bark, LCL- *L. coriacea* leaf, LWB- *L. wightiana* bark, LWL- *L. wightiana* leaf. Experimental results expressed as ± SEM, n=3

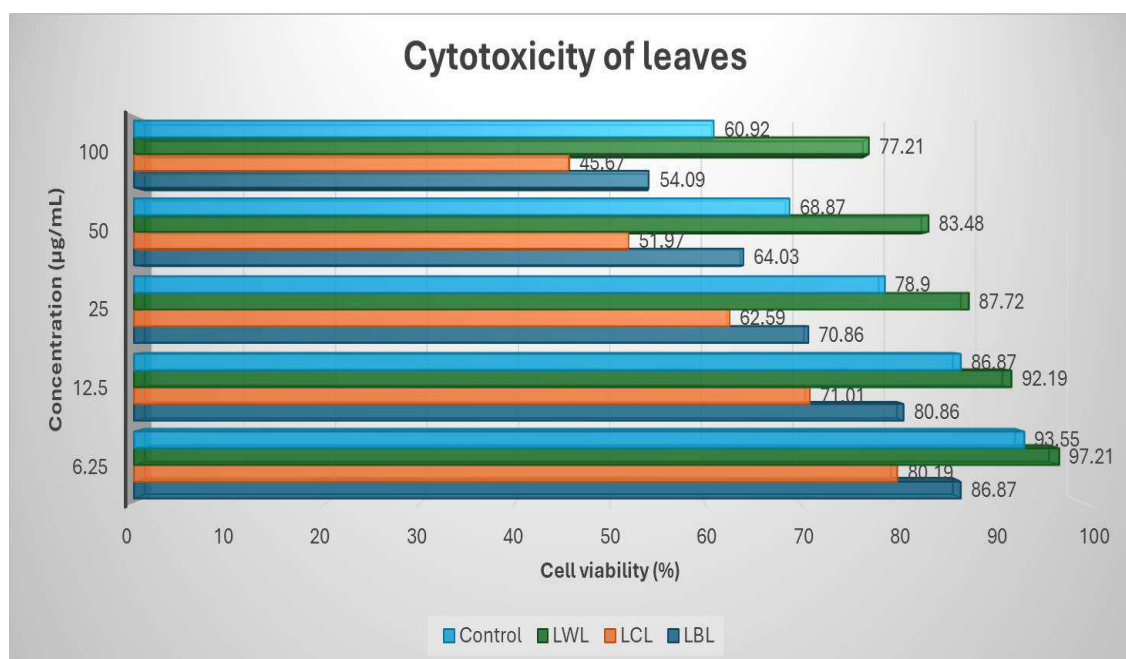


Fig 47 Cytotoxicity of leaf extracts of three *Litsea* spp.

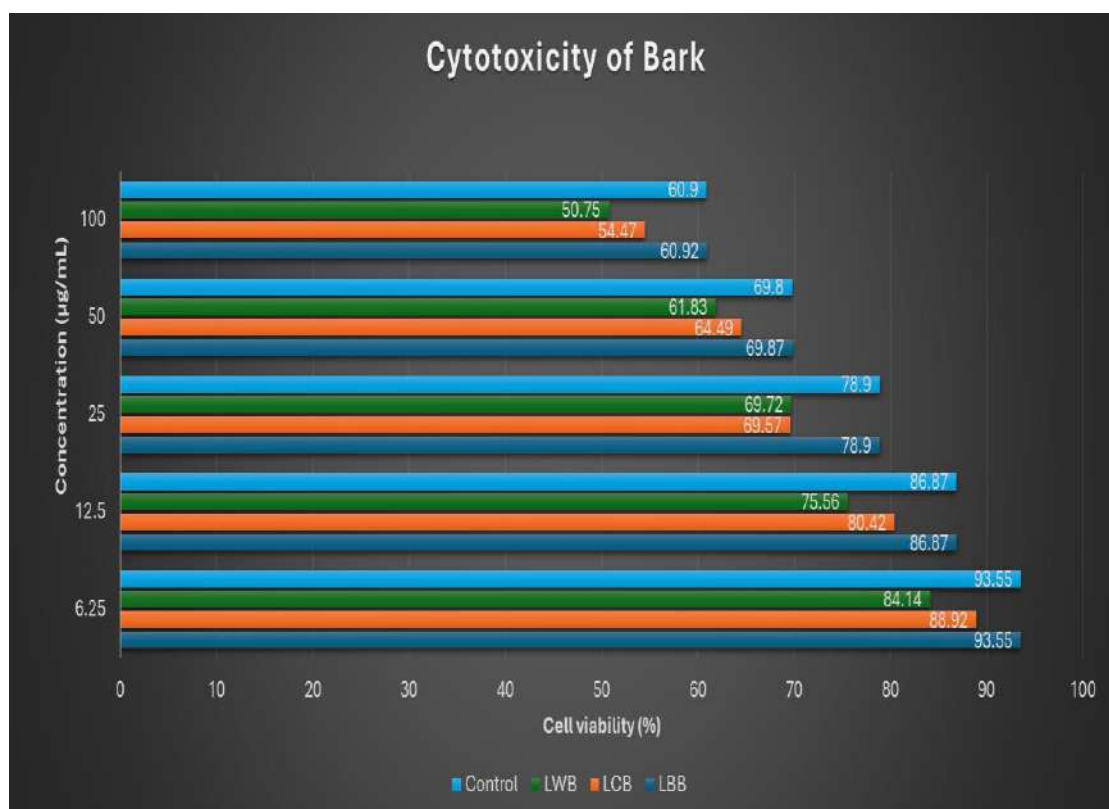


Fig 48 Cytotoxicity of bark extracts of three *Litsea* spp.

From the qualitative phytochemical screening studies, it was found that *L. wightiana* leaves extract contain all the phytochemicals and quantitative assay showed higher content of phenols, flavonoids and tannins when compared to other leaves and bark extracts. Moreover, *L. wightiana* showed significant antioxidant, antibacterial and antidiabetic properties. Hence *L. wightiana* leaves methanolic extract was selected for higher order studies such as anti-inflammatory, gene expression, wound healing and docking studies.

E. ANTIINFLAMMATORY ACTIVITY

Inflammation is a sort of immune response against various types of harmful stimuli. Anti-inflammatory response is a counterbalancing mechanism to regulate and resolve the inflammatory process during an immune response. This study aimed to investigate the anti-inflammatory potential of *L. wightiana* leaves. Both *in vitro* and *in vivo* methods were adopted.

i. *In vitro* anti-inflammatory activity

The anti-inflammatory studies were performed by the *in vitro* method in RAW 264.7 macrophage cell lines. The enzyme inhibition assays were demonstrated by testing the activity of different enzymes like cyclooxygenase, lipoxygenase, myeloperoxidase, and nitric oxide synthase, which are generally involved in the synthesis of proinflammatory mediators. The methanolic extracts of *L. wightiana* leaves were tested for anti-inflammatory activity in selected cell lines. The studies demonstrated dose-dependent inhibition of these enzymes against standard diclofenac sodium as illustrated in Figures 49-52 and Tables 34& 35.

● Cyclooxygenase (COX) activity

Cyclooxygenase enzyme is an important enzyme involved in inflammatory responses. Cyclooxygenase enzyme activity was checked by treating the LPS-induced RAW 264.7 macrophage cells with the methanolic extract of leaves of *L. wightiana*. Diclofenac sodium was used as the standard. The experiment showed a dose-dependent reduction in enzyme activity by the extracts, which indicated the anti-inflammatory potential of the extract. Different concentrations of the sample

(25, 50, 100 $\mu\text{g/mL}$) were taken, the percentage of inhibition of cyclooxygenase function was measured, and the maximum inhibition was found to be $65.44 \pm 0.23\%$ at 100 $\mu\text{g/mL}$. By the same concentration, the standard exhibited a reduction in activity at $85 \pm 0.24\%$.

- **Lipoxygenase (LOX) activity**

Lipoxygenase is a lipid peroxidase enzyme involved in many inflammatory responses and causes various diseases. To check whether *L. wightiana* leaf extracts can act as an anti-inflammatory agent, by inhibiting the LOX, various concentrations of the sample were applied in RAW 264.7 cell lines. The extract inhibited the LOX and the percentage was 17.51 ± 0.005 , 49.88 ± 0.092 , and 64.96 ± 0.43 at varying concentrations like 25, 50 and 100 $\mu\text{g/mL}$. Standard diclofenac sodium exhibited $72 \pm 0.13\%$ of inhibition at 100 $\mu\text{g/mL}$ concentration.

- **Myeloperoxidase (MPO) activity**

MPO is an enzyme which is active in response to pathogenic invasion. In some cases, it produces free radicals and reactive oxygen species that may lead to serious disorders in the cell. The effect of *L. wightiana* leaf extracts was tested for anti-inflammatory activity by checking the inhibition of myeloperoxidase activity using LPS-induced RAW 264.7 macrophage cell lines. Varying concentrations of the sample and the standard diclofenac sodium, like 25, 50, and 100 $\mu\text{g/mL}$ were taken. At 100 $\mu\text{g/mL}$ the MPO enzyme showed reduced activity measured as 0.0129 U/mL of cell lysate. The inhibition of diclofenac was almost like that of the sample.

- **Inducible Nitric oxide synthase (i NOS) activity**

Inhibition of the nitric oxide synthase enzyme using anti-inflammatory agents is widely used in anti-inflammatory treatments. For analyzing the anti-inflammatory effect of *L. wightiana* leaves, the methanolic extracts at the concentrations of 25, 50, and 100 $\mu\text{g/mL}$ were applied in LPS-treated RAW 264.7 cell lines. The standard Diclofenac sodium was also set at the same concentrations to compare the activity. Significant activity was observed, and inhibition of the nitric oxide synthase enzyme was found to be 51.2 % at the concentration of 100 $\mu\text{g/mL}$.

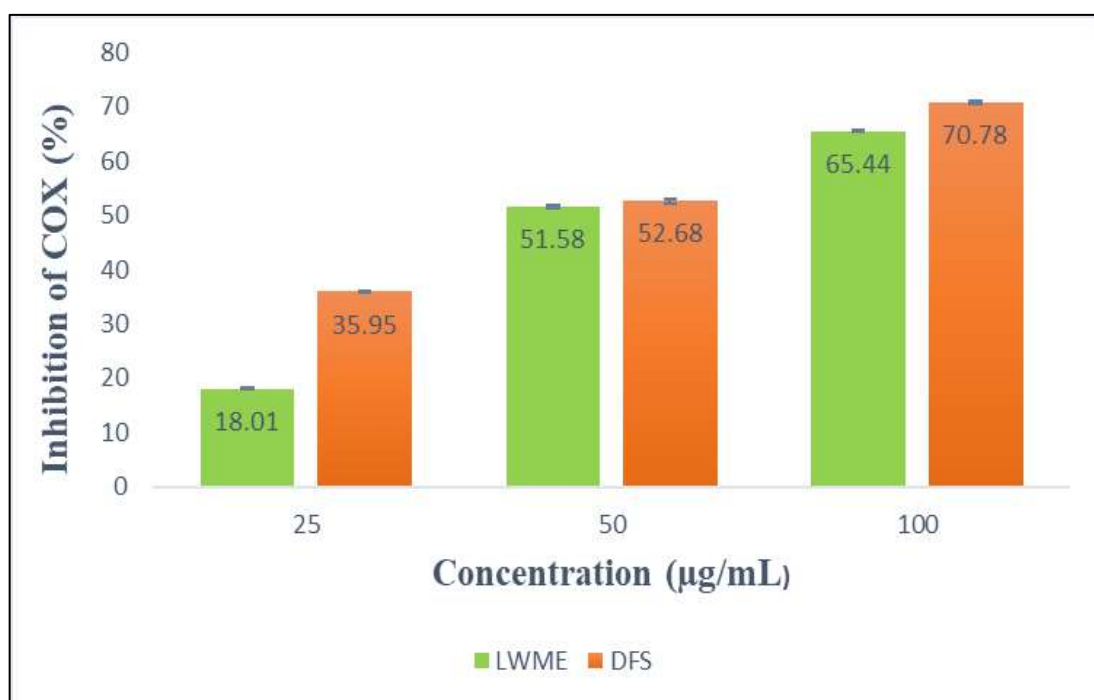


Fig 49 *In vitro* anti-inflammatory activity of LWME by COX inhibition

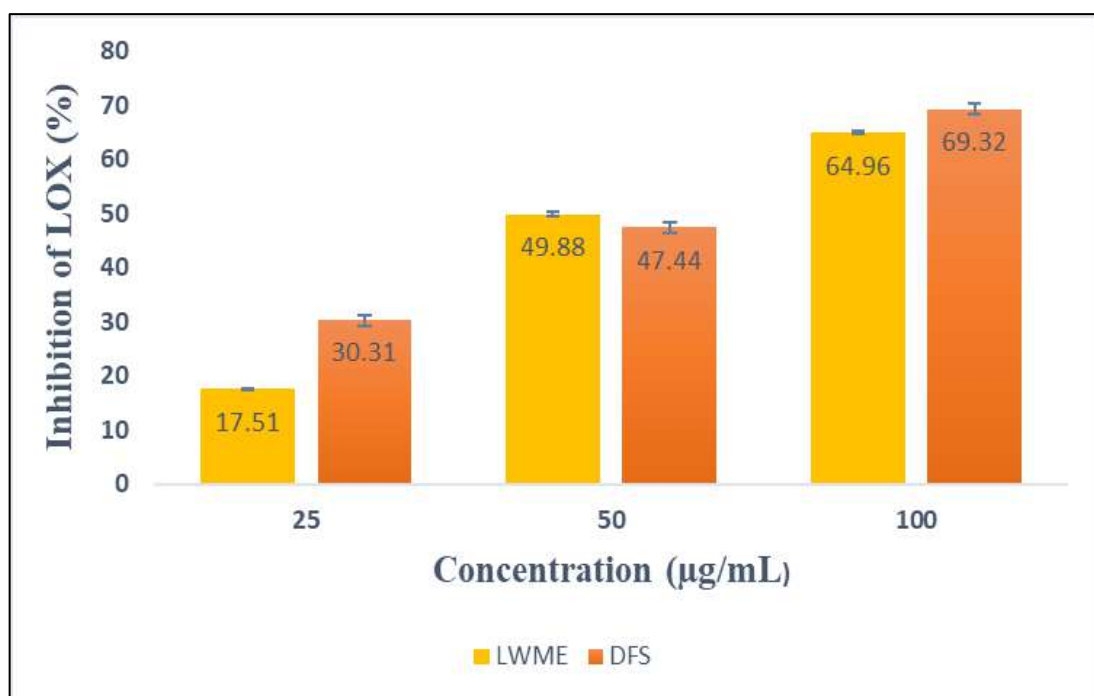


Fig 50 *In vitro* anti-inflammatory activity of LWME by LOX inhibition

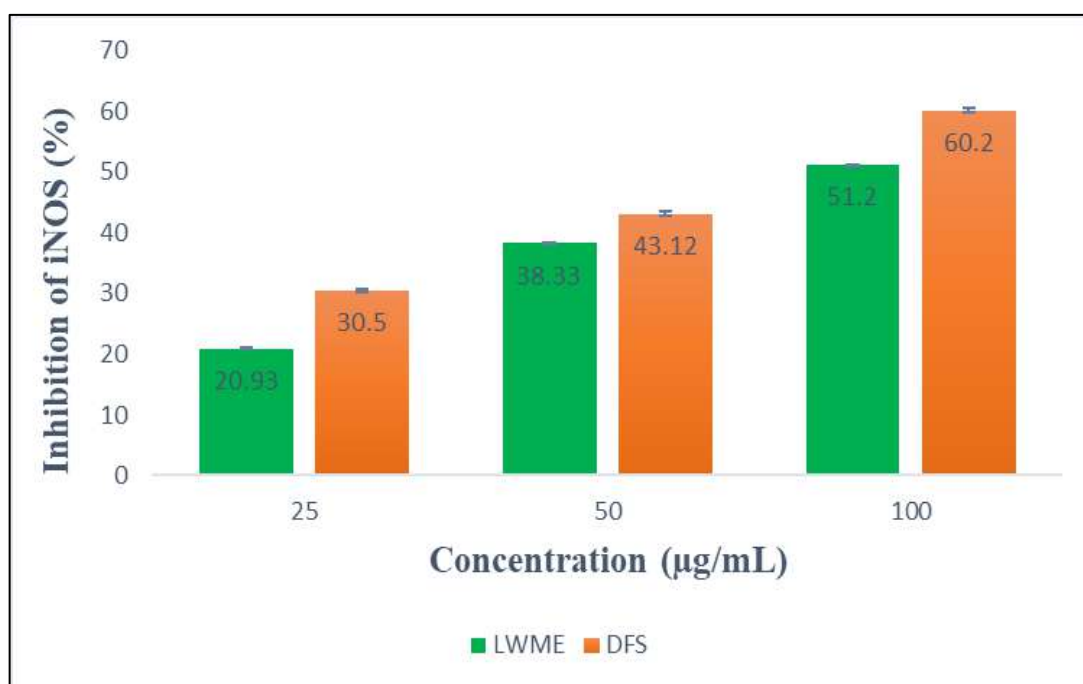


Fig 51 *In vitro* anti-inflammatory activity of LWME by inducible Nitric oxide synthase inhibition

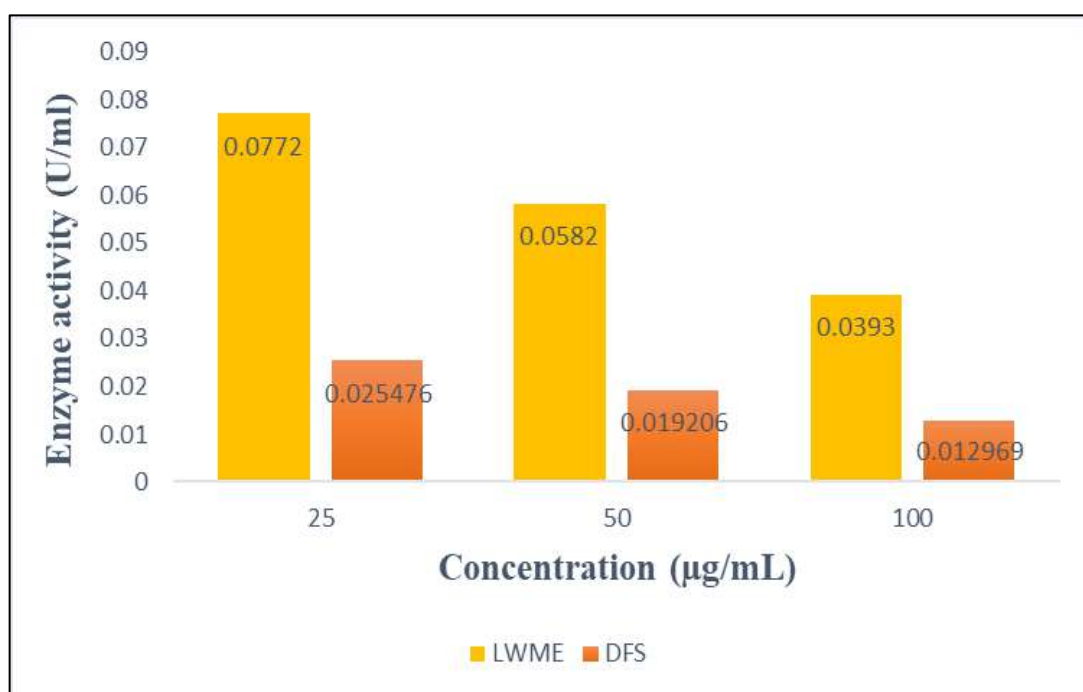


Fig 52 *In vitro* anti-inflammatory activity of LWME by Myeloperoxidase inhibition

- **Cellular Nitrite level**

Evaluation of total cellular nitrite level is utilized as an indicator in inflammatory studies. The higher level of nitrite is always associated with any kind of immune response in cells. Anti-inflammatory agents are commonly used to reduce the nitrite level by inhibiting the nitric oxide synthase enzyme. This experiment studied the potential of *L. wightiana* leaves to reduce the cellular nitrate level. For this, the plant extracts at concentrations of 25, 50, and 100 $\mu\text{g/mL}$ were treated with LPS-stimulated RAW.264.7 macrophage cell lines. The plant extract profoundly reduced the accumulation of NO with respect to increasing concentrations. The extract reduced the amount of NO from 489.55 μg to 208.395 μg (Fig 53).

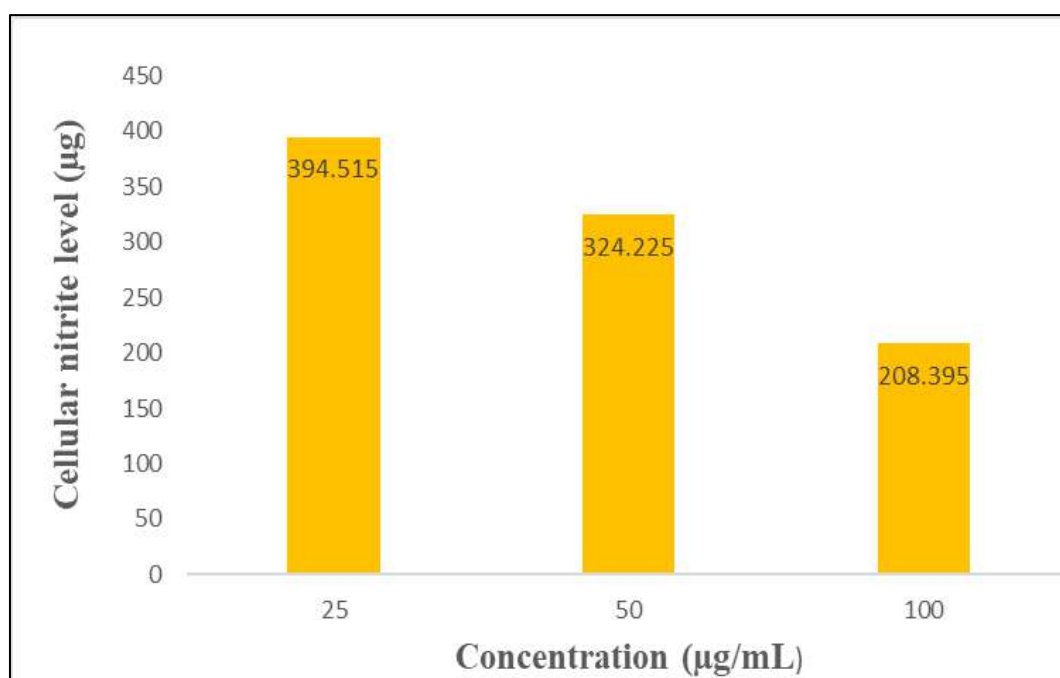


Fig 53 Cellular nitrite level after the treatment with LWME

Table 34 *In vitro* anti-inflammatory activity study by Enzyme inhibition assay

Name of Enzyme	Sample	Concentration (µg/mL)	Enzyme activity /Percentage of Inhibition ± SD
Cyclooxygenase	Extract (LWME)	25	18.01±0.13
		50	51.58±0.23
		100	65.44±0.12
	Standard (DFS)	25	35.95±0.12
		50	52.68±0.43
		100	70.78±0.33
Lipoxygenase	Extract (LWME)	25	17.51±0.15
		50	49.88±0.38
		100	64.96±0.33
	Standard (DFS)	25	30.31±0.29
		50	47.44±0.25
		100	69.32±0.2
Inducible nitric oxide synthase	Extract (LWME)	25	20.93±0.13
		50	38.33±0.15
		100	51.20±0.11
	Standard (DFS)	25	30.50±0.28
		50	43.12±0.34
		100	60.20±0.41

Table 35 *In vitro* anti-inflammatory activity study by Myeloperoxidase inhibition assay

Sample	Concentration (µg/mL)	Enzyme activity ± SD
LWME	25	0.0772±0.13
	50	0.0582±0.55
	100	0.0393±0.34
Standard (DFS)	25	0.0254±0.12
	50	0.0192±0.22
	100	0.0129±0.29

ii. Gene expression studies

To validate the anti-inflammatory effect of *L. wightiana* leaf extracts gene expression studies were carried out. qRT-PCR analysis was used for the study. The results indicated that all the proinflammatory cytokines and transcription factors demonstrated a downhill regulation of genes in LPS-induced RAW 264.7 cells by the extract with increasing concentration, as illustrated in Fig. 54. The extract showed suppression of Tumor Necrosis Factor (TNF)- α , Nuclear Factor kappa B (NFkB), Cyclooxygenase (COX)-2 and expression of Interleukin (IL)-6 (Fig 54).

iii. Docking Study

The molecular docking method was employed to assess the anti-inflammatory potential of the bioactive compounds in *L. wightiana* leaves. Twelve compounds were selected for the study and which were reported with anti-inflammatory activity and these compounds were tested against four target proteins. The overall result showed that, notably, dicentrinone (an alkaloid compound identified by LC/MS analysis) demonstrated favorable binding energy against all target proteins, including Human myeloperoxidase (-8.3), Human inducible Nitric oxide synthase (-9.5), Cyclooxygenase II (-9.2), and 5-Lipoxygenase (-8). These findings position dicentrinone as a promising anti-inflammatory agent. Analysis of interactions revealed that the compound forms a lone hydrogen bond interaction with 1dnu involving GLU102, and 4nos with ASN370. Additionally, it engages van der Waals and π interactions with all other target proteins. Notably, Xanthoplanine, Cubinol, Chalcone, α -cadinol, and similar compounds displayed substantial affinity with the selected proteins. Conversely, molecules such as linalool and hexadecenoic acid exhibited least binding energy and affinity. The binding geometries of the target proteins are illustrated in Figures 55 (i-ii) & 56 (i-ii) and Table 36.

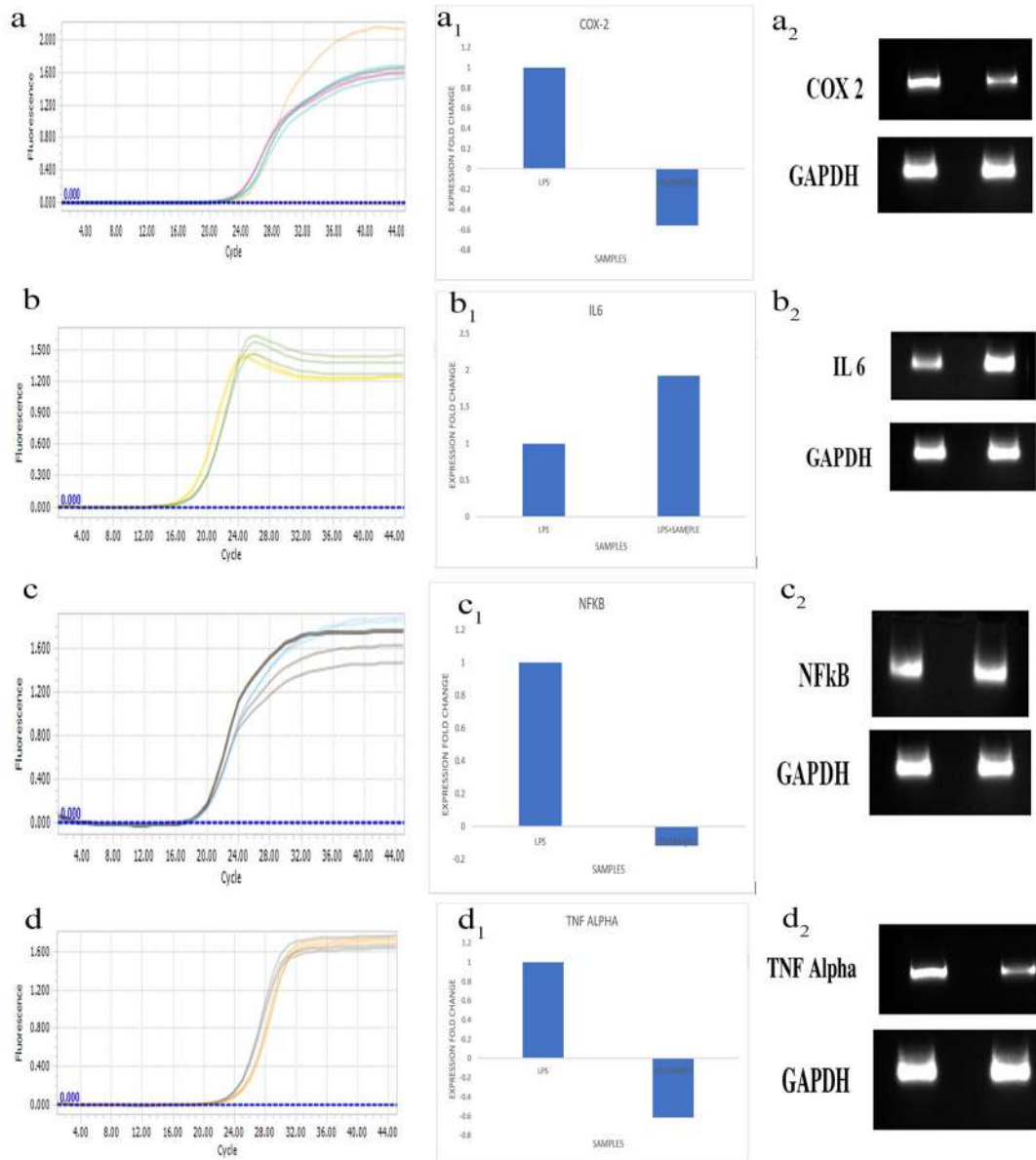


Fig 54 Gene expression analysis of LWME. a, a₁ and a₂. Amplification curve, expression fold change and gel image of COX-2; b, b₁ and b₂. Amplification curve, expression fold change and gel image of IL-6; c, c₁ and c₂. Amplification curve, expression fold change and gel image of NFκB; d, d₁ and d₂. Amplification curve, expression fold change and gel image of TNF Alpha

Table 36 Binding energy of docked proteins with target anti-inflammatory compounds

Compound	Binding Energy (BE) (Kcal/mol)			
	1dnu MPO	4nos iNOS	5ikr COX-2	6n2w LOX
Dicentrinone	-8.3	-9.5	-9.2	-8
Xanthoplanine	-7.8	-9.4	-8.5	-7.3
Chalcone	-7.6	-9.0	-7.5	-6.8
Cubenol	-7.6	-7.1	-7.5	-6.8
α - Cadinol	-7.5	-6.9	-6.9	-6.7
tau-Cadinol	-7.5	-6.9	-6.8	-6.7
1H-Cycloprop[e]azulene,decahydro-1,1,7-trimethyl-4-methylene	-7.3	-6.9	-6.0	-6.2
Carotol	-6.6	-6.6	-5.8	-5.9
9,12-Octadecadienoic acid(Z,Z)-methyl ester	-6.1	-6.5	-5.7	-5.5
Linalool	-5.8	-6.4	-5.3	-5
Decanedioic acid, bis(2-ethylhexyl) ester	-5.7	-6.1	-5.1	-4.7
Hexadecanoic acid-methyl-ester	-5.3	-5.5	-4.9	-4.7

iv. *In vivo* anti-inflammatory study

The *in vivo* anti-inflammatory studies were carried out in animal models using carrageenan induced paw edema model. The *L. wightiana* methanolic leaf extracts showed a remarkable reduction in the size of paw edema, which was treated with carrageenan, an inflammatory substance (Fig 57). The effect of LWME was found to be significant and dose dependent. The drug treatment of 50 mg/kg resulted in a progressive decrease in paw thickness, viz., 3.67 ± 0.04 mm, 3 ± 0.26 mm, 2.33 ± 0.37 mm in the third, fourth and fifth-hour periods, respectively and showed statistically significant at $P < 0.01$ level of significance. Similarly, treatment of the extract with 100 mg/kg exhibited a decline in paw thickness during the second hour after treatment and was significant with a P value < 0.05 . After the fourth hour treatment, the inflammation was reduced to 60.12%, which is higher than the treatment with standard Indomethacin at 58.02% (Table 37 & 38).

Table 37 *In vivo* anti-inflammatory activity of LWME by carrageenan induced paw edema method (Paw thickness)

Groups	Drug Treatment	Paw thickness in mm					
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr
I	Control 0.5%CMC (1 mL/kg)	2.33±0.21	4.50±0.22	5.17±0.31	5.33±0.33	5.17±0.31	4.50±0.22
II	Indomethacin (10 mg/kg)	2.17±0.31	2.83±0.31***	3.17±0.31***	2.50±0.22***	2.17±0.17***	2.17±0.21***
III	LWME (50 mg/kg)	1.83±0.31	3.83±0.31	4.00±0.33	3.67±0.04**	3.00±0.26***	2.33±0.37**
IV	LWME (100 mg/kg)	2.17±0.31	3.17±0.31	3.33±0.17**	3.00±0.26***	2.50±0.22***	2.17±0.21

Values are in Mean±SEM(n=6)

*P<0.05, **P<0.01&***P<0.001 Vs control

Table 38 *In vivo* anti-inflammatory activity of LWME by carrageenan induced paw edema method (% of inhibition)

Groups	% inhibition					
	Drug treatment	1 hr	2 hr	3 hr	4 hr	5 hr
I	Control 0.5% CMC (1 mL/kg)	-	-	-	-	-
II	Indomethacin 10 mg/kg	37.11	38.68	53.09	58.02	51.78
III	LWME 50 mg/kg	14.89	22.63	31.14	41.97	60.12
IV	LWME 100 mg/kg	38.12	42.33	55.43	60.12	65.34

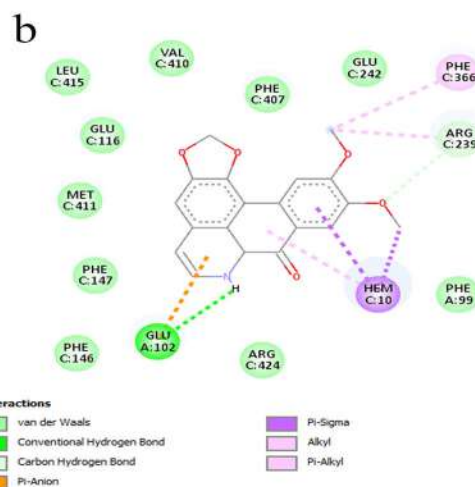
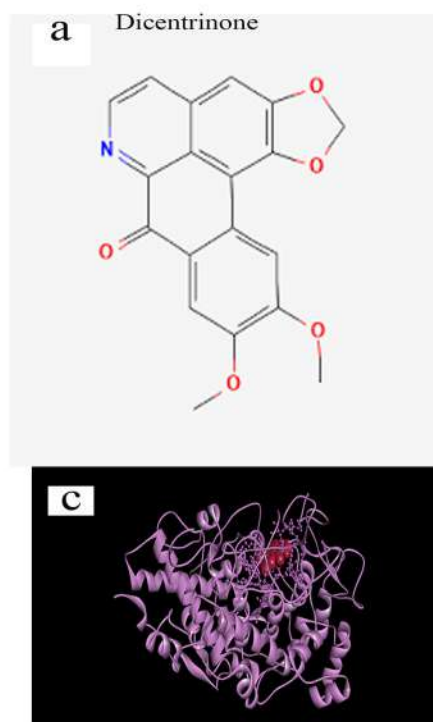


Fig 55(i) Molecular docking of anti-inflammatory compound from LWME with target protein. a. Dicentrinone structure; b. Bound conformation of 1dnu protein and ligand; c. 3D interaction between protein and ligand

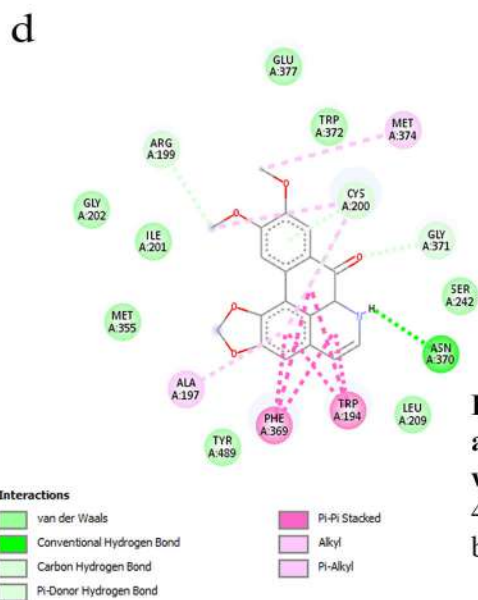


Fig 55 (ii) Molecular docking of anti-inflammatory compound from LWME with target protein. d. Bound conformation of 4nos protein and ligand; e. 3D interaction between protein and ligand

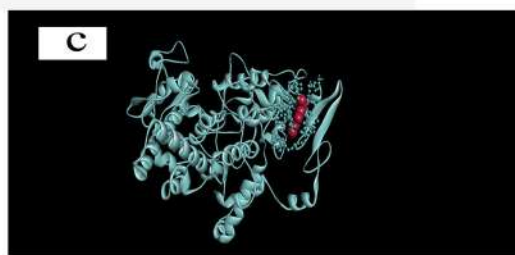
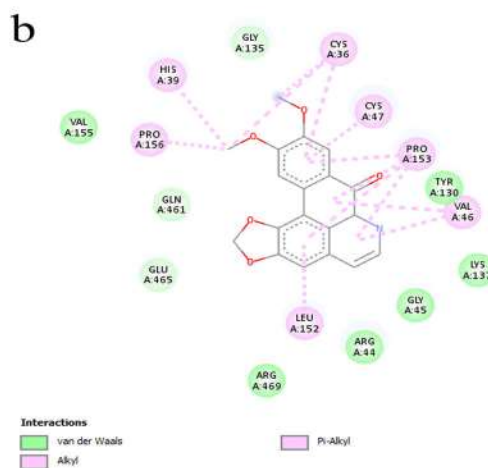
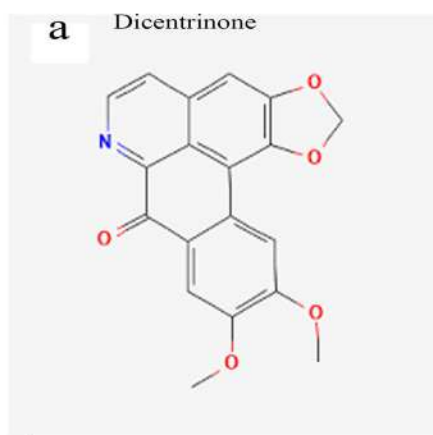


Fig-56(i) Molecular docking of anti-inflammatory compound from LWME with target protein. a. Dicentrinone structure; b Bound conformation of 5ikr protein and ligand; c. 3D interaction between protein and ligand

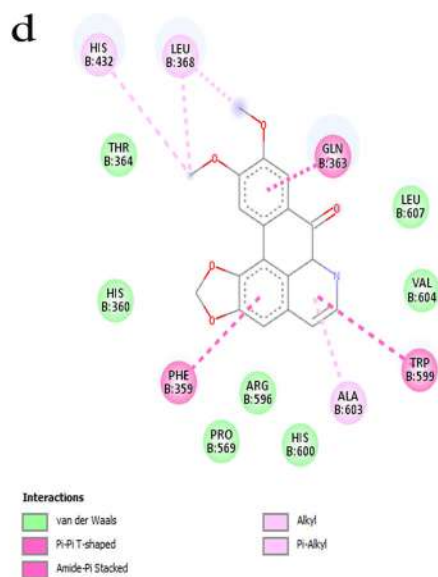


Fig 56(ii) Molecular docking of antiinflammatory compound from LWME with target protein. d. Bound conformation of 6n2w protein and ligand; e. 3D interaction between protein and ligand



Fig 57 *In vivo* anti-inflammatory study by carrageenan induced paw edema in wistar albino rats. a. Paw edema induction; b. LWME administration; c. Measurement of paw thickness

F. WOUND HEALING STUDY

i. *In vitro* wound healing assay

The *in vitro* scratch wound assay by *L. wightiana* methanolic leaf extract (25 µg/mL) showed the maximum (100%) wound closure in scratch wounds in L929 cell lines at 72 hr period which is considered to be a significant wound healing effect when compared with the control (untreated cells) which showed 94.4 % wound closure and cell migration at the same period (Fig 58).

ii. *In vivo* wound healing study

● Excision wound model

The excision wound method in the model animals demonstrated that the methanolic extract of *L. wightiana* leaves resulted in the maximum healing of wounds on 21st day post-wounding (Fig 59). The animals treated with 10% concentration of LMWE extract showed remarkable reduction in the wound area (95.04%) and faster rate of epithelialization showed reduction in wound area (19.67 ± 1.05 mm²). In control animals, it is observed that, after the 21st day, the animals treated with reference control povidone iodine ointment showed complete epithelialization and wound contraction (98.08%). The results were statistically significant at $P < 0.01$ and $P < 0.001$ levels. The results are depicted in Tables 39 & 40.

Table 39. Effect of LMWE on excision wounds in rats (Reduction in wound area)

Groups	Drug Treatment	Wound area mm ²				
		0 Day	3 rd Day	7 th Day	14 th Day	21 st Day
I	Vehicle Control (Simple Ointment)	486.17± 3.07	470.00± 0.77	427.66± 3.54	265.33± 2.01	148.17± 5.98
II	Reference Control Povidone Iodine	483.16± 1.94	430.00± 1.46*	297.00± 2.63***	96.33± 1.38***	6.33± 0.76***
III	5% LMWE	485.67± 2.04	452.67± 2.17	358.83± 2.62*	163.17± 2.87***	43.50± 1.28***
IV	10% LMWE	484.33± 2.59	432.66± 1.49*	322.00± 4.25**	144.50± 1.65***	19.67± 1.05***

Values are in Mean ± SEM; (n = 6)

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ Vs Vehicle Control

Table 40. Effect of LMWE on excision wounds in rats (Percentage of reduction in wound size)

Groups	Drug Treatment	Percentage Reduction in Wound Size (%)			
		3 rd Day	7 th Day	14 th Day	21 st Day
I	Vehicle Control (Simple Ointment)	3.33%	12.03%	45.42%	69.52%
II	Reference Control Povidone Iodine	11.00%	38.52%	80.06%	98.68%
III	5% LMWE	6.79%	26.12%	66.40%	91.04%
IV	10% LMWE	10.67%	33.52%	70.16%	95.94%

• Histopathology

Histopathological examinations of the wound sections stained with hematoxylin revealed a greater assemblage of macrophages with few collagen fibers in the control samples. Complete tissue regeneration was seen in the wound sections treated with the standard, there were more collagen fibers, better-organized blood vessels, and fewer macrophages. The 5% methanolic extract-treated animals had minimal macrophages and moderate collagen deposition, and the animals treated with 10% LMWE had improved healing, including thick collagenation, re-epithelialization, and the absence of macrophages (Fig 60).

iii. Docking Study

To assess the wound healing activity by molecular docking of 4 target proteins, like glycogen synthase kinase-3 β (GSK-3 β), transforming growth factor-beta (TGF- β), c-myc, and β -catenin, with 10 ligand molecules, a docking study was conducted. The ligand compounds were dicentrinone, lauroilsine, corytuberine, reticuline, eudesmin, grandisin, dehydroisoeugenol, 9,12-octadecadienoic acid(z,z) methyl ester, n-hexadecanoic acid, and linalool. The compounds selected were based on their anti-inflammatory and antioxidant potential, and these properties were associated with wound healing potential in plants. The binding energy of docked conformations is given in Table 41. In docking experiments with TGF- β , the results showed that, except linalool, all compounds showed better interaction affinity

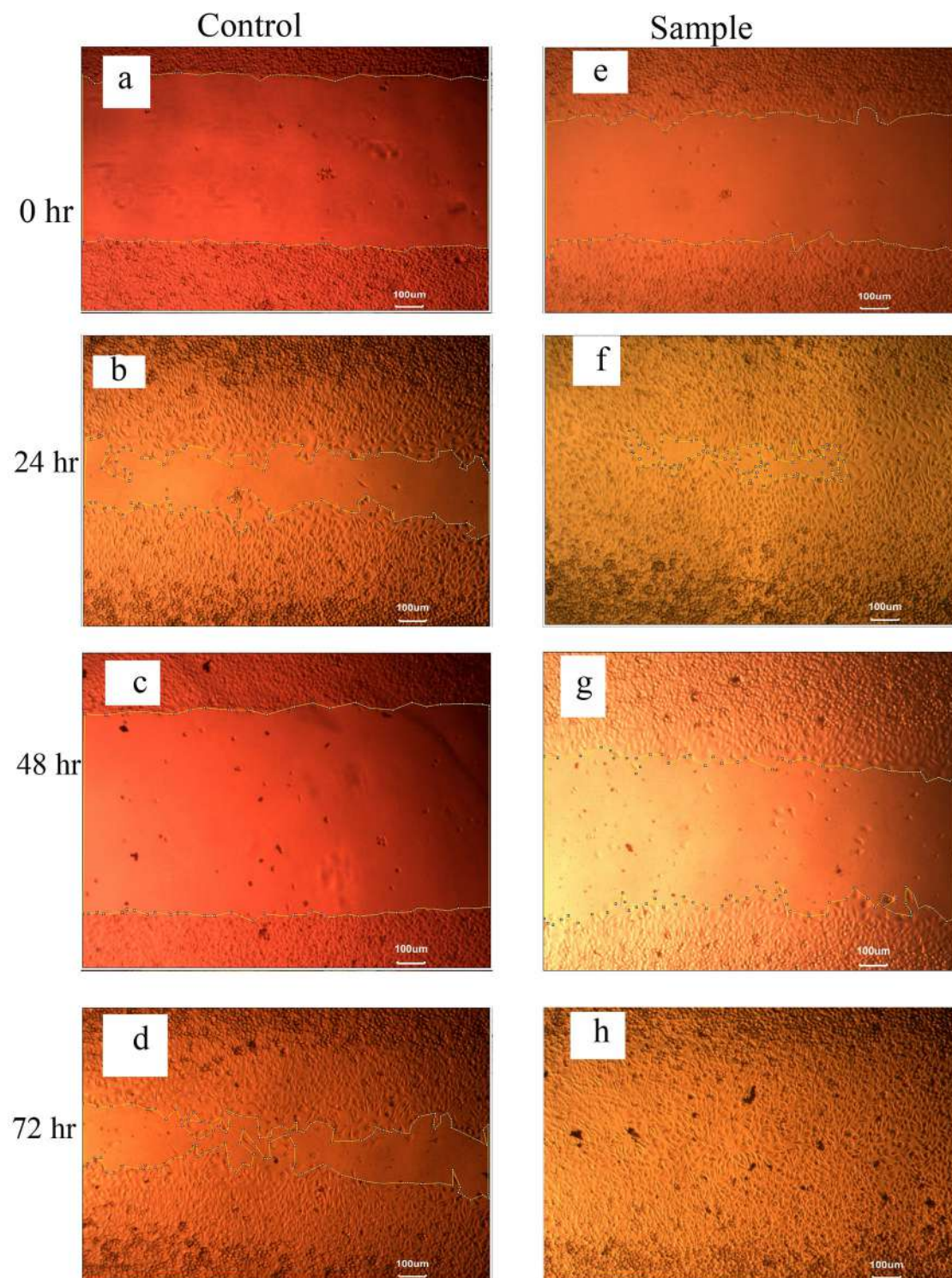


Fig 58 *In vitro* scratch wound healing assay. a-d. Control-cell migration after 72 hr treatment; e-h. Sample- cell migration after treatment with LWME after 72 hours

Drug Treatment	0 Day	3 rd Day	7 th Day	14 th Day	21 st Day
Control (Simple Ointment)	 A	 B	 C	 D	 E
Reference Control Povidone Iodine	 F	 G	 H	 I	 J
5% LMWE	 K	 L	 M	 N	 O
10% LMWE	 P	 Q	 R	 S	 T

Fig 59 *In vivo* wound healing study by LWME- wound contraction on different post excision days(1-21 days) A-E. Control; F-J. Reference control; K-O. LWME (5%); P-T. LWME (10%)

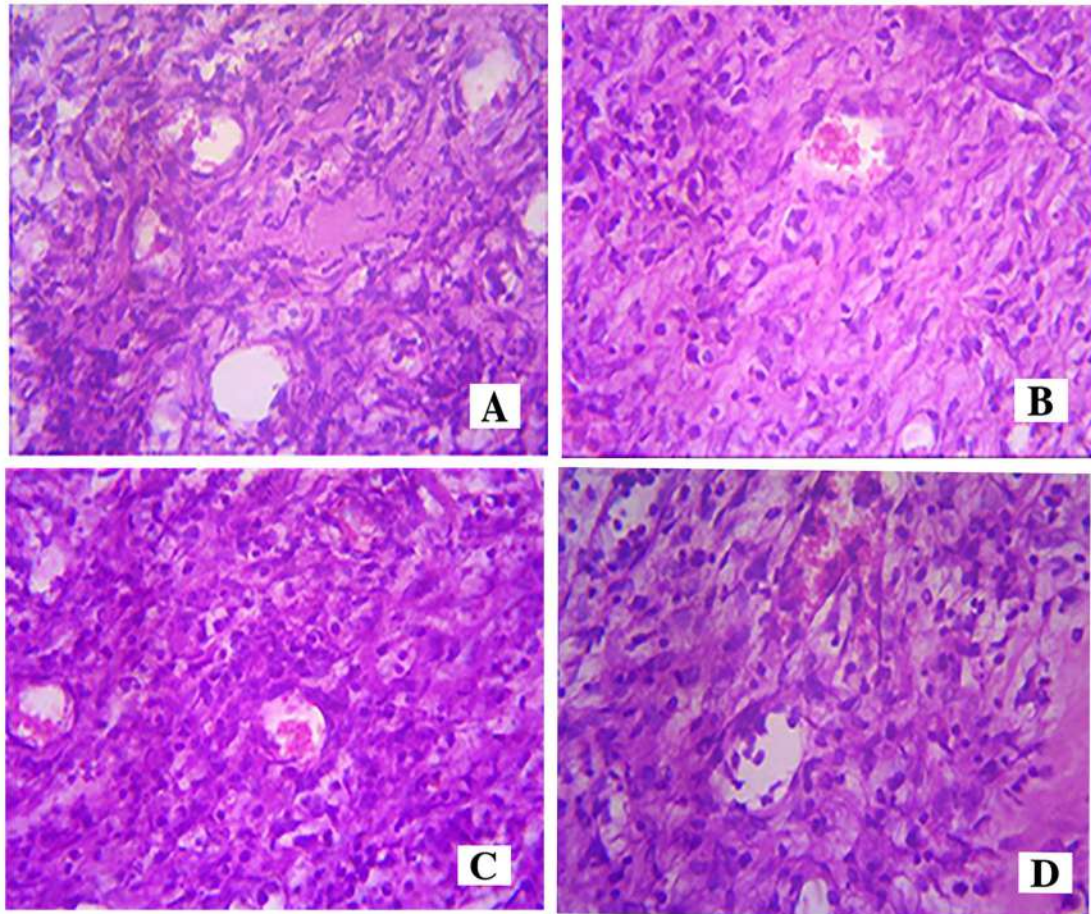


Fig 60 Histopathological study-histological section of granuloma tissue. A. Cells treated with control- incomplete healing; B. Standard-complete healing; C. 5% LWME- moderate healing with increased collagenation and lesser macrophages; D. 10% LWME- better healing with increased collagenation and lesser macrophages

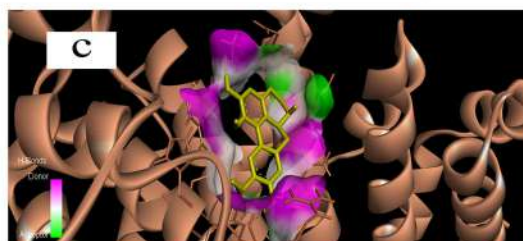
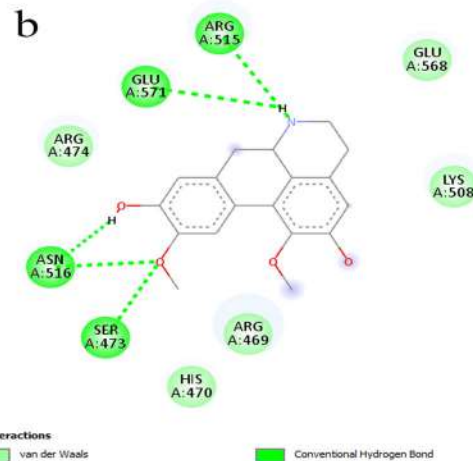
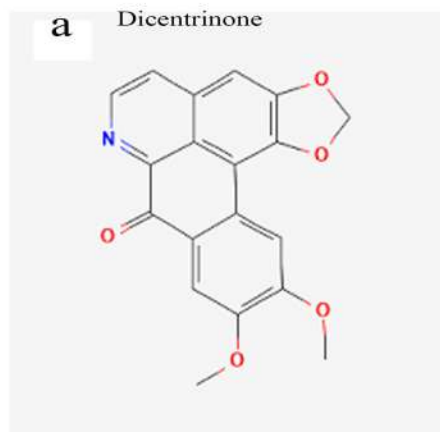


Fig 61(i) Molecular docking of wound healing compound from LWME with target protein. a. Dicentrinone structure; b Bound conformation of 1nkp protein and ligand; c. 3D interaction between protein and ligand

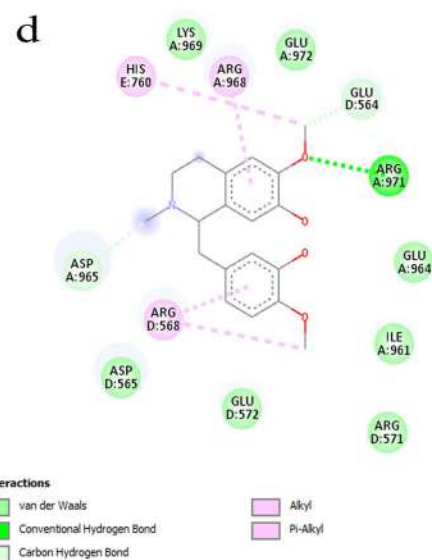
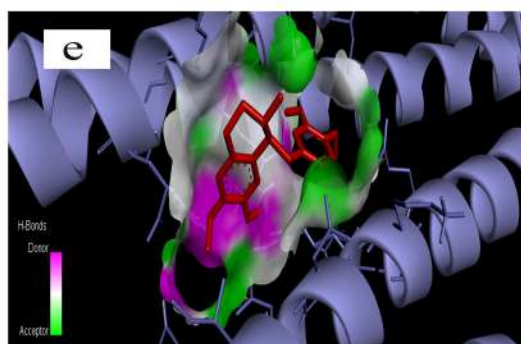


Fig 61(ii) Molecular docking of wound healing compound from LWME with target protein. d. Bound conformation of 1jdh protein and ligand; e. 3D interaction between protein and ligand

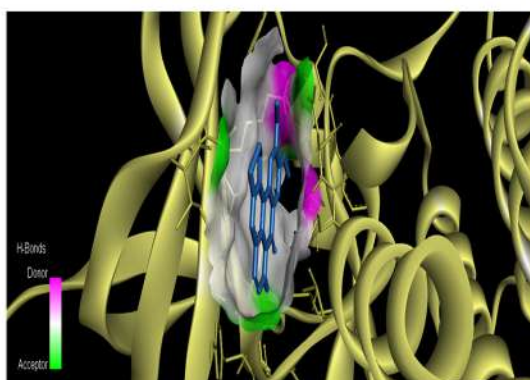
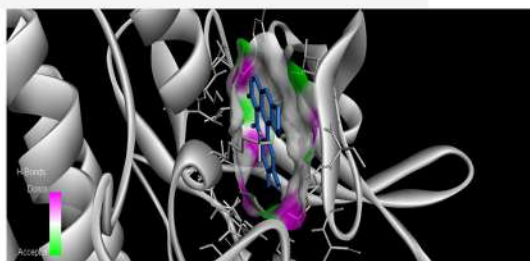
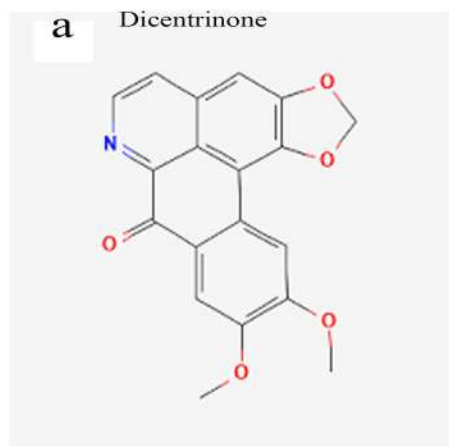


Fig 62(ii) Molecular docking of wound healing compound from LWME with target protein. d. Bound conformation of 5oy4 protein and ligand; c. 3D interaction between protein and ligand

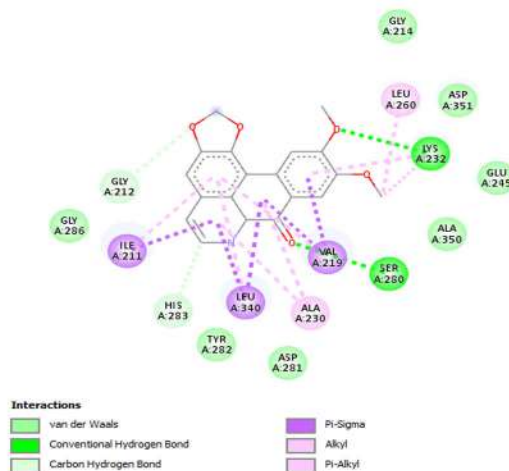
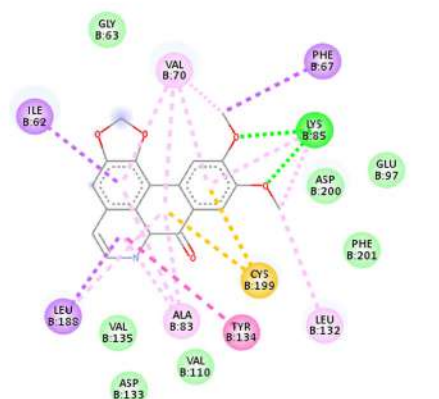


Fig 62(i) Molecular docking of wound healing compound from LWME with target protein. a. Dicentrinone structure; b Bound conformation of 5e8w protein and ligand; c. 3D interaction between protein and ligand



towards the target. From the results, the compound dicentrinone was found to exhibit the highest affinity towards all the selected proteins targets. Docking results with GSK-3 β showed that six among ten molecules showed high affinity towards the target proteins, in which dicentrinone exhibited the highest affinity towards GSK-3 β . Molecular docking with c-myc against ten molecules revealed that six ligands showed high affinity. The docking studies against β -catenine by target compounds showed that six compounds were active against β -catenine, Three-dimensional representation of the binding of compounds with the target proteins and interaction diagrams are given in Figures 61 (i-ii) & 62 (i-ii).

Table 41. Binding energy of docked proteins with target wound healing compounds

Compound	Binding Energy (BE) (Kcal/mol)			
	TGF- β (5e8w)	GSK-3 β (5oy4)	c-myc (1nkp)	β - catenine (1jdh)
Dicentrinone	-10.2	-9.0	-7.7	-6.7
Lauroilsine	-9.6	-8.6	-7.5	-6.6
Reticuline	-9.6	-8.3	-7.3	-6.6
Corytuberine	-9.3	-7.9	-6.9	-6.6
Eudesmin	-8.3	-7.2	-6.7	-6.6
Grandisin	-7.2	-6.8	-6.6	-6.0
Dehydroisoeugenol	-6.8	-5.9	-5.8	-5.2
9,12-octadecadienoic acid (z,z) methyl ester	-6.4	-5.8	-5.4	-5.1
n-hexanoic acid	-6.2	-5.2	-5.3	-4.7
Linalool	-5.6	-5.0	-4.7	-4.7

G. STUDY OF INSECTICIDAL ACTIVITY

The essential oil isolated from *L. coriacea* was exposed to insecticidal activity tests, including contact toxicity, fumigant toxicity, and repellency assays.

i. Contact toxicity

Essential oil isolated from *L. coriacea* leaves was subjected to contact toxicity assay. Different aliquots of oil (5 to 80 ($\mu\text{L}/\text{cm}^2$) prepared in acetone were applied against two stored pests, such as *S. oryzae* and *T. castaneum*, at 24, 48, and 72 hr periods and the efficacy of the oil was evaluated by counting the mortality rate of insects. Maximum mortality of *S. oryzae* insects was observed as $98.8 \pm 0.042\%$ at 72 hrs at maximum concentration. At the same time, the mortality rate of *T. castaneum* was $81.6 \pm 0.063\%$. LD_{50} and LD_{90} and chi-square values were calculated, and the results are depicted in Tables 42 & 43 and Figures 63 & 64.

ii. Fumigant toxicity

Fumigant toxicity assay was conducted to identify the effect of *L. coriacea* essential oil against the target insects *S. oryzae* and *T. castaneum*. It was observed that depending on the different concentrations of essential oil, such as 5, 10, 20, 40, and 80 $\mu\text{L}/\text{L}$ concentrations, *S. oryzae* showed maximum mortality at a rate of $69.2 \pm 0.050\%$, $73.2 \pm 0.95\%$, $83 \pm 0.038\%$, $91.8 \pm 0.085\%$ and $99.2 \pm 0.042\%$, respectively after three days' (72 hr) treatment. At the same concentration and time duration, *T. castaneum* insects showed mortality at a rate of $54.4 \pm 0.077\%$, $62.8 \pm 0.104\%$, $67.8 \pm 0.272\%$, $77.6 \pm 0.095\%$, and $82 \pm 0.129\%$. From the observations, it can be indicated that essential oil showed significant fumigant effects against both the insects, but among them, the maximum effect was towards *S. oryzae*. LC_{50} , LC_{90} , and chi-square values were determined. The results are demonstrated in Tables 44 & 45 and Figures 65 & 66.

iii. Repellency

The repellent activity of *S. oryzae* and *T. castaneum* by the *L. coriacea* essential oil was demonstrated. Essential oils at different concentrations, such as 0.16- 0.63 $\mu\text{L}/\text{cm}^2$ was treated with insects, and after 24 hours it was observed that maximum repellency shown by *S. oryzae* was $88.4 \pm 0.028\%$, $92.4 \pm 0.045\%$, $95.8 \pm 0.055\%$, and 100% and by *T. castaneum* was $71 \pm 0.072\%$, $75 \pm 0.040\%$, $81.4 \pm 0.083\%$, and $85 \pm 0.038\%$, respectively. LC_{50} and LC_{90} values were also recorded. Results are represented in Tables 46 & 47 and Figures 67 & 68.

Table 42 Contact toxicity effect of *L. coriacea* leaves essential oil against *S. oryzae* and *T. castaneum*

Concentration ($\mu\text{L/L}$ air)	% of Mortality (<i>S. oryzae</i>) Mean $\pm$ SD			% of Mortality (<i>T. castaneum</i>) Mean $\pm$ SD		
	24 Hrs	48 Hrs	72 Hrs	24 Hrs	48 Hrs	72 Hrs
5	19.6 $\pm$ 0.09 ^e	40.2 $\pm$ 0.102 ^e	56.8 $\pm$ 0.55 ^e	14 $\pm$ 0.062 ^e	24.6 $\pm$ 0.055 ^e	45.4 $\pm$ 0.040 ^e
10	31.8 $\pm$ 0.085 ^d	54.6 $\pm$ 0.037 ^d	71.6 $\pm$ 0.067 ^d	28.4 $\pm$ 0.116 ^d	32.6 $\pm$ 0.10 ^d	55.8 $\pm$ 0.055 ^d
20	39 $\pm$ 0.089 ^c	67.8 $\pm$ 0.050 ^c	90.2 $\pm$ 0.044 ^c	36 $\pm$ 0.098 ^c	48.8 $\pm$ 0.059 ^c	64 $\pm$ 0.062 ^c
40	44.4 $\pm$ 0.043 ^b	77.4 $\pm$ 0.050 ^b	95.2 $\pm$ 0.042 ^b	40.4 $\pm$ 0.041 ^b	54.2 $\pm$ 0.056 ^b	71.2 $\pm$ 0.049 ^b
80	58 $\pm$ 0.047 ^a	87.2 $\pm$ 0.070 ^a	98.8 $\pm$ 0.042 ^a	54 $\pm$ 0.046 ^a	66.8 $\pm$ 0.079 ^a	81.6 $\pm$ 0.063 ^a

Mean $\pm$ SE of three replications. Mortality of insects was determined by Analysis of Variance (ANOVA), the F value was calculated, followed by LSD at a 0.05% level of significance.

Table 43 Contact toxicity effect of *L. coriacea* leaves essential oil against *S. oryzae* and *T. castaneum* represented by Probit analysis

Time duration (Hrs)	<i>S. oryzae</i> (% mortality)			(X ²)	<i>T. castaneum</i> (% mortality)			(X ²)
	LD50 (LC90) (Log Conc.)	LD ₅₀ (LCL-UCL)	LD ₉₀ (LCL-UCL)		LD50 (LC90) (Log Conc.)	LD ₅₀ (LCL-UCL)	LD ₉₀ (LCL-UCL)	
24	1.125 (2.589)	(0.962-1.254)	(2.260-1.254)	0.201*	1.683(3.273)	(1.527-1.930)	(2.767-4.283)	0.977*
48	0.908(2.043)	(0.745-1.026)	(1.854-2.344)	0.095*	1.436(2.815)	(1.314-1.580)	(2.459-3.452)	0.992*
72	0.621(1.355)	(0.454-0.735)	(1.261-1.484)	0.973*	0.844(2.433)	(0.568-1.010)	(2.111-3.064)	0.808*

LD₅₀- Lethal dose that kills 50% of treated insects, LD₉₀- Lethal dose that kills 90% of treated insects, LCL- Lower confidence limit, UCL- Upper confidence limit, X² – Chi-square value significant at p< 0.05 level.

Table 44 Fumigant effect of *L. coriacea* leaves essential oil against *S. oryzae* and *T. castaneum*

Concentration ($\mu\text{L/L air}$)	Mortality % (<i>S. oryzae</i>)			Mortality % (<i>T. castaneum</i>)		
	8 Hrs	12 Hrs	24 Hrs	8 Hrs	12 Hrs	24Hrs
5	35.8 $\pm$ 0.123 ^e	57.2 $\pm$ 0.055 ^e	69.2 $\pm$ 0.050 ^e	32 $\pm$ 0.088 ^e	45.4 $\pm$ 0.040 ^e	54.4 $\pm$ 0.077 ^e
10	47 $\pm$ 0.051 ^d	61.6 $\pm$ 0.072 ^d	73.2 $\pm$ 0.095 ^d	36.6 $\pm$ 0.073 ^d	52.4 $\pm$ 0.115 ^d	62.8 $\pm$ 0.104 ^d
20	54.6 $\pm$ 0.122 ^c	67.2 $\pm$ 0.010 ^c	83 $\pm$ 0.038 ^b	43 $\pm$ 0.143 ^c	57.6 $\pm$ 0.100 ^c	67.8 $\pm$ 0. 272 ^c
40	62.2 $\pm$ 0.113 ^b	76.6 $\pm$ 0.031 ^b	91.8 $\pm$ 0.085 ^b	58.4 $\pm$ 0.074 ^b	67 $\pm$ 0.096 ^b	77.6 $\pm$ 0.095 ^b
80	66.6 $\pm$ 0.092 ^a	89.2 $\pm$ 0.068 ^a	99.2 $\pm$ 0.042 ^a	64.4 $\pm$ 0.083 ^a	82.8 $\pm$ 0.105 ^a	82 $\pm$ 0.129 ^a

Mean $\pm$ SE of three replications. Mortality of insects was determined by Analysis of Variance (ANOVA), the F value was calculated, followed by LSD at a 0.05% level of significance.

Table 45 Fumigant effect of *L. coriacea* leaves essential oil against *S. oryzae* and *T. castaneum* represented by Probit analysis

Time duration (Hrs)	<i>S. oryzae</i> (% mortality)			(X ²)	<i>T. castaneum</i> (% mortality)			(X ²)
	LC50 (LC90) (Log Conc.)	LC ₅₀ (LCL-UCL)	LC ₉₀ (LCL-UCL)		LC50 (LC90) (Log Conc.)	LC ₅₀ (LCL-UCL)	LC ₉₀ (LCL-UCL)	
8	1.042 (2.170)	(0.916-1.141)	(1.356-0.803)	0.227*	2.183(3.24)	(2.091-2.297)	(2.922-3.935)	4.516*
12	0.656(1.709)	(0.356-0.803)	(1.516-2.124)	8.131*	1.883(2.897)	(1.717-1.983)	(2.671-3.367)	7.710*
24	0.507(1.245)	(0.245-0.651)	(1.157-1.380)	11.887*	1.654 (2.810)	(1.306-1.812)	(2.579-3.350)	1.715*

LC₅₀- Lethal concentration that kills 50% of treated insects, LC₉₀- Lethal concentration that kills 90% of treated insects, LCL- Lower confidence limit, UCL- Upper confidence limit, X² – Chi-square value significant at p< 0.05 level.

Table 46 Repellency effect of *L. coriacea* leaves essential oil against *S. oryzae* and *T. castaneum*

Concentration ($\mu\text{L}/\text{cm}^2$)	<i>S. oryzae</i> Percentage Repellency (PR)					<i>T. castaneum</i> Percentage Repellency (PR)				
	2 Hrs	4 Hrs	8 Hrs	12 Hrs	24 Hrs	2 Hrs	4 Hrs	8 Hrs	12 Hrs	24 Hrs
0.16	59.8 $\pm$ 0.054 ^d	64 $\pm$ 0.078 ^c	75.2 $\pm$ 0.065 ^a	79 $\pm$ 0.050 ^b	88.4 $\pm$ 0.028 ^b	40.6 $\pm$ 0.069 ^d	55 $\pm$ 0.047 ^d	69.8 $\pm$ 0.050 ^d	75.6 $\pm$ 0.195 ^c	71 $\pm$ 0.072 ^d
0.31	67.8 $\pm$ 0.082 ^c	70.2 $\pm$ 0.042 ^b	77 $\pm$ 0.050 ^a	84.2 $\pm$ 0.042 ^a	92.4 $\pm$ 0.045 ^a	62.6 $\pm$ 0.056 ^c	70.6 $\pm$ 0.053 ^c	84.6 $\pm$ 0.029 ^c	85.8 $\pm$ 0.070 ^b	75 $\pm$ 0.040 ^c
0.47	74.2 $\pm$ 0.058 ^b	79.8 $\pm$ 0.042 ^a	82 $\pm$ 0.027 ^a	89 $\pm$ 0.00 ^a	95.8 $\pm$ 0.055 ^a	75.6 $\pm$ 0.031 ^b	82 $\pm$ 0.067 ^b	96.6 $\pm$ 0.027 ^b	94.4 $\pm$ 0.028 ^a	81.4 $\pm$ 0.083 ^b
0.63	87.8 $\pm$ 0.022 ^a	92.8 $\pm$ 0.022 ^a	97 $\pm$ 0.012 ^a	98 $\pm$ 0.00 ^a	100.0.000 ^a	93.8 $\pm$ 0.011 ^a	90.6 $\pm$ 0.028 ^a	97.2 $\pm$ 0.00a	93.8 $\pm$ 0.011 ^a	90.6 $\pm$ 0.028 ^a

Mean $\pm$ SE of three replications. Repellency of insects was determined by Analysis of Variance (ANOVA), the F value was calculated, followed by LSD at a 0.05% level of significance

Table 47 Repellency effect of *L. coriacea* leaves essential oil against *S. oryzae* and *T. castaneum* represented by Probit analysis

Time duration (Hrs)	<i>S. oryzae</i> (% mortality)			(X ²)	<i>T. castaneum</i> (% mortality)			(X ²)
	LC50 (LC90) (Log Conc.)	LC ₅₀ (LCL- UCL)	LC ₉₀ (LCL- UCL)		LC50 (LC90) (Log Conc.)	LC ₅₀ (LCL- UCL)	LC ₉₀ (LCL- UCL)	
2	-0.877(-0.324)	(-1.037—0.780)	(-0.400- -0.209)	0.105*	-0.671(0.169)	(-0.753- 0.611)	(-0.250—0.046)	0.062*
4	-1.085(-0.656)	(-1.369-0.950)	(0.-737- -0.578)	0.908*	-0.839(0.159)	(-1.0240-734)	(-0.266—0.031)	0.599*
8	-3.481(-1.986)	(-4.931-- 0.259)	(-2.120- -0.832)	0.700*	-0.947(0.501)	(-1.111-0.850)	(-0.563—0.421)	0.092*
12	-1.527(-1.022)	(-2.062- -0.458)	(-2.812- -0.096)	0.832*	-1.130(0.468)	(-1.5180.951)	(-0.555—0.351)	0.584*
24	-4.377(-1.691)	(-5.126- -0.288)	(-2.112- -0.053)	0.878*	-1.445(0.160)	(-4.6161.007)	(-0.148—2.287)	0.803*

LC₅₀- Lethal concentration that kills 50% of treated insects, LC₉₀- Lethal concentration that kills 90% of treated insects, LCL- Lower confidence limit, UCL- Upper confidence limit, X²– Chi-square value significant at p< 0.05 level.

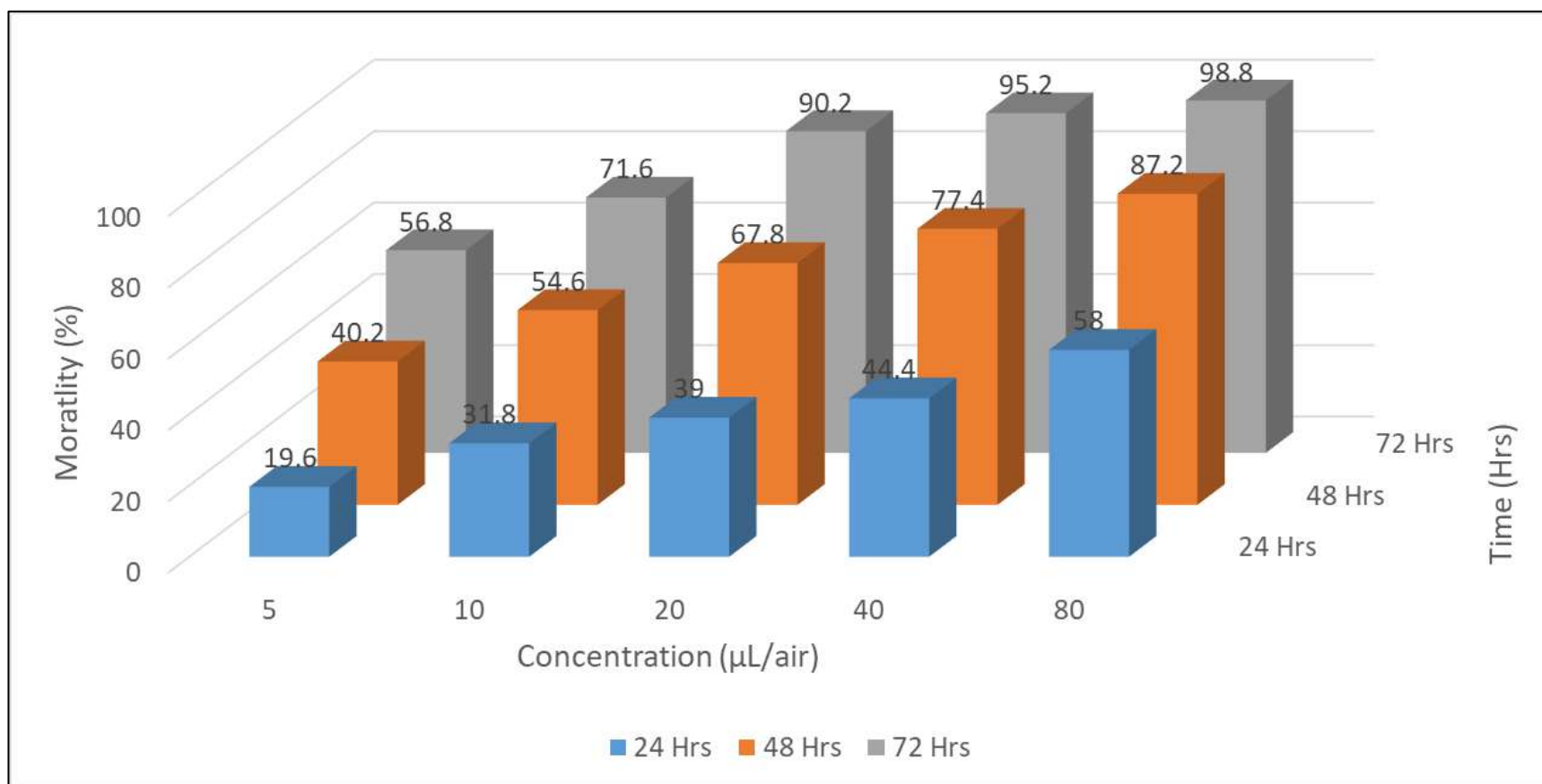


Fig 63 Contact toxicity effect of *L. coriacea* leaves essential oil against *S. oryzae*

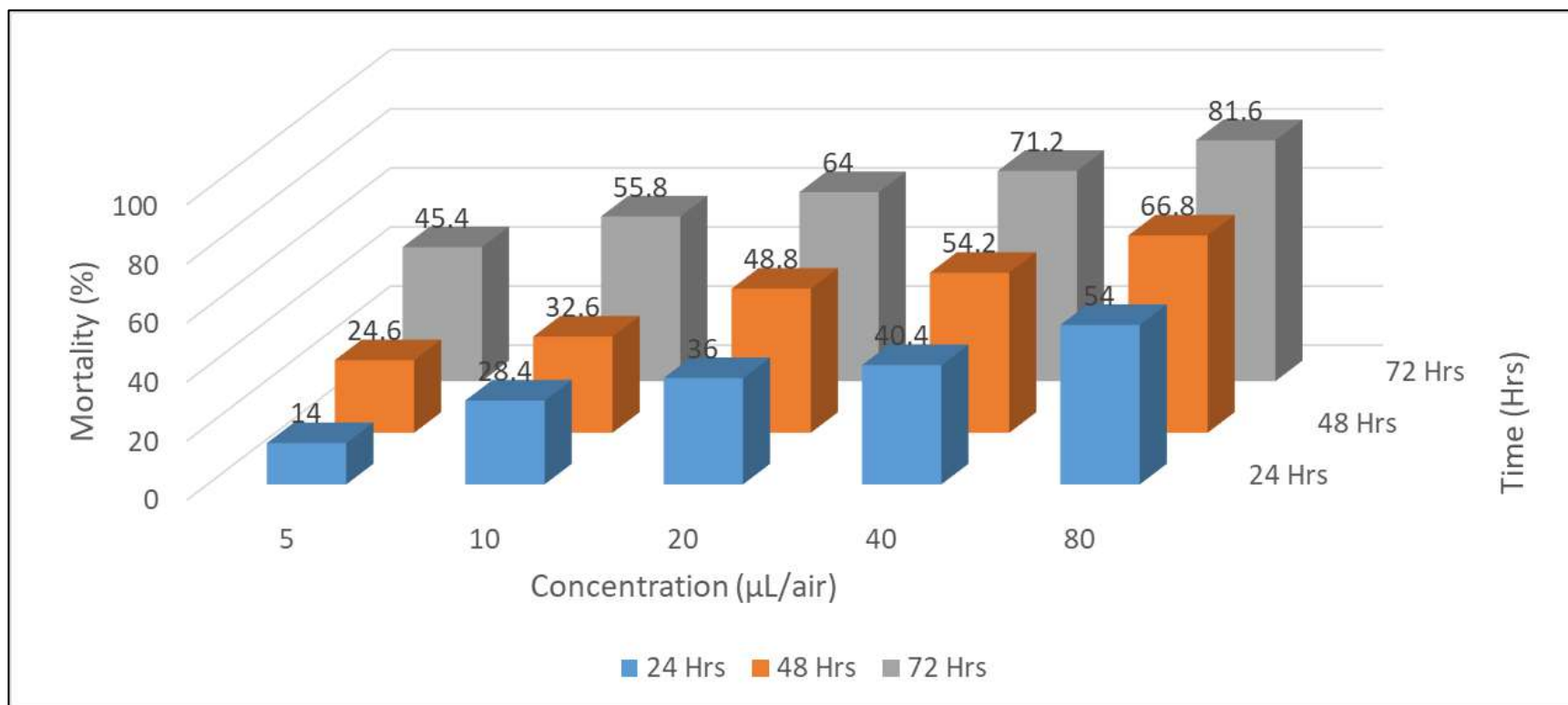


Fig 64 Contact toxicity effect of *L. coriacea* leaves essential oil against *T. castaneum*

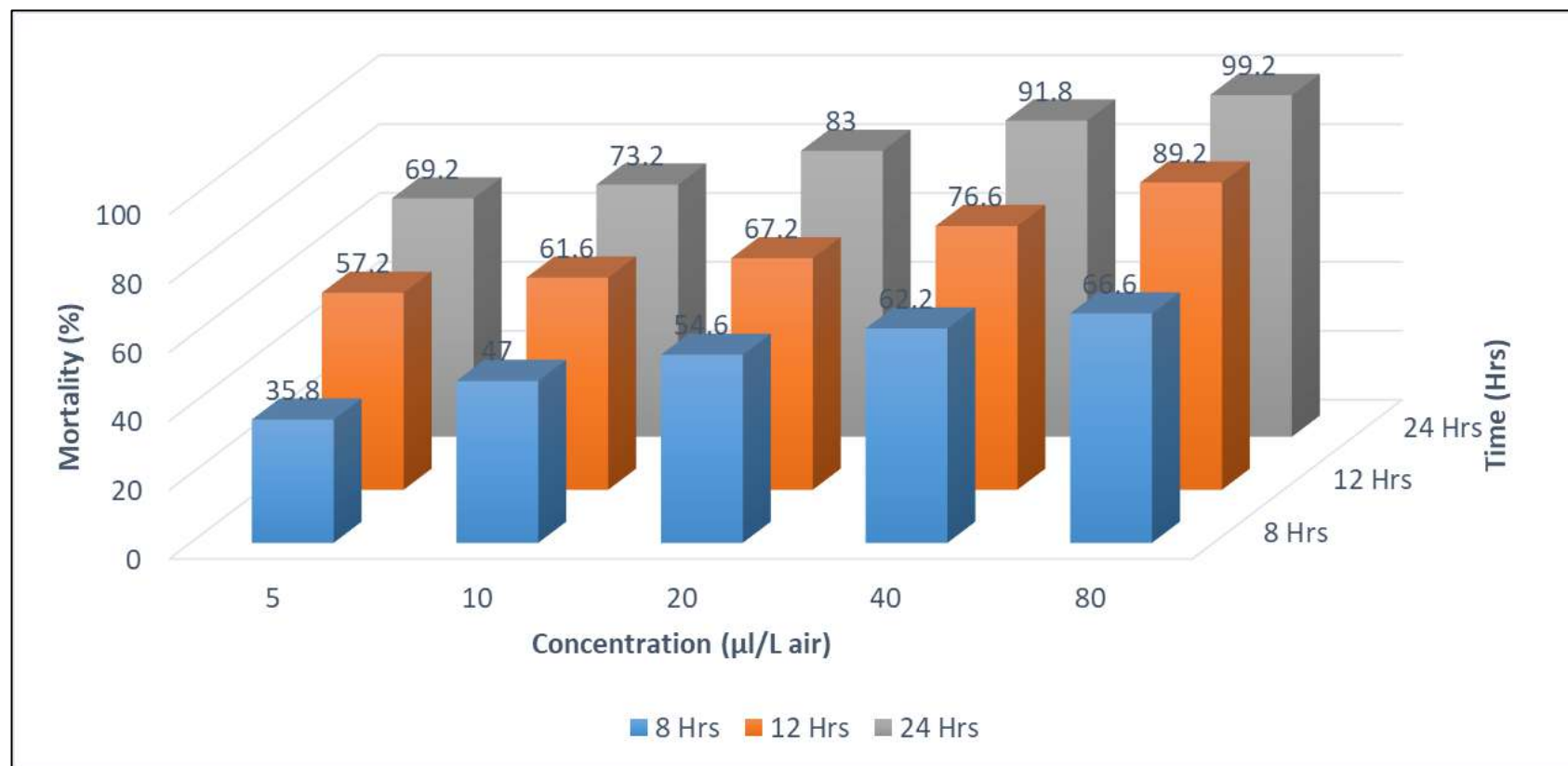


Fig 65 Fumigant toxicity of *L. coriacea* leaves essential oil against *S. oryzae*

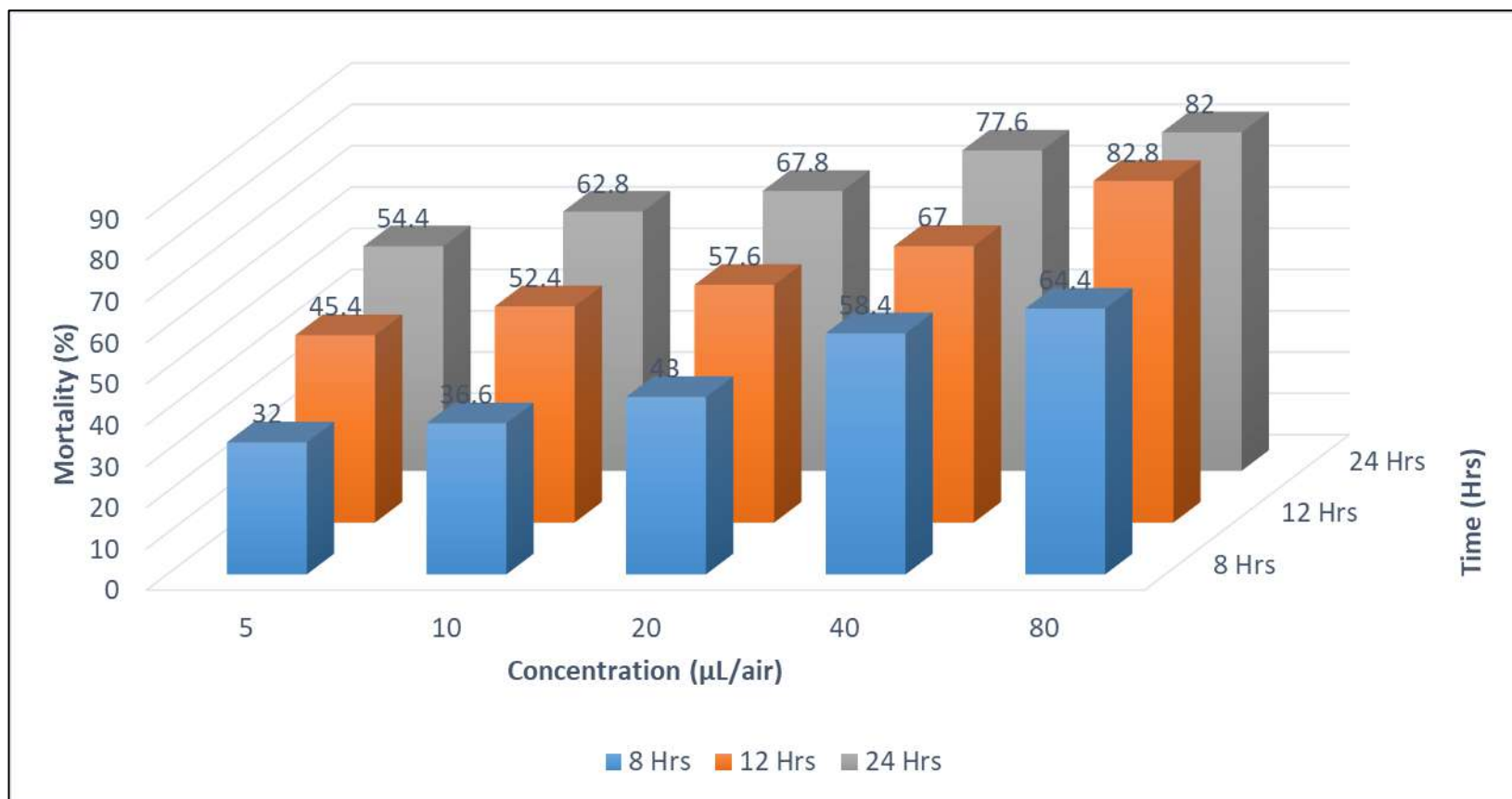


Fig 66 Fumigant toxicity of *L. coriacea* leaves essential oil against *T. castaneum*

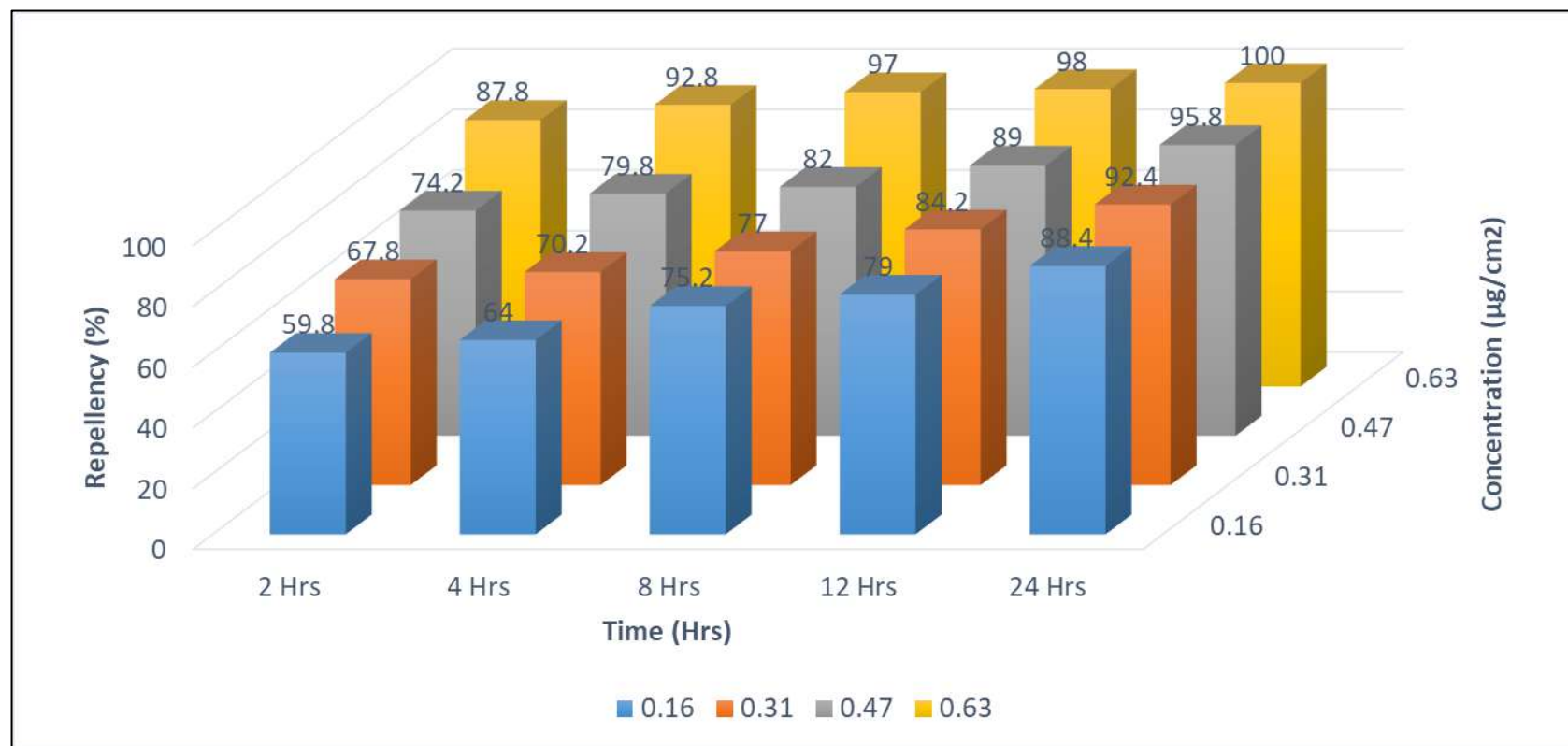


Fig 67 Repellency effect of *L. coriacea* leaves essential oil against *S. oryzae*

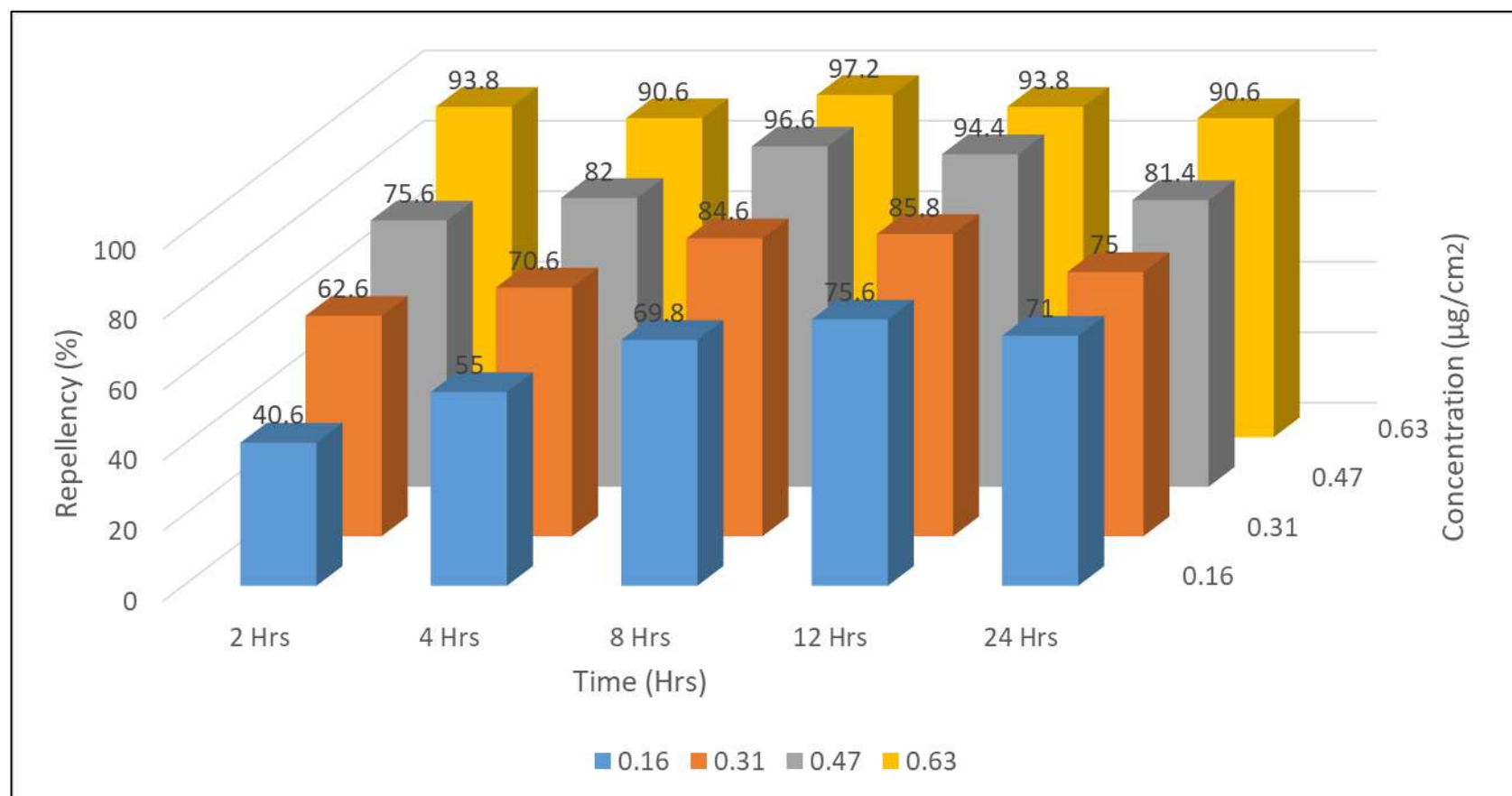


Fig 68 Repellency effect of *L. coriacea* leaves essential oil against *T. castaneum*

CHAPTER V

DISCUSSION

The practice of administering herbal plants as medicine is a traditional healing practice in India. It gained wide acceptance in the form of Ayurveda, in which thousands of plants have been used to treat various ailments. The plant parts like root, leaf, stem, fruit, flower, seed, bark, *etc.*, are used by various medical practitioners in crude form or mixed with other components. As human civilization developed, people became increasingly conscious about their health and have come forward with advanced technologies to develop therapeutic drugs in the field of clinical pharmacology and pharmaceuticals. With the utilization of modern tools and techniques, attempts have been made to design medicines and apply them to incurable diseases. Various phytochemicals have been explored from millions of plants worldwide. These phytochemicals have tremendous pharmacological and nutritional treasures, which are clinically proven. Even though numerous drugs have already been developed, most of the plant properties are yet to be explored. The forests cover mainly hotspots of different biogeographical areas on the Earth, occupied with endemic and rare medicinal plants, in which India contributes significantly to global biodiversity. The evergreen forests of the Indian subcontinent, especially the Southern Western Ghats, are a repository of therapeutically unexplored plant communities.

The Lauraceae family flourishes with abundant medicinal plants, including beneficial trees, shrubs, and herbs. Lauraceae family comprises genus such as *Cinnamomum* and *Litsea* possess several curative species. In the present study, three unexplored species of *Litsea*, viz., *L. bourdillonii*, *L. coriacea*, and *L. wightiana* were selected and phytochemical and pharmacological studies were conducted. The experiments were divided into three parts: Pharmacognostic characterization, phytochemical profiling, and pharmacological studies. As a preliminary phase, pharmacognostic characterization of the three species was done by studying the morphology, anatomy, histochemistry, physicochemical and powder characters.

PHASE 1. PHARMACOGNOSTIC CHARACTERIZATION

i. Morphological study

Morphological traits play a central role in the characterization of plants. These are essential for accurately identifying plant species and serve as a valuable source for analyzing genetic relationships and shared allelic data (Sulassih et al., 2013). Morphological characterization of plants is the primary step to identify the plant species within a specific genus. The present study considered phenotypic features, including habit, nature of stem, leaf, flower, and fruit parameters. The comparative external features of leaf, stem, fruit and flower of three species of *Litsea*, viz., *L. bourdillonii*, *L. coriacea*, and *L. wightiana*, were noted and found that these three species share several common morphological features and differences in their attributes. Srinivas et al. (2021) conducted morphological studies in 12 *Litsea* species distributed in the Karnataka region of the Western Ghats by considering similar features and found comparable results. The characters taken in both studies are used to confine a particular species within a particular genus with respect to morphology. The fruit morphological studies were also compared with the studies of Bhuinya and Mukherjee (2010) in fruits of 17 *Litsea* species from Sikkim.

ii. Anatomical study

Macro and micro anatomy are the initial set of pharmacognostic instruments used to evaluate the quality of medicinal plant components (Andrade et al., 2017). In comparative anatomical studies, the location of the vascular bundles and the presence or absence of secretory canals are significant factors (Ramachandran & Radhakrishnan, 2020). Anatomical characteristics of wood and bark have been extensively utilized to investigate the systematic relationships of members within the Lauraceae family (Stern, 1954). Comparative microscopic characterization of the stems and leaves of *Litsea* species selected for the present study revealed the most distinguishable anatomical features. In stem, there are differences in trichomes, the nature and arrangement of cortical cells, pericyclic fibers and medullary rays. Apart from these differences, they showed resemblances in other microscopic attributes, such as the nature of epidermis, cuticle, vascular tissues, and pith cells.

The three species exhibited remarkable similarity in leaf anatomical features like the nature of epidermis, cuticle, ground tissue, mesophyll tissue, bundle cap, and idioblasts. In the case of palisade and spongy cells, the three plants showed convincing similarity. Irrespective of these similarities, they displayed significant differences in some anatomical traits, particularly trichomes, which were never noticed in *L. coriacea*, but prominent in *L. bourdillonii* and *L. wightiana*. The distribution of idioblasts in the mesophyll appeared slightly different in the three species. The leaves also exhibited variation in the organization of the sclerenchymatous bundle sheath around the vasculature. Vasculature revealed common resemblance but was somewhat different in the three species in the arrangement of bundles, in which *L. wightiana* displayed a notable difference from the other two species Tables 10 & 11.

Petiole anatomical studies of the three *Litsea* species showed similar features, with some significant differences (Table 12). Microscopic characters, like general geometry, nature of epidermis, cuticle, trichome, ground tissue, vasculature, and pith, were observed. Crescent-shaped outline and squarish epidermal cells are commonly noticed in three plants. Absence of trichome was one distinguishable feature of *L. coriacea* from the other two species. Unicellular trichomes were observed in *L. bourdillonii* and *L. wightiana*. A wide cortex with thin-walled parenchyma cells was noticed in three plants, but, specifically, the ground tissue of *L. wightiana* was occupied with numerous scattered sclereids, which were never identified in the other two species. The shape of the vasculature is crescent shaped in *L. bourdillonii* and *L. coriacea*, and horizontal crescent in *L. wightiana*. But only *L. coriacea* displayed two hooks in the upper part of the vasculature. Another difference was in the organization of pith in the three species. *L. bourdillonii* and *L. wightiana* were occupied with wide piths, and *L. coriacea* with a narrow and condensed one.

The results obtained from the anatomical studies can be compared with those in the leaves and stem parts of *L. glutinosa* (Rao et al., 2012). The nature of the cuticle, epidermis and cortical cells are the most significant similar features noticed in the stem. Parenchymatous ground tissue is found in the leaves of the three

presently studied species of *Litsea*. Nizam et al. (2019) investigated the relationship between anatomy and taxonomy by studying petiole anatomy in the *Litsea* complex in Malaysia. The findings highlighted the variation in outline type, trichome type, presence of cuticle, crystals, brachysclereids, sclerenchyma, idioblasts, vascular bundle arrangement, *etc.* The authors opined that the anatomical characteristics hold significant taxonomic value and can serve as a reliable diagnostic tool for identifying genera and species within the Malaysian *Litsea* complex.

iii. Histochemical study

Comparative histochemical localization of phytochemicals in the stem and leaf of *L. bourdillonii*, *L. coriacea*, and *L. wightiana* revealed the presence of starch, lignin, tannin, and fixed oil content in various cells (Table 13). Starch deposition was visible in the cortical and stelar regions of the stem of *L. coriacea*, and the quantity was considerably more than in the other two species. Lignin was present in bundle cap cells and mostly found in the walls of the xylem of the three species. Tannin content was higher in the hypodermal region of the three species, and *L. coriacea* and *L. wightiana* stems had more tannin than *L. bourdillonii*. An increased oil deposit was observed in the hypodermis, cortex, and pith regions of *L. coriacea* and was less or absent in *L. bourdillonii*. Oil content was indicated only in the hypodermal cells and very little in the pith cells of *L. wightiana*.

The localization of histochemicals in the leaves exhibited differences in the three species of *Litsea* selected for the study (Table 14). The starch content was high in the midrib region of *L. coriacea*. The starch content in *L. wightiana* was substantially lower than in *L. coriacea* and was absent in *L. bourdillonii*. The lignin deposition was more in the bundle sheath and xylem tracheid in the three species. The hypodermal cells of *L. wightiana* and the mesophyll cells of *L. coriacea* also had lignin deposition. Tannin content was high in the hypodermis and the midrib region of the leaf of *L. wightiana* compared to *L. coriacea* and *L. bourdillonii*. The fixed oil content was high in the cortical region and mesophyll of *L. coriacea*. Oil content was very little in the leaf of *L. wightiana* and completely absent in *L. bourdillonii*. The histochemical study of stem and leaf samples proved that the

deposition of starch, lignin, tannin, and oil differs significantly across the three species. In comparison to the other two species, *L. coriacea* had higher oil content in the hypodermis, cortex, leaf midrib, and the pith region of the stem. Lignin deposition was prevalent in the bundle cap and xylem tracheid of the stem of *L. coriacea* than in the other species.

An increased amount of tannin deposition was observed in the cortical region of the stem of *L. wightiana* than *L. coriacea*. The presence of lignin, tannin, and oils in leaves and wood samples was observed in histochemical studies in *L. glaucescens*. The study also revealed the presence of phenolic compounds, oils, and non-structural carbohydrates (Tapia-Torres et al., 2014). The differences in the distribution of histochemicals in the leaf and bark samples of the three *Litsea* species investigated in this study may be useful in identifying these plants based on histochemicals.

iv. Physicochemical characterization

A comparative study of the physicochemical characteristics, including water-soluble extractive, alcohol-soluble extractive, total ash, acid-insoluble ash, sulphated ash values, and crude fiber content, was conducted. The results demonstrated that the leaf and bark samples exhibited significant differences in physicochemical parameters. This is the first study conducted so far, to determine the physicochemical parameters in *Litsea* plants. Generally, physicochemical studies in plants are utilized for the identification, authentication, standardization, and quality control of plant products, and to ensure the safety of plant-derived products. Extractive values are valuable for assessing the chemical constituents of a crude drug and estimating specific components that are soluble in a particular solvent (Sharma et al., 2009). The physicochemical analysis of *Acacia ferruginea* (Jeevitha et al., 2021), *Moringa oleifera* (Belay & Sisay, 2014), *Eclipta alba* (Kumar et al., 2020), and *Woodfordia fruticosa* (Grover et al., 2014) are some of the studies used for the standardization of the plants concerned.

V. Powder characterization

The powder characterization of the stem bark explored the distribution of cork cells, cortical cells, fibers, stone cells, starch grains, and crystals in the three samples studied, and the results displayed differences in powder features, such as the shape of cells in sectional view, fiber characters, nature of sclereids and starch grains. It has been found that the fibers are long with pointed ends and narrow lumen in *L. coriacea* and *L. wightiana* but blunt ended in *L. bourdillonii*. The powder microscopic characterization of *L. glutinosa*, revealed that the fibers are thick-walled with forked ends and the presence of rectangular or squarish type sclereids (Rao et al., 2012), which were not found in any other species in the present study. In some contexts of taxonomical disputes, the powder microscopic studies help identify the specific species of *Litsea*.

Pharmacognostic traits help identify and distinguish the members of the Lauraceae family. Several studies have been carried out in the Lauracean members particularly in the genus *Litsea*. Singh et al. (2015) reported that the oil cells and the mucilage cells, typically found distributed with rays, parenchyma, and fibres, are the distinctive traits of the Lauraceae family. In *Litsea* species, uniquely prismatic crystals are located within fibers and rays as revealed by powder microscopy.

PHASE II. PHYTOCHEMICAL PROFILING

i. Qualitative and quantitative phytochemical screening

Extraction is a fundamental process aimed at concentrating and isolating bioactive compounds from plant materials. Extraction methods play a crucial role in obtaining compounds from plant tissues for further analysis. For the present study, the Soxhlet extraction method was employed. The Soxhlet extraction technique is a widely utilized method in analytical practices (Zygler et al., 2012). It is one of the simple and effective methods as it completes the extraction with the minimum amount of solvent (Gopalasatheeskumar, 2018). Like the extraction method, it is also important to consider the solvent used. Various solvents, like ethanol, methanol, hexane, chloroform, and diethyl ether, are used in hot extraction. Among these

solvents, methanol is identified as the most effective solvent, which is employed in various extraction procedures by researchers. High polarity, low boiling point, and compatibility are the added advantages of methanol. Hence, the present study also utilized methanol as a solvent for extraction.

Phytochemicals serve secondary functions in plants such as the activation of defense mechanisms, acceleration of growth, provision of color, odour and flavour (Molyneux et al., 2007). Bioactive phytochemicals play a key role in various pharmacological experiments. The results of qualitative phytochemical analysis of the leaf and bark extracts evidenced the occurrence of phenols, flavonoids, tannins, and glycosides in *L. bourdillonii*, *L. coriacea*, and *L. wightiana*. Sterols and quinones were found in the leaves and absent in the bark samples of the selected species. Terpenoids were found to be distributed in all leaf samples and the bark of *L. wightiana*. Fatty acid was observed in the leaves of *L. bourdillonii* and *L. coriacea* and was absent in the other samples. Saponins were found in the leaves of *L. bourdillonii* and the bark of *L. wightiana*.

The study revealed the presence of important phytochemicals such as flavonoids, phenols, tannins, glycosides, terpenoids, alkaloids, saponins, and fatty acids in studied samples. This result is comparable to the phytochemical screening of *L. glutinosa* leaves (Bhowmick et al., 2014) and bark (Parikh & Rangrez, 2012) and confirms the presence of alkaloids, flavonoids, phenols, tannins, glycosides, saponins, terpenoids, and steroids. Qualitative phytochemical screening of *L. monopetala* also revealed the presence of alkaloids, tannins, steroids, flavonoids, and reducing sugars (Ali Ahmed et al., 2012).

The quantitative analysis of methanolic extracts indicated that the amount of flavonoid content of the leaf samples was in the order *L. wightiana* > *L. bourdillonii* > *L. coriacea*, and the bark samples *L. wightiana* > *L. coriacea* > *L. bourdillonii*. The concentration of tannin in the leaves was in the order *L. wightiana* > *L. bourdillonii* > *L. coriacea*, and in bark samples *L. bourdillonii* > *L. wightiana* > *L. coriacea*. The phenol content in the leaf and bark samples was in the order *L. wightiana* > *L. bourdillonii* > *L. coriacea*. The comparative quantitative

analysis revealed that the total phenolic content was the highest in *L. wightiana* leaves ($119.14 \pm 0.26 \mu\text{g}/\text{mg}$) and the lowest in *L. coriacea* bark ($8.14 \pm 0.20 \mu\text{g}/\text{mg}$). Phenolics play a major proactive role in preventing heart diseases and cancer (Kahkonen et al., 1999). Plants possess phenolics that show high antioxidant activity, and this assumption is validated by the experiments in *Tridax procumbens* (Habila et al., 2010), which revealed that high antioxidant activity corresponds to total phenolic content present in the sample. The present study results also testified the same.

Flavonoids are recognized for their antioxidant properties and have proved their ability to suppress tumor initiation, promotion, and progression (Kim et al., 1994). Tannins have been reported to specifically inhibit the replication of HIV (Kashiwada et al., 1992). Tannins, as natural polyphenol complexes with proteins, function as dietary supplements that aid in reducing oxidative damage to cell membranes, lipids, proteins, and nucleic acids.

ii. Liquid chromatography and mass spectrometric analysis

Liquid chromatography-mass spectrometric analysis (LC/MS) is an analytical tool widely used in phytochemical profiling. These techniques help identify and quantify the chemical compounds in plant extracts. The principle or steps involved are as follows. Primarily, the sample is dissolved in a liquid solvent and passed through a chromatographic column. Based on the interaction of compounds with the stationary phase and their solubility in the mobile phase get separated. The separated compounds exit the column at different retention times. The eluted compounds are ionized to form charged particles. These ions are analyzed based on their mass/charge ratio using a mass analyzer. The resulting mass spectra provide the molecular structure of the specific compounds capable of identification and quantification (Gika et al., 2014).

Liquid chromatographic and mass spectrometric analysis of selected species of *Litsea* revealed various classes of phytochemicals in both positive and negative ionization modes. The compounds belong to phenols, flavonols, alkaloids, terpenoids, sterols, fatty acids, and quinones. Major compounds identified from the

L. wightiana leaf extract include chalcone, xanthoplanine, dicentrinone, corytuberine, grandisin, palmitic acid, (+)-eudesmine, dehydroisoeugenol, and laurilitsine. Chalcone is a compound that exhibits diverse biological properties such as anti-inflammatory, antidiabetic, antioxidant, and antituberculosis efficacies (Zhuang et al., 2017). It has also been reported to possess antiparasitic, antiangiogenic activity and cytotoxicity (Valadares et al., 2009). Corytuberine is an aporphine alkaloid and has demonstrated cytotoxicity against SMMC-7721 tumor cells (Zetler, 1988). It also has anti-inflammatory, antioxidant, and anti-rheumatic activities (Do Santos, 2018). Dicentrinone, isolated from *Ocotea leucoxydon* extracts, exhibits inhibition against human topoisomerase 1 (Zhou et al., 2000) and its anti-tumor efficacy has been experimentally demonstrated by inhibiting protein disulfide isomerase (PDI) (Zhao et al., 2021). Xanthoplanine, an isoquinone alkaloid, hinders the CrKL-STAT-5 complex in the inflammatory process by diminishing the polarization of macrophages (Shi et al., 2020). Grandisin, a neolignan compound, possesses chemoprotective, cytotoxic, anti-angiogenic, anti-inflammatory, and anti-leishmanial potentials (Patel, 2022). It has been found effective for vasodilation and was isolated and purified from *Piper tectoniifolium* (Marques, 2022). Another significant compound identified is eudesmin, which has demonstrated anticancer properties on lung cancer cells by inducing apoptosis (Jiang et al., 2017). Laurilitsine, an aporphine alkaloid, inhibits HIV integrase activity (Mohan et al., 2020). Isocorydine, another aporphine alkaloid identified by LC/MS, targets the drug-resistant cancer stem cells by inducing PDCD4-related apoptosis and inhibiting cell growth in hepatocellular carcinoma cell lines through cell cycle arrest and apoptosis. Linalool, a terpene alcohol, exhibits diverse biological potentials, including anticancer, anti-inflammatory, anti-hyperlipidemic, antibacterial, antinociceptive, analgesic, anxiolytic, anti-depressive, and neuroprotective activities (Pereira et al., 2018). Dehydroisoeugenol, a neolignan compound, possesses biological activities like antioxidant, anti-inflammatory, antimicrobial, and anticarcinogenic effects. Barrera & Suarez (2010) examined the anti-inflammatory effect of the aporphine alkaloids from the leaves of *Pleurothyrium cinerum* and *Ocotea macrophylla* and found the inhibitory effect against COX-1, COX-2 and 5-

LOX enzymes. Hence the presence of the above-mentioned compounds attributes significant bioactivities to the leaves of *L. wightiana*.

LC/MS analysis of leaf methanolic extracts of *L. coriacea* and *L. bourdillonii* revealed similar bioactive compounds such as columbin, shogaol, gingerol, chlorogenic acid, piperlongumine, pipermonaline, apigenin, p-cymene, hexamethylquercetagenin, myristic acid, and lauric acid. Most of these compounds possess significant bioactive properties. Columbin is a diterpenoid with anti-inflammatory and anti-angiogenic properties (Abdelwahab et al., 2012). Gingerol has antioxidant, anti-inflammatory, antibacterial, and cardiovascular protective effects (Gao et al., 2024). Phenolics such as gallic acid, syringic acid, demethoxycurcumin, dihydroxybenzoic acid, caffeic acid, and chlorogenic acid help decrease various diet-related disorders, together with antioxidant, antibacterial, and hypotensive activities (Li et al., 2023). Shogaol, a phenolic compound, possesses anticancer effects (Zhu et al., 2013). Piperlongumine and pipermonaline are alkaloid compounds with neuroprotective, antidiabetic, and antitumor effects (Zhu et al., 2021). Apigenin is a flavone compound with antioxidant, anti-inflammatory, and antimicrobial properties (Liu et al., 2024). P-cymene is a monoterpene compound that possesses antioxidant, anti-inflammatory, anti-tumor, and antimicrobial properties (Balahbib et al., 2021).

According to the data obtained from the current study, there are observed some similarities between the composition of major compounds in *L. wightiana*, *L. bourdillonii*, and *L. coriacea* and other *Litsea* species, such as *L. cubeba* and *L. glutinosa*, from which isocorydene and laurilitsine were identified (Dai et al., 2024). When comparing the three species selected for the study, *L. coriacea* and *L. bourdillonii* showed greater similarity in phytoconstituents than *L. wightiana*.

iii. Gas chromatography and mass spectrometric analysis

Gas chromatography and mass spectrometric analysis is an analytical technique that combines the features of gas chromatography and spectrometry to identify the volatile constituents in a sample. It detects the trace level of compounds and identifies the chemical structure of the compounds. It is a very effective

technique in analyzing mixtures with multiple components, such as plant extracts and essential oils. The basic principles and steps involved in GC/MS include vaporizing the sample and carrying it by an inert gas through a chromatographic column. The column separates the components of the mixture based on the interaction with the stationary phase, and each compound remains in the column at a specific time known as retention time. The separated compounds are ionized, typically by electron impact, to produce charged fragments. The ions are sorted and detected depends on mass-to-charge (m/z) using a mass analyzer. Molecular fingerprints of each compound is provided in the form of mass spectra.

Gas chromatography and spectrometric analysis of methanolic extracts of *L. wightiana* leaves revealed certain volatile phytochemicals. The major compound found was Oleic acid (65.16%) followed by n-Hexadecanoic acid (36.56%), 11-Octadecanoic acid methyl ester (13.22%), Hexadecanoic acid methyl ester (10.49%), Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)(1 α ,4 α ,8 α)- (5.99%), Copaene (5.68%), and Globulol (5.52%) (Table 23). These compounds were reported to exhibit antioxidant, anti-inflammatory, anti-proliferative, anti-microbial, anticancer, insecticidal, hepatoprotective, neuroprotective, and cytotoxicity potentials (Keskes et al., 2017). Specifically, hexadecanoic acid methyl ester demonstrates antioxidant, anti-inflammatory, and cytotoxic properties, while 10-octadecanoic acid methyl ester provides antibacterial and antifungal characteristics, along with reducing blood cholesterol levels (Belakhdar et al., 2015; Ravi & Krishnan, 2017). Hexadecanoic acid is also noted for its anticancer properties (Khan & Javaid, 2022). Furthermore, 9,12,15-octadecanoic acid (Z, Z) methyl ester and globulol contribute antioxidant properties (Premathilaka & Silva, 2016; Rodriguez et al., 2019). In addition to antioxidant attributes, n-hexadecanoic acid exhibits pesticide, antipsychotic, anti-androgenic, and hypocholesterolemic activities (Tyagi & Agarwal, 2017). In the GC/MS analysis of methanolic leaves extract of *L. bourdillonii* and *L. coriacea* couldn't detect the volatile compounds. This may be due to species specific metabolic pathways, tissue specific localization of volatile compounds or environmental conditions that influence the generation of secondary metabolites.

PHASE III. PHARMACOLOGICAL STUDY

i. Antimicrobial activity

Antimicrobial studies focus on evaluating the ability of plant extracts to kill or inhibit microorganisms, including bacteria, fungi and viruses. These studies are conducted using various methodologies such as agar well diffusion, disc diffusion, broth dilution, antibiofilm activity experiments, *etc.*

In the current study, the antibacterial activity of the leaf and bark extracts of the three *Litsea* species were examined by agar well diffusion method. Bacteria such as *E. coli*, *P. aeruginosa*, and *S. aureus* were used, and streptomycin, the common antibiotic, was taken as the standard. The antibacterial activity studies in *L. bourdillonii*, *L. coriacea*, and *L. wightiana* showed significant antibacterial activity with inhibition zones ranging from 11-18 mm compared to the previous studies in other *Litsea* species. Sandipkumar & Bhatt (2012) studied the antibacterial properties of *L. polyantha* and observed the zone of inhibition against *E. coli* (8.75 ± 0.25 mm) and *P. aeruginosa* (7 ± 1 mm). Pradeepa et al. (2011) reported the inhibitory activity of *L. glutinosa* against *E. coli* (16.40 ± 0.55 mm). The MIC of *L. wightiana* leaf was 191.401 µg/mL against *E. coli* and 488.124 µg/mL against *P. aeruginosa*. Mandal et al. (2000) studied the antibacterial effect of the methanolic bark extract of *L. glutinosa* against 16 bacteria using the agar diffusion method and reported the inhibitory effect of the extract against both Gram-positive and Gram-negative bacteria, with the inhibition zone range of 6.5–13.5 mm.

Tripathi and Shukla (2018) conducted a study to evaluate the antifungal effect of *L. cubeba* plant extract against root-rotting fungus *Botryodiplodia theobromae* and obtained promising results. An antimicrobial study was conducted in *L. glutinosa* at different solvent extracts against pathogenic bacteria and fungi. Among them, *P. aeruginosa* and *C. albicans* exhibited good antimicrobial effects (Bakht et al., 2019). But, in the present study, antifungal assay in the leaf and bark of the three *Litsea* species showed no significant activity against the target fungal strains *C. albicans* and *A. niger*.

ii. Antioxidant Activity

Oxidative stress occurs due to the imbalance of free radicals, reactive oxygen species (ROS), which can potentially damage tissues, ultimately leading to aging and other consequences, mainly chronic diseases. Plants rely on a robust antioxidant system, comprising enzymes and metabolites, to mitigate oxidative damage. Among the antioxidants, ascorbic acid, glutathione, *etc.*, are very effective in neutralizing free radicals. Secondary metabolites like polyphenols, flavonoids, terpenoids, *etc.*, play an irreplaceable role in detoxifying free radicals formed during various environmental stress conditions (Llaurado Maury et al., 2020).

Natural antioxidants are extensively found in both food sources and medicinal plants. Plants serve as rich reservoirs of phytochemicals, including antioxidants vital for their physiological processes, adaptation to environmental stresses, and provide health benefits to humans. Different categories of flavonoids are sufficiently present in most fruits and vegetables. Quercetin, myricetin, kaempferol, hesperidine, catechins, gallic acid, apigenin, luteolin, and many more compounds are effective natural antioxidants (Xu et al., 2017).

The present study assessed the antioxidant potential of leaf methanolic extracts of the three *Litsea* species by various methods like DPPH assay, hydroxy radical scavenging assay, superoxide radical scavenging assay and ferrous reducing power assay.

● DPPH free radical scavenging assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay is commonly practiced to examine the antioxidant activity of a substance, typically plant extracts, foods or synthetic compounds. This assay provides a simple, quick, and reliable way to measure the capacity of a sample to counteract free radicals, which are reactive chemicals that can harm cells and have a role in aging and illness. The DPPH molecule is a stable free radical and in solution this has a deep violet color. When an antioxidant donates a hydrogen atom or electron to DPPH, the

radical is reduced, turning it colorless or pale yellow. In the present study, all the extracts showed a dose-dependent radical scavenging potential, in which *L. wightiana* leaves exhibited high antioxidant activity with an IC_{50} value of 30.32 $\mu\text{g/mL}$ when compared to the standard ascorbic acid (IC_{50} - 54.53 $\mu\text{g/mL}$), an antioxidant which is commonly employed as the standard in DPPH assays. Hence, the *L. wightiana* leaf extract was selected for further antioxidant assays such as hydroxyl radical scavenging assay, superoxide radical scavenging assay, and ferrous reducing power assays.

- **Hydroxyl radical scavenging assay**

Hydroxyl radical scavenging assay is a method to evaluate the antioxidant capacity of a substance in neutralizing hydroxyl radicals. Hydroxyl radicals are highly reactive species that can cause oxidative damage to biomolecules, leading to cellular damage and various diseases. This assay measures the ability of antioxidants to inhibit or scavenge hydroxyl radicals, highlighting their protective potential. In the current analysis, *L. wightiana* leaf extracts were subjected to measure the antioxidant efficacy by the above-mentioned method by setting gallic acid as the standard antioxidant and justified the result with IC_{50} values of 271.255 $\mu\text{g/mL}$ and 533.504 $\mu\text{g/mL}$ of extract and the standard respectively.

- **Superoxide radical scavenging assay**

In Superoxide radical scavenging assay, an antioxidant counteracts and nullify the effect of the superoxide radicals, which are reactive oxygen species (ROS) produced during cellular processes like oxidative phosphorylation. These radicals can impair the biological systems, contributing to serious health disorders in the form of cancer, neurodegenerative disorders, and cardiovascular issues. Hence, the superoxide radical scavenging capability of the *L. wightiana* leaf extract was examined for its antioxidant potential and proved the same with an IC_{50} value of 1052.27 $\mu\text{g/mL}$, which is comparable with the IC_{50} value of 567.07 $\mu\text{g/mL}$ of the standard antioxidant ascorbic acid.

- **Ferrous reducing antioxidant power assay (FRAP)**

FRAP assay is an antioxidant assay which evaluates the antioxidant potential of a substance based on its ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). This reduction is an indirect indicator of a substance's electron-donating capacity, a crucial property of antioxidants. In the present study, *L. wightiana* leaf extracts were subjected to the FRAP assay and found an increased reduced capacity with respect to an increase in concentration. It was observed that the absorbance by the extract at 700 nm ranged from 0.135 ± 0.047 to 0.488 ± 0.11 , and the standard quercetin was from 0.347 ± 0.005 to 0.878 ± 0.005 . These values indicated a good result and confirmed the antioxidant potential of the *L. wightiana* leaf extracts.

The antioxidant activity of plant extracts is always correlated with the phenolic content. The present study also justifies the statement. The reducing power of extracts is the main indicator of antioxidant activity and is indicated by the polyphenols causing reduction (Devi & Meera, 2010). In *L. floribunda* the reducing power of leaf and stem bark is caused due to the presence of tannins and flavonoids (Devika et al., 2016). The present study results is comparable with the antioxidant activity of four *Litsea* plants viz., *L. monopetala*, *L. glutinosa*, *L. assamica*, and *L. leata* (Choudhury et al., 2013). The total flavonoids and tannins were found higher in the leaf than in the bark extracts. The study highlights a positive correlation between the quantity of phytochemicals and antioxidant activity. Four different kinds of phenolic compounds were identified in *L. monopetala* bark (Arfan et al., 2008). Plant flavonoids, which have antioxidant activity *in vitro*, have been suggested to act as antioxidants *in vivo* in various investigations (Shin et al., 2008). Polyphenols and flavonoids found in nature can help reduce peroxidation, low-density lipoprotein oxidation, and cause atherosclerosis and heart diseases (Samak et al., 2009). The genus *Litsea*, according to Agrawal et al. (2009), contains many secondary metabolites. The current research also supported the assertion. Through LC/MS and GC/MS analysis of the leaf extracts, several phenolic and flavonoid compounds associated with antioxidant activity in *L. bourdillonii*, *L. coriacea* and *L. wightiana* were identified. Many therapeutic plants have a significant level of

phenolic compounds, and there is a positive linear relationship between total phenolic content and plant antioxidant activity (Maizura et al., 2011).

iii. Antidiabetic activity

Plants work as antidiabetic agents by leveraging their natural bioactive compounds to regulate blood sugar levels, enhance insulin function, and combat diabetes-related complications. These compounds act through diverse mechanisms, making plants a valuable source for managing diabetes naturally or as complementary therapies. Enzyme inhibition assay of alpha-amylase and alpha glucosidase enzymes are the two methods commonly employed to evaluate the antidiabetic potential of plants.

The alpha-amylase enzyme inhibition method is used to assay the antidiabetic potential of plant extracts by assessing their ability to inhibit the activity of alpha amylase, an enzyme responsible for breaking down starch to glucose. This inhibition causes a reduction in postprandial blood sugar levels, making it an available tool in diabetic management. Acarbose, a popular antidiabetic drug, is very effective in regulating the uncontrolled activity of alpha-amylase (Cheng et al., 2005). Alpha-glucosidase enzyme inhibition assay is another common method adopted in antidiabetic assays. This method utilizes the ability of plant extracts to inhibit the alpha-glucosidase enzyme, which is critical for carbohydrate digestion in the small intestine. These enzymes hydrolyze disaccharides and oligosaccharides into glucose, which is absorbed into the bloodstream. Inhibiting this enzyme can delay glucose release and absorption, thereby managing high glucose levels. Plant and plant products can act as competitive inhibitors of these enzymes and work effectively as potent antidiabetic agents.

In the present investigation, the antidiabetic activity of the methanolic leaf and bark extracts of the three species of *Litsea* was evaluated by enzyme inhibition assays. From the alpha-amylase inhibition assay, it can be inferred that, among the extracts, *L. wightiana* leaves showed the highest inhibition with an IC₅₀ value of 134.67 µg/mL, followed by *L. wightiana* bark (148.83 µg/mL), *L. bourdillonii* bark (176.20 µg/mL), *L. bourdillonii* leaf (219.06 µg/mL), *L. coriacea* leaf (228.95

µg/mL), and *L. coriacea* bark (412.90 µg/mL). All the extracts showed a concentration-dependent antidiabetic activity compared to acarbose, the antidiabetic drug (IC₅₀ 45.41 µg/mL). The alpha-glucosidase enzyme inhibition assays also showed almost similar results, with enhanced inhibition of the enzyme with increased concentration of extracts. Different extracts showed variation in the activity, such as *L. wightiana* leaf with an IC₅₀ value of 50.30 µg/mL followed by *L. coriacea* bark (88.771) µg/mL, *L. wightiana* bark (99.32 µg/mL), *L. bourdillonii* leaf (164.09 µg/mL), *L. coriacea* leaf (176.763 µg/mL), and *L. bourdillonii* bark (217.779 µg/mL). The experiment was compared with the antidiabetic activity of acarbose, with an IC₅₀ value of 20.23 µg/mL.

Phytochemicals play a significant role in managing diabetes due to their diverse mechanisms of action. These compounds, including flavonoids, alkaloids, terpenoids, saponins, and polyphenols, exhibit antidiabetic activity by targeting various pathways involved in glucose metabolism, insulin function, and oxidative stress. The potent role of two flavonoid glycosides as effective antidiabetic agents from *L. glutinosa* leaf was confirmed by *in vivo* experiments (Bulbul et al., 2024). An *in vivo* antidiabetic study conducted in diabetic rats proved that the ethanolic extract enriched with flavonoids in *L. coreana* could mitigate hyperglycemia (Lu et al., 2010). It is reported that the presence of flavonoid and tannin compounds in the extract is accountable for the antidiabetic activity of the plants (Al-ishaq et al., 2019). Phytochemical screening of some *Litsea* species, namely *L. elliptica*, *L. ferruginea*, *L. firma*, and *L. graciae*, together with alpha-amylase and alpha-glucosidase inhibitor assay, revealed the presence of alkaloids, flavonoids, phenols, tannins, saponins, *etc.* and direct correlation with antidiabetic activity (Rijai et al., 2023). The antidiabetic studies on *L. cubeba*, conducted by similar methodology, exhibited a significant effect (Chakraborty & Mandal, 2018). The *in vitro* antidiabetic studies in *L. petiolata* also showed efficient activity (Ambarwati et al., 2021). All the above-cited reviews concurred with the present study results. So, the study could confirm the inevitable role of phytochemicals in controlling diabetic-related disorders, and it ascertained that the *Litsea* species studied can be included in the list of antidiabetic medications.

iv. Cytotoxicity

Cytotoxicity refers to the ability of a substance to kill cells or reduce their viability. Cytotoxicity studies are generally employed in plant extracts to assess their potential as therapeutic agents, particularly in cancer research. On the other hand, it is a critical parameter in evaluating the safety and therapeutic potential of various compounds contained in plant extracts. Cell viability is an important indicator of cytotoxicity tests and can determine the two-sided benefits of this assay. Many plant extracts contain phytochemicals that can selectively target and kill cancer cells. Cytotoxicity assays help to identify these compounds and evaluate their effectiveness. But, before plant extracts are used in pharmaceuticals, it is essential to ensure that they are non-toxic to normal healthy cells.

MTT assay is an effective method generally adopted to test the cytotoxicity effects of plant extracts in human cancer cell lines by *in vitro*. In the present research, cytotoxicity studies of leaf and bark extracts of three *Litsea* species by MTT assay showed no significant cytotoxicity effect on SK-MEL cell lines. Even though all the extracts exhibited a dose-dependent decrease in cell viability, no extract could express a significant level of cytotoxicity to recommend the plants for cancer research. Among the extracts, only *L. coriacea* leaf extract showed moderate cytotoxicity with 45.67% viability, and all other extracts showed 50% or above cell viability at maximum concentration (100 µg/mL). The results were compared with the cytotoxicity effects of isoquinoline alkaloids identified from *L. cubeba*. All the compounds exhibited high toxicity in different cell lines such as HepG2, SK-MEL-2, MCF-7, SGC 7901, and SK-OV-3 (Zhang et al., 2012). However, the *in vitro* cytotoxicity studies in *L. glutinosa* are in line with the present study results, as no significant cytotoxicity was observed (Sumithregowda et al., 2017). Plants with low cytotoxicity are generally associated with therapeutic potentials like antioxidant, anti-inflammatory, and immune modulating. They are also potential agents in cosmetics and dietary supplements with no toxic effects.

v. Anti-inflammatory activity

Inflammation is a crucial protective mechanism that helps maintain the integrity of an organism when faced with physical, chemical, or infectious damage. However, it is often observed that the inflammatory response to severe injuries mistakenly harms healthy tissue (Lunardelli et al., 2006). Inflammation is a biological reaction caused by the immune system to protect against harmful stimuli, such as infections, injuries, or toxins. While inflammation is a vital defense mechanism, excessive or chronic inflammation may lead to various illnesses, mainly include arthritis, cardiovascular issues, asthma, and autoimmune disorders. Anti-inflammatory activity refers to the ability of a substance to reduce or inhibit inflammation in the body. Plant extracts exhibit anti-inflammatory activity through various mechanisms, primarily driven by bioactive phytochemicals. Phytochemicals inhibit the production of cytokines like TNF- α , IL-1 β , and IL-6, which are key drivers of inflammation. TNF- α is a key pro-inflammatory cytokine produced by various immune cells. Its production rate and blood concentration reflect the severity of the immune response (Beutler & Cerami, 1989). Proinflammatory cytokines play a critical role in mediating and regulating inflammation and immunity.

During organ injury caused by endotoxins, TNF- α is considered the primary mediator, promoting the secretion of other cytokines like IL-1 and IL-6, which are predominantly produced by immune cells and play a vital role in the inflammatory process (Roshak et al., 1996). Bioactive compounds regulate NF- κ B and MAPKs, which are involved in the inflammatory response (Merecz-Sadowska et al., 2020). Plant extracts can reduce the activity of enzymes like cyclooxygenase (COX) and lipoxygenase (LOX), enzymes that produce inflammatory mediators like prostaglandins and leukotrienes (Rodriguez-Yoldi, 2021). Many secondary metabolites found in medicinal plants, which primarily include terpenoids and flavonoids, are recognized as significant anti-inflammatory agents. Notably, monoterpenoids such as camphene, borneol, and α -pinene have been identified for their anti-inflammatory properties (Lin et al., 2008).

- ***In vitro* Anti-inflammatory activity**

Enzyme inhibition assays are generally used to evaluate the anti-inflammatory activity of plant extracts *in vitro*. Cyclooxygenase (COX), lipoxygenase (LOX), myeloperoxidase (MPO), and inducible nitric oxide synthase (iNOS) enzyme activities are tested by these assays.

- **Enzyme inhibition assay**

Cyclooxygenase-2 (COX-2) has a crucial role in inflammatory processes and is strongly expressed in cell types such as macrophages and mast cells, particularly when triggered by pro-inflammatory cytokines (Needleman & Isakon, 1997). In the current investigation, the anti-inflammatory potential of *L. wightiana* leaves extract was evaluated by checking the potential of extract to suppress cyclooxygenase, lipoxygenase, and inducible nitric oxide synthase enzymes and myeloperoxidase enzymes in lipopolysaccharide-induced RAW 264.7 cells. The results revealed significant activity, as evidenced by the dose-dependent inhibition of enzymes such as cyclooxygenase (IC_{50} , $19.42 \pm 0.08 \mu\text{g/mL}$), lipoxygenase ($16.21 \pm 1.2 \mu\text{g/mL}$), and nitric oxide synthase ($36.56 \pm 0.012 \mu\text{g/mL}$). Ultimately, iNOS inhibition caused the reduction of cellular nitrite levels. Myeloperoxidase enzyme activity was also inhibited by the extract and was measured as 0.0129 U/mL of cell lysate. The observed IC_{50} values indicated the potency of the methanolic leaves extract of *L. wightiana* in mitigating the inflammatory processes. Thus, blocking these enzymes may aid in the treatment of diseases associated with oxidative stress and inflammation. Comparatively, the leaf extract of *L. quinqueflora* enriched with polyphenols including gallic acid, sinapic acid, p-coumaric acid, and umbelliferone, demonstrated robust anti-inflammatory action with an IC_{50} value of $23.59 \mu\text{g/mL}$ (Jose & Anilkumar, 2021). Similarly, anti-inflammatory studies conducted on *Psidium guajava* and *Syzygium cumini* leaf extracts also confirmed the attenuation of COX-2, LOX, iNOS, and cellular nitrite levels (Deepa et al., 2017).

vi. Gene expression study

In the context of immunological and inflammatory processes, cytokines serve as mediators and pro-inflammatory substances. In this study, the mRNA expression level of specific pro-inflammatory cytokines, namely interleukin IL-6, TNF- α and COX-2, along with the transcription factor NF- κ B, were assessed to determine their impact on the activity of inflammatory enzymes. The results indicated that all the cytokines demonstrated a downhill regulation of genes in LPS-induced RAW264.7 cells except IL-6 as illustrated in Figure 49. Even though IL-6 functions as a potent target in anti-inflammatory studies, sometimes this gets expressed due to its pleiotropic nature. Because of this property this gene may counteract in cell during anti-inflammatory action (Linge et al., 2022). TNF- α , IL-8, IL-6, IL-1, and COX-2 are recognized as pro-inflammatory mediators that contribute a significant role in chronic inflammatory disorders (Kim et al., 2007). The transcription factor NF- κ B contributes to intracellular signaling pathways associated with cytokine production (Ghosh, 1998). A study involving glycosides of dihydrostilbene isolated from *L. coreana* leaves revealed mild anti-inflammatory action by inhibiting TNF- and IL-1 production in RAW264.7 cells stimulated with lipopolysaccharides (LPS) (Tang et al., 2013). Furthermore, gene expression studies conducted in *L. cubeba* root extracts demonstrated the suppression of mRNA of iNOS and COX-2 in LPS-induced RAW 264.7 cells, with IC₅₀ for TNF- α inhibition measured at $28.2 \pm 0.9 \mu\text{M}$ and $15.0 \pm 1.0 \mu\text{M}$ respectively (Lin et al., 2016).

vii. *In vivo* anti-inflammatory activity

Carrageenan-induced paw edema method is a widely used *in vivo* model for studying inflammation. It is a commonly employed method to assess the anti-edematous effects of natural products, as it responds to cyclooxygenase (COX) inhibitors and serves as a method to evaluate the efficiency of Nonsteroidal Anti-inflammatory Drugs (NSAIDs). This method involves injecting carrageenan, a polysaccharide, into the paw of animals to induce localized inflammation and edema. The extent of inflammation can be measured by the paw volume. When anti-inflammatory drugs are administered to the test groups, their efficacy is determined

by comparing the reduction in paw swelling with that of the control groups. In the present study, *L. wightiana* methanolic leaf extracts showed a remarkable reduction in the size of paw edema induced by carrageenan, an inflammatory substance. The effect of *L. wightiana* methanolic leaves extract was significant and dose dependent. The drug treatment of 50 mg/kg resulted in a progressive decrease in paw thickness, viz. 3.67 ± 0.04 mm, 3 ± 0.26 mm, 2.33 ± 0.37 mm in the third, fourth and fifth-hour periods, respectively and found statistically significant at $P < 0.01$ level of significance (Table 37).

Similarly, the treatment of extract with 100 mg/kg exhibited a decline in paw thickness during the second hour after treatment and was significant at a P value < 0.05 . After the fourth hour of treatment, the inflammation was reduced to 60.12% which is higher than standard indomethacin (58.02%) (Table 38). These results are comparable with the findings of the anti-inflammatory experiments in *Ocimum gratissimum* where the reduction in paw thickness was observed in animals treated with the extract concentration of 100 mg/kg and 500 mg/kg, respectively (Ajayi et al., 2017). The anti-inflammatory effect of the methanolic extracts was higher than the extracts in other solvents due to the presence of bioactive constituents like terpenoids or flavonoids in plants (Lin et al., 2008). Several phytochemicals, including steroids, flavonoids, alkaloids, terpenoids, glycosides, tannins, and phenolic compounds are well known for their anti-inflammatory property (Agarwal & Rangari, 2003; Amresh et al., 2007). In the present study, phytochemical screening of *L. wightiana* leaves also revealed the possession of all the above-mentioned phytochemicals.

viii. Docking studies

In silico molecular docking is a computational technique to study the interaction between a ligand, such as a bioactive compound and a target protein. It helps understand how a compound binds to a specific site on a protein, which is crucial for drug discovery and development. In anti-inflammatory studies, docking is used to evaluate the potential of natural or synthetic compounds to inhibit

inflammatory mediators like cyclooxygenase (COX), lipoxygenase (LOX), or cytokines such as TNF- α and IL-6.

Major steps involved in the molecular docking process are the preparation of target proteins, saving the protein structures in .pdf file format, preparation of ligands by sketching the appropriate conformations. Docking method validation by checking the capability of appropriate binding of native ligand to the corresponding protein using docking programs like the Auto dock, and recreation of the orientation and position of ligands in the expected crystal structure. The final step is the implementation of the docking of natural ligands and target protein molecules by Auto dock Vina program. After docking identification, the best-docked conformation is identified based on the lowest binding energy. Protein- ligand interaction factors, including hydrogen and other bonds, are then determined using Discovery Studio Visualizer software (Herowati & Widodo, 2017).

In silico molecular docking method was employed to assess the anti-inflammatory potential of bioactive compounds in *L. wightiana* leaves. Twelve phytochemicals identified by LC/MS analysis were selected and all the compounds were reported with anti-inflammatory activity (Singh Grewal et al., 2017). The overall result showed that dicentrinone, an alkaloid compound, demonstrated favorable binding energy against all target proteins, including human myeloperoxidase (-8.3), human inducible nitric oxide synthase (-9.5), cyclooxygenase II (-9.2), 5-lipoxygenase (-8), respectively (Table 36). These findings position dicentrinone as a promising anti-inflammatory agent. Analysis of interactions revealed that the compound forms a lone hydrogen bond interaction with 1dnu involving GLU102, and 4 nos with ASN370. Additionally, it engages Van der Waals and π interactions with all other target proteins. Notably, xanthoplanine, cubinol, chalcone, α -cadinol, and similar compounds displayed substantial affinity with the selected proteins. Conversely, molecules such as linalool and hexadecanoic acid exhibited the least binding energy and affinity. Molecular docking studies of garcinol from *Garcinia* have proven its potential as an effective anti-inflammatory agent by suppressing LPS-induced iNOS and COX-2 expression (Kalita et al., 2022). Peralta et al. (2024) conducted *in silico* docking studies of three

compounds, namely pinocembrin, scopoletin, and 5,7,3,4-tetrahydroxy isoflavone, from *L. guatemalensis* targeting anti-inflammatory activity and obtained profound results. Gogoi et al. (2012) conducted docking studies to prove that the genus *Litsea* possesses great potential to inhibit COX-2 enzymes. Thirteen bioactive compounds isolated from different *Litsea* species were utilized in the study.

ix. Wound Healing Study

A wound is formed due to the disruption of cellular and anatomical continuity in living tissue by physical, chemical, or biological agents. Normal wound healing is a gradual and slow process that involves the release of clotting factors, platelet aggregation, cytokine production by inflammatory cells, and fibroblast mobilization for collagen deposition. The wound healing mechanism involves processes, like epithelialization, contraction, and connective tissue implantation. Production, deposition, and maturity of new collagens are the main attributes of healing process (Agarwal et al., 2009; Murthy et al., 2013). Synthetic drugs composed of antibiotics, anti-inflammatory drugs, hydrogels, and dressings are used for enhanced healing. Since time immemorial, human beings have utilized several plant parts for faster wound healing. Saini et al. (2016) reported numerous plants used for wound healing in traditional medicine. *Aloe vera*, *Asparagus racemosus*, *Ocimum sanctum*, *Centella asiatica*, and *Curcuma longa* are well-known among them. The wound healing activity of various *Litsea* species has been studied, and positive results were reported (Jamaddar et al., 2022). However, the wound-healing activity of *L. wightiana* leaves has not yet been reported in any literature. The purpose of the current study is to understand the wound healing capacity of *L. wightiana* leaves. Both *in vitro* and *in vivo* methods were adopted for the study.

Scratch wound assay is a widely used *in vitro* method to study cell migration in two-dimensional environments. This technique involves creating a cell-free zone within a confluent cell monolayer by physically excluding cells or removing them through mechanical, thermal, or chemical processes. For the present study, scratch wounds were created in L929 cell lines, and the methanolic extracts of *L. wightiana* leaves were applied. It was noticed that the sample exhibited faster wound closure

within given periods (24 hr, 48 hr and 72 hr) compared to that of the control. A similar study was conducted on *Aristolochia saccata* (Bolla et al., 2019), and the exposure of methanolic leaf extract to scratched L929 cells induced wound healing.

In vivo wound Healing Experiments involve testing the efficacy of plant extracts on living organisms, typically animal models, to evaluate their potential in promoting wound repair and tissue regeneration. These studies are significant in understanding the biological effects of plant-derived compounds on the wound healing process, including reduction of inflammation, collagen synthesis, and tissue regeneration.

In the present *in vivo* experiments, the ointment formulations of methanolic leaves extract of *L. wightiana* (LWME 5% and LWME 10%) lead to fast wound healing in Wistar albino rats after treatment of excision wounds with respect to the control group. The wound healing was monitored for 21 days, during which the change in the size of the wound and its epithelialization were noted. The wound region was then examined histopathologically. Arunachalam and Parimelazhagan (2013) conducted similar *in vivo* experiments to test the wound healing potential of *Ficus amplissima*. Similar inferences were obtained, which led to the assumption that the phenol compounds present in the plants may be responsible for the activity. In both experiments, ointment formulations of plant extracts were applied on animal wounds, and the wound healing process by wound contraction period and epithelialization was checked. A further analysis was done histopathologically. Both results concurred and joined in proving the wound healing potential of the tested extracts.

- **Histopathology**

The histological examinations of the groups treated with 5% and 10% *L. wightiana* leaf methanolic extracts showed higher collagen deposition and fibroblast distribution, indicating a wound healing mechanism similar to the one observed in *Artemisia absinthium* (Boudjelal et al., 2020). Saleem et al. (2021) evaluated the wound healing efficiency of *Bougainvillea glabra* extracts in Wistar albino rats, and 5% w/w ointment made using the extract led to an increased rate of wound

contraction, epithelialization, and collagenation by histopathological analysis. This review also supports the validity of the present work in *L. wightiana*. High content of flavonoids and polyphenols was observed in both plant extracts.

The main bioactive compounds, like flavonoids, chlorogenic acids, *etc.*, are responsible for the wound healing effect (Pang et al., 2017). The topical application of tannin is superior in excision wound healing (Rane & Mengi, 2003). Compounds with antibacterial or free radical scavenging activities are crucial in promoting wound healing. *In vitro* investigations have revealed that several conventional plant-based preparations contain antioxidant and wound healing properties (Suntar et al., 2012; Shah & Amini, 2017). Most of the traditionally used medicinal plants contain phytochemicals like alkaloids, phenols, tannins, terpenes, steroids, flavonoids, glycosides, and fatty acids, that have an important role in the wound healing process (Rex et al., 2018). Flavonoids, saponins, phenols, tannins and essential oils are the phytoconstituents of medicinal plants renowned for their wound-healing potential (Addis et al., 2020). The phytochemical profiling showed the presence of phenolic, flavonoid, and alkaloid compounds in *L. wightiana* (Habeeba & Rasmi, 2023) which may be the cause of the enhanced wound healing effect demonstrated by the plant and may contribute to a cost-effective drug to cure wounds in animals.

• Docking studies

Molecular docking is an effective method for examining the dynamic behaviour of ligand-bound proteins in the protein-binding pockets (Mangoni et al., 1999). In wound healing studies, docking provides a molecular-level understanding of how bioactive compounds interact with specific proteins involved in the wound healing process. Molecular docking studies associated with wound healing activity are generally practiced using the involvement of some proteins in the wound healing process. Molecular docking based on *in silico* studies proved that *Moringa oleifera* seed extract could target TNF- α , TGF- β , and IL- β by specific metabolites in it by interacting with the active sites of three-wound healing-associated proteins (Shady et al., 2022). The comparative molecular docking analysis of isolated lupeol and standard drug nitrofurazone against glycogen synthase kinase-3 β (GSK-3 β) protein

within the context of the Wnt signaling pathway corroborated the wound healing potential of lupeol (Harish et al., 2008). In the present study, the wound healing potential of *L. wightiana* leaf extracts was verified by *in silico* characterization. Ten phytochemicals identified from *L. wightiana* leaves, reported to possess wound healing activity, were selected as ligands and docked against the targeted proteins, and promising results were obtained. The compounds like dicentrinone, lauroilsine, coryuberine, reticuline, eudesmin, grandisin, dehydroisoeugenol, 9,12-octadecadienoic acid(*z,z*) methyl ester, and n-hexanoic acid showed better affinity towards the target proteins with least binding energy. The docking study revealed a good binding affinity of compounds selected for study against all four target proteins including GSK-3 β , TGF- β , β -catenin and c-myc. All the compounds showed better binding energy in which dicentrinone was in the first position against GSK -3 β , TGF- β , β -catenin and c-myc. Reticuline also showed least binding energy with c-myc and lauroilsine with β -catenine.

The antioxidant potential has been established as a therapeutic indicator of enhancing the wound healing properties of plant extracts (Subhashini & Arunachalam, 2011). Numerous therapeutic plants with encouraging wound healing properties have been shown to have strong antioxidant capacity. The antioxidant potential of *L. wightiana* leaf extracts has already been proven.

x. Analysis of essential oil by GC/MS and GC-FID analysis

Essential oils are aromatic plant oils that retain the natural fragrance and beneficial properties of their source plants. They are widely used in aromatherapy, cosmetics, pharmaceuticals, and food industries due to their antimicrobial, antioxidant, and other therapeutic effects. The exploration of plant-based natural products, especially essential oils (EOs), emerged as alternative pest-control solutions as a response to the challenges posed by the prolonged use of synthetic insecticides. Essential oils can act as natural insecticides due to the occurrence of bioactive compounds like terpenoids, phenolics, alkaloids, *etc.*, that cause neurotoxicity, respiratory interference, growth inhibition, and deterrent and repellent properties in insects. The compounds identified from plants and explored as insect-

repellent candidates include limonene, citral, β -caryophyllene, and methyl salicylate (Guarino et al., 2025). Essential oils derived from plants have been extensively studied for their potential as natural insecticides against stored pests. *T. castaneum* is a popular stored grain pest causing massive destruction of cereal crops worldwide. Several plant-derived natural insecticides were found effective against this harmful pest. *Cymbopogon citratus* active essential oil was found very effective against *T. castaneum*, and this essential oil mainly consists of citral, neral, citronellol, D-lemonene, geraniol, and geranyl acetate, which has biofumigant properties (Devi et al., 2020). *S. oryzae*, another stored grain pest, commonly called rice weevil, is famous for its harmful involvement and destructive damage to several crops worldwide. Several studies have been carried out to eradicate this harmful organism, where naturally occurring bioactive insecticides made up of essential oils from various plants were found to cause adverse effects on these pests. Most of the essential oils evaluated for their effectiveness against *S. oryzae* belong to plant families such as Lamiaceae, Lauraceae, Piperaceae, Verbenaceae, etc. (Opiyo et al., 2022). Terpenoids and phenylpropanoids extracted from essential oils of plant parts are safe to use because they exhibit moderate toxicity or are even non-toxic to mammals and other vertebrates (Isman & Seffrin, 2014).

In the current study, the essential oil analysis and insecticidal activity of the essential oil of *L. coriacea* leaves against stored grain insect pests such as *S. oryzae* and *T. castaneum* were conducted. Contact toxicity, fumigant toxicity, and repellent activity against the two insects were assessed. Essential oil extraction was carried out by hydro distillation method using the Clevenger apparatus. The extracted oil was subjected to GC/MS analysis together with GC-FID for the identification and quantification of compounds. As result some significant bioactive compounds, mainly terpenoids such as eucalyptol, endo-Borneol, linalyl acetate etc. were identified (Table 24). In the present study, the insecticidal activity of *L. coriacea* leaf essential oil was determined by contact toxicity, fumigant toxicity, and repellency assays.

xi. Bioactivity of essential oils

● Contact toxicity

It is a common method used to assess the insecticidal activity of plant essential oils by recording their ability to kill the insects upon direct contact. This method tests the efficacy of essential oils against various insect pests. In this assay, essential oils are applied directly to the insects and the exposed ones are kept under observation for a specific duration. Mortality rates or behavioral changes are recorded to determine the effectiveness of oils (Kim & Lee, 2014). The present contact toxicity assay against *S. oryzae* and *T. castaneum* confirmed the role of oil concentration and time in insect mortality. *L. coriacea* essential oil at 80 $\mu\text{g}/\text{cm}^2$ caused $98.8 \pm 0.042\%$ mortality of *S. oryzae* and $66.8 \pm 0.079\%$ mortality of *T. castaneum* insects at 72 hours after the treatment.

● Fumigant toxicity

Fumigant toxicity assay is another method commonly employed together with contact toxicity tests to evaluate the insecticidal properties of plant-derived essential oils. It involves exposing insects to the vapor of essential oils in a sealed environment to assess their lethal effect. In the present fumigant assay, the *L. coriacea* essential oil at 80 $\mu\text{g}/\text{mL}$ caused $99.2 \pm 0.042\%$ mortality of *S. oryzae* after 24 hours. On the other hand, the oil showed mortality of *T. castaneum* at $82.8 \pm 0.015\%$ at the same concentration and time. The fumigant toxicity of essential oils extracted from rosemary and lemon plants was evaluated against the red flour beetle, *T. castaneum*. The findings revealed that rosemary essential oil exhibited significantly higher toxicity than lemon essential oil (Lee et al., 2002). Essential oils typically exhibit fumigant toxicity due to the high content of monoterpenes, which are primarily responsible for their fumigant toxicity effects (Koul, 2004).

● Repellency assay

It is a widely used method for examining insecticidal activity by evaluating the ability of plant essential oils to repel insects. Filter papers are divided into treated and untreated areas, and the movement of the exposed insects towards the untreated

area is considered as the best indicator of repellent property. In the present repellency test, *L. coriacea* oil showed a 100% repellency effect towards *S. oryzae* and a $90.6 \pm 0.028\%$ repellency effect towards *T. castaneum* at a maximum concentration of $0.63 \mu\text{L}/\text{cm}^2$ after 24 hours.

In the present study, the insecticidal activity studies of *L. coriacea* essential oil, including contact, fumigant, and repellency assay results, were compared with the experiments of Rani (2012) and concluded that the studies share similar results. The present study looked at the analysis of essential oil and study of insecticidal activity of the leaf essential oil of *L. coriacea* against stored grain insects such as *S.oryzae* and *T. castaneum*. The experimental results were compared with the study results of Cosimi et al. (2009), and it was found that compounds like linalyl acetate, linalool, and terpineol were predominant in both samples. Repellency tests were carried out in both studies and obtained almost similar findings. The insecticidal activity study of *L. salicifolia* essential oil also showed repellency against *S. zeamis* and *T. castaneum*, and this also supports the present study.

Essential oil analysis of *L. cubeba* revealed the presence of compounds such as eucalyptol (2.07%), linalool (2.36%), and α -terpineol (0.67%) (Yang et al., 2014). Identical compounds were also recognized from *L. coriacea* essential oil and the composition included eucalyptol (2.12%), linalool (41.06%), and α -Terpineol (75.81%). α -Terpineol was identified as the most repellent compound from the essential oil of *Baccharis salicifolia* (Garcia et al., 2005). The contact toxicity effect in both experiments was positive. In *L. cubeba*, toxicity against *L. serricone* and *L. bostrichophila* was determined with LD₅₀ values of $11.76 \mu\text{g}/\text{adult}$ and $20.15 \mu\text{g}/\text{cm}^2$ and in *L. coriacea*, the toxicity effect with LD₅₀ values against *S. oryzae* and *T. castaneum* was $4.173 \mu\text{L}/\text{cm}^2$ and $6.983 \mu\text{L}/\text{cm}^2$ respectively.

CHAPTER VI

SUMMARARY AND CONCLUSIONS

Nature has endowed us with a remarkable diversity of plants, which play a fundamental role in medicine and the preservation of human health. Plants have been an invaluable source of medicinal compounds for centuries, and most potent drugs originate from natural plant substances. Their rich chemical diversity allows one to harness a wide range of bioactive compounds with the potential to combat diseases, alleviate symptoms, and enhance overall well-being. Recognizing the significance of plants in medicine underscores the importance of preserving biodiversity and exploring the untapped potential of the plant kingdom for future medical advancements. The study of plant-derived compounds known as phytochemistry has led to the development of countless medications widely used in modern healthcare. Additionally, plant-based remedies such as herbal supplements and botanical extracts remain popular among individuals seeking alternative or complementary treatments. Moreover, ongoing research into plants' potential medicinal properties is uncovering new compounds with therapeutic applications, offering hope for innovative treatments and a deeper understanding of complex interactions between plants and human health. In the era of advancing technology and drug discovery, plants remain an invaluable resource in improving health care and well-being.

The genus *Litsea* holds several medicinal species with significant therapeutic properties, and most of them have been explored for their pharmaceutical potential. However, the three *Litsea* species selected for the present study, viz., *L. bourdillonii*, *L. coriacea* and *L. wightiana*, are unexplored. To reveal the hidden pharmaceutical potential of these plants, phytochemical studies and pharmacological evaluation have been carried out. As a part of the work, some preliminary pharmacogenetic characterization was also carried out to differentiate plants.

Morphological study

Comparative macromorphological features of *L. bourdillonii*, *L. coriacea* and *L. wightiana* were carried out and found that the three species showed maximum similarity while exhibiting some slight differences. Similar features like phyllotaxy, the nature of the stem, inflorescence, flower, fruit type, fruit color, fruit shape, *etc.* Variations in morphological features were observed in leaf characters such as tip, margin, shape, length, leaf lamina, the size of the fruit, and perianth tube. Morphological examination of the three species gives knowledge about identification and diversity, offering valuable information for scientific research and conservation efforts.

Anatomical and histochemical studies

The anatomical characters of the leaf, stem, bark, and petiole of *L. bourdillonii*, *L. coriacea*, and *L. wightiana* were examined. The study has provided crucial insights into their anatomical characteristics. In the analysis of each species, distinct anatomical features were identified, such as variation in tissue arrangements, cell types, and vascular patterns. Additionally, histochemical staining of leaf and stem parts revealed differences in the distribution of specific compounds within plant tissues like lignin, starch, tannin, and fixed oils. The abundance of distribution of different chemicals varied in the three species. Starch deposition in leaves was observed in the midrib region of *L. coriacea* than that of *L. bourdillonii*, and starch was absent in *L. wightiana*. In the stem, starch was present abundantly in the cortex and stele of *L. coriacea* compared to the other two species. Lignin content was found in leaf and stem tissues of all three species, with variation in the cells in which they are distributed. Tannin was present in the leaf and stem hypodermal region and was found to be high in *L. wightiana* compared to *L. bourdillonii* and *L. coriacea*. Fixed oils were distributed mainly in *L. coriacea* leaf and were completely absent in *L. bourdillonii*. The presence of oil was very low in stem when compared to the leaf.

Physicochemical and powder characterization

Physicochemical parameters such as water-soluble extractive, alcohol-soluble extractive, total ash, acid-insoluble ash, sulphated ash values and crude fiber content were tested in the leaves and bark of three species of *Litsea* selected for the study. Comparative physicochemical characters were noticed in three species. Water-soluble extractive (10.36%) and alcohol-soluble extractive (10.76%) values in *L. wightiana* were higher than the other two species. Sulphated ash content in the leaf of *L. bourdillonii*, *L. coriacea*, and *L. wightiana* were 3.94%, 1.96% and 4.13% and acid-insoluble ash in the leaf were 0.94%, 0.54%, and 0.88%. In bark samples, the sulphated ash contents were 15.86%, 9.62%, and 4.98%, and acid-insoluble ash was 0.54%, 0.34%, and 0.59% in *L. bourdillonii*, *L. coriacea* and *L. wightiana*, respectively. Crude fiber content was found high in *L. wightiana* leaves (4.40%) and bark (6.83%) respectively when compared to all other samples.

Phytochemical profiling

Preliminary qualitative phytochemical profiling of the methanolic extracts of the leaf and bark extracts of *L. bourdillonii*, *L. coriacea*, and *L. wightiana* revealed the presence of various classes of bioactive compounds including phenols, flavonoids, tannins, and glycosides in all samples. Sterols and quinones were observed only in leaf samples. All phytochemicals screened were found in *L. wightiana* leaves. Alkaloids were present in all samples except *L. wightiana* bark, and terpenoids were absent in *L. bourdillonii* and *L. coriacea* bark extracts. Leaf and bark extracts were subjected to quantitative estimation of flavonoids, phenols, and tannins. Among bark samples, the flavonoid content was higher in *L. wightiana*, ($32 \pm 0.37 \mu\text{g}/\text{mg}$). Phenol and tannin content were high in *L. bourdillonii* ($79 \pm 0.43 \mu\text{g}/\text{mg}$ and $173 \pm 0.25 \mu\text{g}/\text{mg}$, respectively). When leaf samples were analyzed, the flavonoid, phenol and tannin quantities were estimated more in *L. wightiana* ($37 \pm 0.41 \mu\text{g}/\text{mg}$, $119.14 \pm 0.26 \mu\text{g}/\text{mg}$ and $294.66 \pm 0.42 \mu\text{g}/\text{mg}$), respectively.

Liquid chromatographic mass spectrometric (LC/MS) Q-TOF analysis of *L. wightiana*, *L. coriacea*, and *L. bourdillonii* leaf extracts identified bioactive compounds belonging to various categories such as flavonoids, phenols, tannins,

glycosides, alkaloids, saponins, *etc.* Most of them proved their pharmacological efficacy, such as antioxidants, antibacterial, antidiabetic, anti-inflammatory, analgesic, wound healing, and insecticidal properties. Significant compounds identified by LC/MS analysis from *L. wightiana* leaves include chalcone, corytuberine, dicentrinone, xanthoplanine, laurilitsine, reticuline, grandisin, linalool, and the notable compounds from *L. coriacea* composed of luteolin, caffeic acid, columbin, magnoflorine, chlorogenic acid, gingerol, shogaol, hetisine, diosgenin, aucubin, piperlongumine, pentosalen, apigenin, arbutin, *etc.* From *L. bourdillonii*, compounds like senkirkine, berberine, p-cymene, and gallic acid were identified. Similar compounds were identified from *L. coriacea* and *L. bourdillonii* consisted of myristic acid, shogaol, columbin, p-cymene, chlorogenic acid, aucubin, apigenin, hexamethyl quercetagenin, syringic acid, *etc.* Gas chromatographic and spectrometric analysis of *L. wightiana* leaves extract revealed a variety of volatile compounds such as copaene, globulol, cubenol, tau-cadinol, hexadecanoic acid methyl ester, n-Hexadecanoic acid, 9,12-octadecanoic acid (z,z)-methyl ester, naphthalene esters, *etc.* GC/MS analysis of *L. coriacea* essential oil revealed volatile constituents like eucalyptol, linalool, endoborneol, α -terpineol, cyclohexanol esters, linalyl acetate *etc.*

Antimicrobial activity

The antibacterial and antifungal efficacy of leaf and bark extracts of three species of *Litsea* were carried out. Among the studied extracts *L. wightiana* bark and *L. bourdillonii* bark exhibited a significant inhibitory effect towards gram-negative bacteria than gram positive bacteria. But the extracts showed no significant antifungal activity against the fungal strains such as, *C. albicans* and *A. niger*.

Antioxidant activity

The antioxidant potential of leaf and bark extracts of the *L. bourdillonii*, *L. wightiana*, and *L. coriacea* was performed by DPPH Assay. *L. wightiana* leaf extracts showed the highest antioxidant activity among other samples. Hence, *L. wightiana* leaf extracts were further subjected to hydroxyl radical scavenging

activity, superoxide radical scavenging activity, and ferrous reducing power assays. All the assays gave positive results with significant antioxidant activity.

Antidiabetic activity

Antidiabetic potential of three *Litsea* species was investigated by alpha amylase and alpha glucosidase enzyme inhibition assays, and the efficacy of leaf and bark extracts as potential antidiabetic medicines.

Cytotoxicity

In vitro cytotoxicity experiments were carried out to examine the toxic effects of *Litsea* plant extracts in SK-MEL cell lines, and no efficient cytotoxicity implied the pharmacological relevance of the species studied.

Anti-inflammatory activity

The anti-inflammatory potential of *L. wightiana* leaves extract was evaluated using both *in vitro* and *in vivo* methods. An *in vitro* assay using an enzyme inhibitory assay proved that the extract effectively inhibited the functions of enzymes such as cyclooxygenase, lipoxygenase, myeloperoxidase, and nitric oxide synthase. As a result of the inhibition of nitric oxide synthase, the level of nitrite in the cell was found to decrease. This result laid the foundation for *in vitro* anti-inflammatory activity. The anti-inflammatory activity was confirmed by gene expression studies. It was also found that the genes responsible for producing proinflammatory mediators and cytokines were downregulated.

To identify the anti-inflammatory compounds behind the activity, molecular docking was conducted. Various compounds obtained in GC and LC/MS analysis, which were reported to be anti-inflammatory, were docked against target proteins like cyclooxygenase-2, 5-lipoxygenase, human myeloperoxidase and human inducible nitric oxide synthase, and it was found that the alkaloid compound dicentrinone showed shorter binding energy and more affinity towards target compounds compared to other phytochemicals.

To give additional supportive evidence for this study, an *in vivo* anti-inflammatory experiment was carried out in model animals using the carrageenan-induced paw edema model and promising results were obtained.

Wound healing activity

Wound healing efficacy of *L. wightiana* leaves extract was evaluated by *in vitro* scratch wound healing assay and *in vivo* excision wound model experiment in Wistar albino rats. Histopathological examinations were also conducted to investigate the actual wound healing mechanism in animal tissues. Encouraging findings were obtained, which established the wound-healing potential of the extract.

Essential oil analysis and Bioactivity studies

Essential oil from leaves of *L. coriacea* was isolated by the hydrodistillation method using the Clevenger apparatus. Isolated oil was subjected to GC/MS and GC-FID analysis, and important volatile constituents, mainly monoterpenoids, were identified. The essential oil was again subjected to insecticidal activity studies. The experiment was against two stored pests, namely, *S. oryzae* and *T. castaneum*. Contact toxicity, fumigant toxicity and repellent properties were studied and confirmed the insecticidal potential of *L. coriacea* leaf oil.

Phytochemical profiling and pharmacological studies have greatly expanded the knowledge of medicinal plants and their potential application in healthcare. These studies enable the identification of specific bioactive components responsible for the health benefits, allowing for the targeted development of herbal medicines and dietary supplements. Additionally, they contribute to the sustainable utilization of plant resources by promoting biodiversity conservation.

Generally, plant essential oils are more affordable than conventional insecticides, which encourages the advancement and development of botanical alternatives. The present study seeks the possibility of essential oil as effective insecticides or repellents, or fumigants against stored pests like *S. oryzae* and moderate activity against *T. castaneum*. The findings of insecticidal activity studies,

including contact, fumigant and repellent efficacies of *L. coriacea* essential oil, were proved and substantiated by statistical tests. This study is significant because the use of natural insecticides can mitigate adverse effects such as toxicity to other non-targeted organisms, damage to environment and insecticide resistance associated with synthetic chemicals. In this regard, biological insecticides derived from plants offer a sustainable solution, being effective, biodegradable, selective and posing minimal risk development in the insect population, thereby ensuring greater environmental safety.

The present study provides an insight into the pharmacological potential of three species of *Litsea*, viz., *L. bourdillonii*, *L. coriacea*, and *L. wightiana*. The study reveals the antibacterial, antioxidant, antidiabetic, anti-inflammatory, wound healing and insecticidal properties of *Litsea* species selected for the study. Since these species are unexplored till the time, this study unravels the bioactivities of these endemic species of *Litsea* distributed in the Southern Western Ghats, India. This investigation is a comprehensive analysis of phytochemistry and pharmacological aspects of these species. Even though several aspects of the pharmacology of these trees have been studied, more bioactivities are yet to be explored. Further investigations and in-depth studies may contribute a lot more to scientific society and healthcare management.

CHAPTER VI

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Physicochemical parameters such as water-soluble extractive, alcohol-soluble extractive, total ash, acid-insoluble ash, sulphated ash values and crude fiber content were tested in the leaves and bark of three species of *Litsea* selected for the study. Comparative physicochemical characters were noticed in three species. Water-soluble extractive (10.36%) and alcohol-soluble extractive (10.76%) values in *L. wightiana* were higher than the other two species. Sulphated ash content in the leaf of *L. bourdillonii*, *L. coriacea*, and *L. wightiana* were 3.94%, 1.96% and 4.13% and acid-insoluble ash in the leaf were 0.94%, 0.54%, and 0.88%. In bark samples, the sulphated ash contents were 15.86%, 9.62%, and 4.98%, and acid-insoluble ash was 0.54%, 0.34%, and 0.59% in *L. bourdillonii*, *L. coriacea* and *L. wightiana*, respectively. Crude fiber content was found high in *L. wightiana* leaves (4.40%) and bark (6.83%) respectively when compared to all other samples.

Phytochemical profiling

Preliminary qualitative phytochemical profiling of the methanolic extracts of the leaf and bark extracts of *L. bourdillonii*, *L. coriacea*, and *L. wightiana* revealed the presence of various classes of bioactive compounds including phenols, flavonoids, tannins, and glycosides in all samples. Sterols and quinones were observed only in leaf samples. All phytochemicals screened were found in *L. wightiana* leaves. Alkaloids were present in all samples except *L. wightiana* bark, and terpenoids were absent in *L. bourdillonii* and *L. coriacea* bark extracts. Leaf and bark extracts were subjected to quantitative estimation of flavonoids, phenols, and tannins. Among bark samples, the flavonoid content was higher in *L. wightiana*, ($32 \pm 0.37 \mu\text{g}/\text{mg}$). Phenol and tannin content were high in *L. bourdillonii* ($79 \pm 0.43 \mu\text{g}/\text{mg}$ and $173 \pm 0.25 \mu\text{g}/\text{mg}$, respectively). When leaf samples were analyzed, the flavonoid, phenol and tannin quantities were estimated more in *L. wightiana* ($37 \pm 0.41 \mu\text{g}/\text{mg}$, $119.14 \pm 0.26 \mu\text{g}/\text{mg}$ and $294.66 \pm 0.42 \mu\text{g}/\text{mg}$), respectively.

Liquid chromatographic mass spectrometric (LC/MS) Q-TOF analysis of *L. wightiana*, *L. coriacea*, and *L. bourdillonii* leaf extracts identified bioactive compounds belonging to various categories such as flavonoids, phenols, tannins,

glycosides, alkaloids, saponins, *etc.* Most of them proved their pharmacological efficacy, such as antioxidants, antibacterial, antidiabetic, anti-inflammatory, analgesic, wound healing, and insecticidal properties. Significant compounds identified by LC/MS analysis from *L. wightiana* leaves include chalcone, corytuberine, dicentrinone, xanthoplanine, laurilitsine, reticuline, grandisin, linalool, and the notable compounds from *L. coriacea* composed of luteolin, caffeic acid, columbin, magnoflorine, chlorogenic acid, gingerol, shogaol, hetisine, diosgenin, aucubin, piperlongumine, pentosalen, apigenin, arbutin, *etc.* From *L. bourdillonii*, compounds like senkirkine, berberine, p-cymene, and gallic acid were identified. Similar compounds were identified from *L. coriacea* and *L. bourdillonii* consisted of myristic acid, shogaol, columbin, p-cymene, chlorogenic acid, aucubin, apigenin, hexamethyl quercetagenin, syringic acid, *etc.* Gas chromatographic and spectrometric analysis of *L. wightiana* leaves extract revealed a variety of volatile compounds such as copaene, globulol, cubenol, tau-cadinol, hexadecanoic acid methyl ester, n-Hexadecanoic acid, 9,12-octadecanoic acid (z,z)-methyl ester, naphthalene esters, *etc.* GC/MS analysis of *L. coriacea* essential oil revealed volatile constituents like eucalyptol, linalool, endoborneol, α -terpineol, cyclohexanol esters, linalyl acetate *etc.*

Antimicrobial activity

The antibacterial and antifungal efficacy of leaf and bark extracts of three species of *Litsea* were carried out. Among the studied extracts *L. wightiana* bark and *L. bourdillonii* bark exhibited a significant inhibitory effect towards gram-negative bacteria than gram positive bacteria. But the extracts showed no significant antifungal activity against the fungal strains such as, *C. albicans* and *A. niger*.

Antioxidant activity

The antioxidant potential of leaf and bark extracts of the *L. bourdillonii*, *L. wightiana*, and *L. coriacea* was performed by DPPH Assay. *L. wightiana* leaf extracts showed the highest antioxidant activity among other samples. Hence, *L. wightiana* leaf extracts were further subjected to hydroxyl radical scavenging

activity, superoxide radical scavenging activity, and ferrous reducing power assays. All the assays gave positive results with significant antioxidant activity.

Antidiabetic activity

Antidiabetic potential of three *Litsea* species was investigated by alpha amylase and alpha glucosidase enzyme inhibition assays, and the efficacy of leaf and bark extracts as potential antidiabetic medicines.

Cytotoxicity

In vitro cytotoxicity experiments were carried out to examine the toxic effects of *Litsea* plant extracts in SK-MEL cell lines, and no efficient cytotoxicity implied the pharmacological relevance of the species studied.

Anti-inflammatory activity

The anti-inflammatory potential of *L. wightiana* leaves extract was evaluated using both *in vitro* and *in vivo* methods. An *in vitro* assay using an enzyme inhibitory assay proved that the extract effectively inhibited the functions of enzymes such as cyclooxygenase, lipoxygenase, myeloperoxidase, and nitric oxide synthase. As a result of the inhibition of nitric oxide synthase, the level of nitrite in the cell was found to decrease. This result laid the foundation for *in vitro* anti-inflammatory activity. The anti-inflammatory activity was confirmed by gene expression studies. It was also found that the genes responsible for producing proinflammatory mediators and cytokines were downregulated.

To identify the anti-inflammatory compounds behind the activity, molecular docking was conducted. Various compounds obtained in GC and LC/MS analysis, which were reported to be anti-inflammatory, were docked against target proteins like cyclooxygenase-2, 5-lipoxygenase, human myeloperoxidase and human inducible nitric oxide synthase, and it was found that the alkaloid compound dicentrinone showed shorter binding energy and more affinity towards target compounds compared to other phytochemicals.

To give additional supportive evidence for this study, an *in vivo* anti-inflammatory experiment was carried out in model animals using the carrageenan-induced paw edema model and promising results were obtained.

Wound healing activity

Wound healing efficacy of *L. wightiana* leaves extract was evaluated by *in vitro* scratch wound healing assay and *in vivo* excision wound model experiment in Wistar albino rats. Histopathological examinations were also conducted to investigate the actual wound healing mechanism in animal tissues. Encouraging findings were obtained, which established the wound-healing potential of the extract.

Essential oil analysis and Bioactivity studies

Essential oil from leaves of *L. coriacea* was isolated by the hydrodistillation method using the Clevenger apparatus. Isolated oil was subjected to GC/MS and GC-FID analysis, and important volatile constituents, mainly monoterpenoids, were identified. The essential oil was again subjected to insecticidal activity studies. The experiment was against two stored pests, namely, *S. oryzae* and *T. castaneum*. Contact toxicity, fumigant toxicity and repellent properties were studied and confirmed the insecticidal potential of *L. coriacea* leaf oil.

Phytochemical profiling and pharmacological studies have greatly expanded the knowledge of medicinal plants and their potential application in healthcare. These studies enable the identification of specific bioactive components responsible for the health benefits, allowing for the targeted development of herbal medicines and dietary supplements. Additionally, they contribute to the sustainable utilization of plant resources by promoting biodiversity conservation.

Generally, plant essential oils are more affordable than conventional insecticides, which encourages the advancement and development of botanical alternatives. The present study seeks the possibility of essential oil as effective insecticides or repellents, or fumigants against stored pests like *S. oryzae* and moderate activity against *T. castaneum*. The findings of insecticidal activity studies,

including contact, fumigant and repellent efficacies of *L. coriacea* essential oil, were proved and substantiated by statistical tests. This study is significant because the use of natural insecticides can mitigate adverse effects such as toxicity to other non-targeted organisms, damage to environment and insecticide resistance associated with synthetic chemicals. In this regard, biological insecticides derived from plants offer a sustainable solution, being effective, biodegradable, selective and posing minimal risk development in the insect population, thereby ensuring greater environmental safety.

The present study provides an insight into the pharmacological potential of three species of *Litsea*, viz., *L. bourdillonii*, *L. coriacea*, and *L. wightiana*. The study reveals the antibacterial, antioxidant, antidiabetic, anti-inflammatory, wound healing and insecticidal properties of *Litsea* species selected for the study. Since these species are unexplored till the time, this study unravels the bioactivities of these endemic species of *Litsea* distributed in the Southern Western Ghats, India. This investigation is a comprehensive analysis of phytochemistry and pharmacological aspects of these species. Even though several aspects of the pharmacology of these trees have been studied, more bioactivities are yet to be explored. Further investigations and in-depth studies may contribute a lot more to scientific society and healthcare management.

CHAPTER VII

RECOMMENDATIONS

Litsea species selected for the present study were identified to have higher therapeutic potential and can be recommended for various ailments. Phytochemical and pharmacological studies proved bioactivities, like antibacterial, antioxidant, antidiabetic effects of *L. bourdillonii*, *L. coriacea* and *L. wightiana*. The three species were enriched with active phytoconstituents like alkaloids, flavonoids, phenols, glycosides, saponins, *etc.*, in leaf and stem bark. The quantitative study confirmed that the quantity of phenols, flavonoids and tannins are present more in the leaves of *L. wightiana*. Hence, the high-order activities like anti-inflammatory and wound-healing efficacy of plants using both *in vitro* and *in vivo* methods were studied, and positive results were obtained. The biological effect of *L. wightiana* is promising and encouraging, and it can contribute more to the scientific world. Essential oil analysis and insecticidal activity studies revealed the potential of *L. coriacea* as biocontrol agents. This research revealed valuable potentialities of the selected plants. A study on the other aspects will disclose more useful results.

The results and reports as part of the present investigation

- Morphological studies of the three selected species were conducted, which will be helpful for the public in identifying and collecting plants.
- Anatomical and histochemical studies will also be beneficial to distinguish the plants, with the knowledge of internal structure and chemical constituents.
- The study has given information about the physicochemical and powder features of leaf and bark samples of the selected plants.
- Phytochemical profiling substantiated that the leaves contain more phytochemicals than the stem bark. The GC/MS and LC/MS Q-TOF studies contributed to identifying volatile and non-volatile compounds.

- GCMS/GC-FID revealed the compounds in *L. coriacea* leaves essential oil
- The antioxidant efficacy of leaf samples was revealed by the study, which can be utilized to mitigate the illness caused by free radicals.
- The plants possess moderate antibacterial and cytotoxicity effects.
- The study revealed the antidiabetic potential of the plants.
- The anti-inflammatory potential of *L. wightiana* leaves was explored by *in vitro* and confirmed by *in vivo* studies, gene expression analysis and molecular docking methods. This may contribute to the treatment of inflammatory diseases.
- The wound-healing capability of *L. wightiana* leaves was demonstrated by *in vitro* and *in vivo* methods. Histopathological experiments provided additional evidence for the wound healing process. Extracts were prepared as an ointment and applied to the experimental animals, and the same can be applied in humans.
- The essential oil isolation and its bioactivity studies are another aspect of this work. These studies proved that the essential oils of *L. coriacea* can be used to eradicate stored grain pests such as *S. oryzae* and *T. castaneum*, which pose severe threats to stored food products.

Recommendations for further research scope are

- Isolation and study of detailed bioactivities of the phytochemicals of three *Litsea* plants.
- Nanoparticle studies and synthesis of nanoparticles using effective phytoextracts of *Litsea* plants.
- Clinical trials of ointment made of *L. wightiana* leaf extracts, patenting, production and marketing of ointment.

- Since the plants are proven with significant bioactivities and included in the Nearly Threatened (NT) category of IUCN, develop promising propagation techniques to conserve the three medicinally important *Litsea* species endemic to the Southern Western Ghats.

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