

**Mycochemical characterization and bioactivity screening
of *Microporus affinis* (Blume & T. Nees) Kunt. and
M. xanthopus (Fr.) Kunt.**

Thesis
submitted to the University of Calicut
for the award of the degree of
DOCTOR OF PHILOSOPHY IN BOTANY

by
ANEESH S.



**CELL AND MOLECULAR BIOLOGY DIVISION
DEPARTMENT OF BOTANY
UNIVERSITY OF CALICUT
KERALA - 673635, INDIA
OCTOBER 2025**



**UNIVERSITY OF CALICUT
DEPARTMENT OF BOTANY**

CALICUT UNIVERSITY P.O., 673 635
KERALA, INDIA

Dr JOHN E. THOPPIL
Senior Professor
Cell & Molecular Biology Division

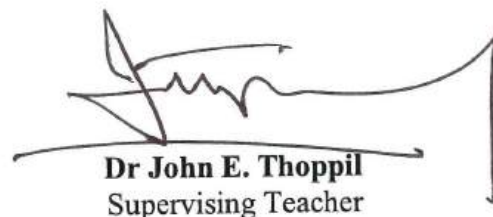
Phone: 0494-2407406, 407
Mob : 09847767193
E-mail: jethoppil@gmail.com

Date: 14-10-2025

CERTIFICATE

This is to certify that the thesis entitled “**Mycochemical characterization and bioactivity screening of *Microporus affinis* (Blume & T. Nees) Kunt. and *M. xanthopus* (Fr.) Kunt.**” submitted to the University of Calicut, for the award of the degree of DOCTOR OF PHILOSOPHY IN BOTANY is a record of original research work done by Aneesh S., during the period of study (2016-2025) at the Cell and Molecular Biology Division, Department of Botany, University of Calicut under my supervision and guidance and it has not formed the basis for the award of any degree or diploma. I also certify that the contents in the thesis are subjected to plagiarism check using the software iThenticate and no text or data is reproduced from other works.

C. U. Campus



Dr John E. Thoppil
Supervising Teacher

Dr. John E. Thoppil
Senior Professor
Cell & Molecular Biology Division
Department of Botany
University of Calicut-673635



UNIVERSITY OF CALICUT
DEPARTMENT OF BOTANY
CALICUT UNIVERSITY P.O., 673 635
KERALA, INDIA

Dr JOHN E. THOPPIL
Senior Professor (Retd.)
Cell & Molecular Biology Division

Phone: 0494-2407406, 407
Mob: 09847767193
E-mail: jethoppil@gmail.com

Date: 23/06/2026

CERTIFICATE

I certify that no corrections were pointed out by the adjudicators in thesis entitled "Mycochemical characterization and bioactivity screening of *M. affinis* (Blume & T. Nees) Kunt. and *M. xanthopus* (Fr.) Kunt." submitted by Mr Aneesh S. Hence a hard copy of the thesis with the original content along with a soft copy is hereby submitted to the University.

C. U. Campus

Dr JOHN E. THOPPIL

Supervising Teacher

Dr. John E. Thoppil
Emeritus Professor
Department of Botany
University of Calicut-673635



DECLARATION

I hereby declare that the work presented in the thesis entitled “**MYCOCHEMICAL CHARACTERIZATION AND BIOACTIVITY SCREENING OF *MICROPORUS AFFINIS* (BLUME & T. NEES) KUNT. AND *M. XANTHOPUS* (FR.) KUNT.,**” is based on the original work done by me under the guidance of Dr John E. Thoppil, Senior Professor (Retd), Department of Botany, 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 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.

Aneesh S. Anusky

Name and Signature of the scholar

Dr John E. Thoppil

Name and Signature of the supervising teacher

Place: CU Campus

Date: 15-10-2025

Dr. John E. Thoppil
Senior Professor
Cell & Molecular Biology Division
Department of Botany
University of Calicut-673635



UNIVERSITY OF CALICUT CERTIFICATE ON PLAGIARISM CHECK

1.	Name of the Research Scholar	Aneesh S	
2.	Title of thesis / dissertation	Mycochemical characterization and bioactivity screening of Microporus affinis (Blume & T. Nees) Kunt. and M. xanthopus (Fr.) Kunt.	
3.	Name of the Supervisor	Dr John E. Thoppil	
4.	Department/Institution	Department of Botany, University of Calicut, Malappuram	
5.	Similar content (%) identified	Non Core	Core
		Introduction/ Theoretical overview/Review of literature/ Materials & Methods/ Methodology	Analysis/Result/Discussion / Summary/Conclusion/ Recommendations
		9	7
	Acceptable maximum limit (%)	10	10
6.	Software used	iThenticate	
7.	Date of verification	08.10.2025	

*Report on plagiarism check, specifying included/excluded items with % of similarity to be attached.

Checked by (with name, designation & signature)

Name and signature of the Researcher

Name and signature of the Supervisor.

Aneesh S.

Dr John E. Thoppil

Dr. John E. Thoppil
Senior Professor
Cell & Molecular Biology Division
Department of Botany
University of Calicut-673635

The Doctoral Committee* has verified the report on plagiarism check with the contents of the thesis, as summarized above and appropriate measures have been taken to ensure originality of the Research accomplished herein.

Name & Signature of the HoD/HoI (Chairperson of the Doctoral Committee)

(Dr. A. Yusuf)

*In case of languages like Malayalam, Tamil etc..on which no software is available for plagiarism check, a manual check shall be made by the Doctoral Committee, for which an additional certificate has to be attached.

Head, Department of Botany
University of Calicut
Calicut University P.O.
Kerala, India - 673 635



ACKNOWLEDGEMENT

First and foremost, I would like to praise and thank God, the Almighty, who has showered countless blessings, knowledge, opportunities, and strength to withstand the challenging situations throughout the PhD endeavour. It is a humbling experience of gratification to look back and thank those who have helped me to accomplish my long-cherished dream. It was a long and bumpy ride, but the support, motivation, and encouragement of many made this journey worthwhile.

I would like to express my heartfelt gratitude from the bottom of my heart to my research supervisor, beloved and most respected Dr John E. Thoppil, Senior Professor (Retd), Department of Botany, University of Calicut, for his patience, unwavering support, insightful advice, caring, and guidance throughout the completion of my thesis. His thoughtful feedback, regular monitoring, and constant encouragement have not only honed my skills but also enriched the quality of this thesis. His understanding of students' family circumstances, health, and work pressure must be greatly acknowledged. It is my privilege to be one of his students. The time and energy invested in me is always weighed in gold. His supervision was a gift to me, and he was a tremendous boon to my research endeavour. This venture would not have been possible without him. Thanks a lot, Dear Sir.

I extend my sincere thanks to Prof. C. C. Harilal, Head, Department of Botany, University of Calicut, for providing facilities for the completion of my thesis work. I extend my gratitude to Prof. Santhosh Nampy, Prof. V. V. Radhakrishnan, and Prof. Jos T. Puthur, former heads of the Department of Botany, University of Calicut.

I thankfully acknowledge all the teaching faculties and non-teaching staff of the Department of Botany for their support during the tenure of my Ph.D. I am immensely thankful to Dr P. Manimohan for his valuable suggestions during the collection of materials. I express my deep sense of gratitude to Dr T. K. Arunkumar, Associate Professor, Zamorin's Guruvayurapan College, Calicut, for his patience,

support, timely response, and authenticating the study materials. I also appreciate his expertise in the field of macrofungi, especially bracket fungi.

I am greatly indebted to Dr P. Balakrishnan and Dr Jyothilekshmi P., who have inspired me a lot to pursue in the field of study and to become a botanist as I am today. Their constant guidance, valuable suggestions, proper advice, friendly approach, moral support, and encouragement molded me to overcome challenges and develop a deeper connection to the world of Botany.

I express my sincere thanks to Mrs Geethakumari, Dr Zareena Viji, Dr Divya, Ms Smitha, Dr Kishore, Dr Rajesh, Prof. Sethumathavan, Dr George Joseph, Dr C. M. Joy, and Dr Jacob Varghese. Special mention to Dr V. J. Dominic, Dr M. S. Francis, Dr Mollykutty and Dr T. C. Thankachan for their valuable advice, support, guidance, suggestions, and inspirations, which helped me to pursue the field of Botany.

I am grateful to my teachers, Dr Jyothilakshmi P. and Dr M. S. Francis, for encouraging me to pursue a career in Botany rather than accept a position as Agricultural Demonstrator with the Spices Board of India. Their timely guidance was instrumental to my success.

I express my thanks to Dr Suma, Head, Department of Plantation Crops, Agricultural University, Mannuthy, Thrissur, for relieving me for joining M. Phil., which made this journey possible.

I thankfully acknowledge Mr Pavithran (Retired Principal, GFVHSS Cheruvarhur, Kasaragod) for relieving me for PhD admission in the Department of Botany.

I am greatly indebted to Mr Laison T. J., Principal, and Mrs Julin Joseph, Manager, RMHSS Aloor, Thrissur for permitting me to convert the mode of research into part time.

I express my heartfelt thanks to Dr Deepnalatha, Assistant Professor, Department of Botany, University of Calicut, and Dr Anil Kumar, Assistant Professor,

Govt College, Mahi, for suggestions, guidance, and support rendered during the initial stages of research, especially for material collection.

Financial assistance provided by the Government of Kerala in the form of E-grantz is duly acknowledged.

I am greatly thankful to the Department of Forest, Govt of Kerala, for granting forest permission to collect fungal materials, and special mention to Mr Asharaf, Range officer, Thusharagiri Wildlife Sanctuary, Kozhikode, for extra care and support during sample collection.

I am grateful to the Advanced Instrumentation Research Facility, Jawaharlal University of Delhi, for GC/MS analysis. I also acknowledge the Sophisticated Analytical Instrument Facility, Indian Institute of Technology, Bombay, for HR-LC/MS analysis.

I thank the National Centre for Cell Science, Research Institute, Pune, Maharashtra, for giving cell lines.

I am greatly thankful to Dr Rajesh and Dr Nithin, Centre for Molecular Biology and Applied Science, Thiruvananthapuram, for providing laboratory facilities for cell line studies.

I thank the Director and staff of the Central Sophisticated Instrumentation Facility, University of Calicut, for providing laboratory facilities.

I thank Dr. Nasirudheen T., Assistant Librarian at CHMK Library, for conducting the plagiarism check. I would like to thank the Librarian, Department of Botany, University of Calicut. I also appreciate the support of Dr Prakasan, former Librarian, Department of Botany, University of Calicut.

I thank the staff of the Mushroom Division, Indian Institute of Horticultural Research, Bangalore, for permitting me to visit the institution and the library. I am also grateful to the staff of M. S. Swaminathan Research Foundation, Wayanad, for the help rendered in data collection. Special mention to Mr Salim for the help rendered by him. I thank Mr Prakasan, a member from the tribal community, for

introducing me into the tribal colonies of Muthanga, Chithalayam, and Thirunelli of Wayanad district, which helped me to discuss with the elders of tribal groups regarding medicinal importance and toxic nature of the study materials. I am greatly indebted to Mrs Ammaniyamma, a 90-year-old woman from the Chithalayam tribal area, for patiently hearing and revealing ideas of mushroom usage.

I express my sincere thanks to Pondicherry University, Goa University, MG University, Kottayam, the Indian Institute of Forest Research, Dehradun, Uttarakhand, and Bharathiar University, Coimbatore, SD College, Alapuzha, Bishop Moore College, Mavelikara, Alapuzha, and Providence College, Kozhikode, for providing me the platform for paper presentations.

I extend my heartfelt thanks to Mr Rajesh and other staff, Bina Photostat, Villunniyal, for the technical support in documenting this thesis.

I am deeply indebted to my labmates, Dr Renjana, Dr Sandhya Vincent Neelamkavil, Dr Seemadevi, Dr Sumitha, Dr Anjana, Dr Neethu Kannan, Dr Prajitha, Dr Sajitha Menon, Dr Rubeena, Dr Preetha Mol, Dr Niranjana, Dr Nushiba Nasser, Mrs Sameena, Mrs Divyalakshmi, Ms Hiba, Mrs Thasnim, Mrs Sheethal, for support, suggestions, and care. I extent my deep sense of gratitude to my dear Dr Sinitha and Dr Archana for being my good motivators. Their encouragement made the challenging moments much easier. My emotional attachments towards them will always be there, and I cannot thank them enough for their presence and affection. This lab has rendered a truly rewarding experience, and that is largely due to the positive atmosphere created by Dr Ramyasree, Dr Reshmi, and Dr Aswathi. Their support has lifted my spirits through this arduous research. Thank you for making the lab a fun and inspiring place to be. Energy and friendship rendered by them made this journey incredibly enjoyable. I express my sincere gratitude to Mrs Thasni, Ms Krishna and Ms Mirsha for their moral support and a fresh perspective when I was stuck on. Once again, I am extremely thankful to all my labmates for the teamwork and shared expertise.

I am so happy to have friends like Dr Manu Philip, Dr Veena, Dr Neethu, Dr Farhad, and Dr Surekha. Thanks a lot, being my constant source of joy and strength, making the good times better and the tough times easier.

I am forever grateful for the good times and kindness of Mr Rakesh and Mr Dhanesh. Thanks for always showing up when it counts and for providing a shoulder to lean on when life gets hard.

I express my whole hearted thanks to my buddies, Dr. Shinoj, Dr. Vishnu Mohan, Mr. Anu, Dr. Binil, Dr. Rajeesh, Dr. Vishnu, Dr. Akhil, Dr. Syam Radh, Dr. Sarath, Mr. Nicemon, Mr. Nikhil, Mr. Noble, Mr. Irfan, and Mr. Sasi for their support, friendship, and the many memorable moments we have shared. I am truly grateful for your encouragement and camaraderie. Thank you for recognizing my strengths and providing encouragement throughout my research tenure.

I extend my thanks to Dr Faisal, Dr Hareesh, Mr Vinod, Mr Riyas, Mr Abhishek, Dr Nitheesh Roy, Mr Ajeesh, Dr Nitheesh, Dr Shahina, Dr Greeshma, Dr Sanoj, Mr Prasanth, Dr Janeesha, Mr Anwar, and Dr Alfred. My in-depth gratitude and love towards Mr Abhimanyu, Mr Sayanth, Mr Ajmal, Mr Binoy, Mr Vyshnav, Mr Sangeeth, Mr Visal, Mr Pradhyumnan, Mr Ansil, Mr Sumith, Mr Tijo, Mr Vishnu, and Mr Deepak. I extend thanks to Mr Dheeraj, Mr Ashok, Mr Abhilash, and Mr Alan.

I thank Mrs Arunima for the prediction that I'd pursue a Ph.D, which was so encouraging and truly helped me through it.

I would like to thank all my colleagues who have suffered a lot, and their support and encouragement made the completion of this journey much better. I appreciate my students at RMHSS Aloor, Thrissur for their understanding and cooperation during the final stages of my thesis work.

I thank my cousins Appu, Kathu, Chakku, Ammu and Achu for their kindness, support, and encouragement during the toughest times.

I would like to offer my profound thanks to my family for their endless love, support, and patience during this challenging journey. Their belief in me was my

constant strength, and without them, this achievement would not be possible. I cannot find words to express my gratitude towards my family members for everything they have done for me. Their unwavering support has been my foundation, and your encouragement has carried me through every difficult moment during the past several years. This Ph.D. is a testament to their love and belief in me, as it is to my own efforts. Thanks a lot, my dear Achan and Amma. Special thanks to my dear wife Aswathi for tolerating my constant talk about my research and for being my rock and always being there to listen. I am so grateful for her endless patience, sacrifice, and understanding during the long hours and stressful moments of my research tenure. I want to express my deepest gratitude to my dear sister Anu, who was a constant confidante and unwavering wall of love and support during the challenges of my doctoral studies. Thanks to my sister, whose unwavering belief and constant encouragement were a vital source of my PhD journey. I extend my thanks to Santhoshettan, Abhi, and Achu for support and care. I express my whole hearted thanks to Arunettan and Amma for their moral support. I am grateful to my father-in-law, who cared deeply about my career but he is not here to witness this moment. I thank him for leading with strength, wisdom, and love.

Finally, I sincerely thank everyone who contributed, in any capacity, to the completion of this Ph.D. thesis.

Aneesh S.

ABSTRACT

Mycochemical characterization and bioactivity screening of *Microporus affinis* (Blume & T. Nees) Kunt. and *M. xanthopus* (Fr.) Kunt.

Bracket fungi are valuable in research for their potential to develop new pharmaceuticals. In this study, the Polyporaceae family of Basidiomycetes was explored for its bioactivities. *Microporus*, a less studied genus in Polyporaceae, has yet to be examined in detail for medicinal impacts. This research focuses on *M. xanthopus* (Fr.) Kunt. and *M. affinis* (Blume & T. Nees) Kunt., distinguished by the lateral stipe in *M. affinis* and the central stipe in *M. xanthopus*.

Two species of *Microporus* were screened for chemical constituents. Chemical characterization was done in four sections: preliminary qualitative mycochemical (fungal chemical) screening, quantitative estimation (measuring the amount of specific chemicals), determination of volatile chemical constituents through GC/MS analysis (Gas Chromatography/Mass Spectrometry), and identification of non-volatile chemicals through HR-LC/MS analysis (High Resolution Liquid Chromatography/Mass Spectrometry). Terpenoids, steroids, and alkaloids were observed in both species in preliminary studies. Glycosides and saponins were reported only in *M. xanthopus*. Alkaloids, terpenoids, carbohydrates, and proteins were determined quantitatively. GC/MS analysis of *M. xanthopus* revealed 45 compounds, while *M. affinis* had 46. In both species, fatty acids were the leading class, and linoleic acid had the highest peak area. HR-LC/MS in *M. xanthopus* reported 31 compounds, while *M. affinis* had about 9 mycoconstituents. Different types of fungal sterols (a group of steroids) with a wide spectrum of bioactivities have also been reported.

Because of its diverse chemical constituents, *Microporus* was studied for bioactivities such as antioxidant (combating cell damage from oxidants), cytotoxic (toxic to cells), antiproliferative (preventing cell growth), and neuroprotective (protecting nerve cells) effects. Special emphasis was given to extended neuroprotective studies of *M. xanthopus*, confirmed by techniques like ROS (Reactive Oxygen Species) measurement using DCFDA (a fluorescent dye), Annexin V-FITC apoptosis (detection of programmed cell death using a specific marker) using flow cytometry (cell analysis technique), autophagy (cellular self-digestion), indirect ELISA (a lab method to detect proteins), and gene expression studies using qRT-PCR (quantitative Reverse Transcription Polymerase Chain Reaction).

The fungal extracts were subjected to free radical scavenging assays, like DPPH, hydroxyl radicals, nitric oxide, and reducing power. In this scenario, *M. affinis* was found to be more potent than *M. xanthopus* even though both species are having antioxidant potential. Oxidative damage has a key role in the aging process and is linked to diseases like Cancer, Alzheimer's, and Parkinson's. Free radical scavengers can help to mitigate these effects. So, the study was carried out on cytotoxic, antiproliferative, and neuroprotective areas. Cytotoxic efficiency was tested in *Allium cepa*, together with the degree of mitotic index and percentage of chromosomal aberrations. Fungal extract was tested *in vitro* for antiproliferation on the DLD1 cell line (colorectal cancer) using the MTT assay. Comparatively, the lower LC₅₀ value of *M. affinis* indicates its higher cytotoxic potential towards the DLD1 cell line than that of *M. xanthopus*. It was compared with the normal cell line L929. The degree of apoptosis was confirmed by the AO/EtBr double staining method. The presence of terpenoids in *Microporus* supports neuroprotective activities. Hence, the fungal extracts were subjected to the *in vitro* study of neuroprotective efficacy on IMR-32 (human neuroblastoma cell line). Cell viability was assessed by using the MTT assay.

Neurodegeneration was induced by using β -amyloid. Apoptosis determination using the AO/EtBr method clearly revealed the neuroprotective efficacy of *M. xanthopus*. A dose dependent increase in cell viability was noticed in *M. xanthopus* only. So, further validations on neuroprotection were carried out in *M. xanthopus* only. From the study conducted, it was clear that the increased ROS level could be controlled by using fungal extract on amyloid treated cells. It indirectly supports the recovery of cells with neurodegeneration. In indirect ELISA, the primary antibodies, ChAt, Glutamate Receptor 2, Tau, and alpha synuclein were used. HRP conjugate was used as the secondary antibody. In a study of Annexin V- FITC, it was clear that the sample treatment was able to reduce the late apoptotic cells, which indicates its potential role in neuroprotection. The effect of the parkin gene was also compared with the housekeeping gene actin. The present study proved that the increased cell viability, decreased apoptosis, and lower ROS levels are good for neuroprotection. Even though it is positively supporting neuroprotection, *in vivo* studies are also required to substantiate the neuroprotective efficacy of this fungal material.

Keyword: Basidiomycetes, *Microporus*, Mychochemical, Bioactivity, Antioxidant, Anticancer, Neuroprotection

സംഗ്രഹം

മൈക്രോപോറസ് അഫിനിസ്, മൈക്രോപോറസ് സാന്തോപ്പസ് എന്നിവയുടെ രാസസ്വഭാവ രൂപീകരണവും ജൈവപ്രവർത്തന പരിശോധനയും.

ഗവേഷണ മേഖലയിൽ ബ്രാക്കറ്റ് ഫംഗസുകൾ ഔഷധ നിർമ്മാണത്തിനായി ചെലുത്തുന്ന സ്വാധീനം വിലപ്പെട്ടതാണ്. ബസിഡിയോമൈസറ്റുകൾ എന്ന വിഭാഗത്തിലെ പോളിപോറേസി കുടുംബത്തിൽപ്പെട്ട മൈക്രോപോറസ് അഫിനിസ്, മൈക്രോപോറസ് സാന്തോപ്പസ് എന്നിവയുടെ രാസ സ്വഭാവ രൂപീകരണം, ജൈവ പ്രവർത്തന പരിശോധന എന്നീ കാര്യങ്ങളിൽ ഈ ഗവേഷം ശ്രദ്ധ കേന്ദ്രീകരിക്കുന്നു.

ഫംഗസുകളിലെ രാസവസ്തുക്കളുടെ പ്രാഥമിക വിലയിരുത്തൽ, രാസവസ്തുക്കളുടെ അളവ് കണക്കാക്കൽ, ബാഷ്പശീല ജൈവ സംയുക്തങ്ങളുടെ ജി. സി /എം. എസ് വിശകലനം, അസ്ഥിരമല്ലാത്ത ജൈവസംയുക്തങ്ങളുടെ എച്ച്. ആർ-എൽ. സി/എം. എസ് വിശകലനം എന്നീ നാലു വിഭാഗങ്ങളിലായാണ് രാസ സ്വഭാവരൂപീകരണം നടത്തിയത്. പ്രാഥമിക പഠനങ്ങളിൽ രണ്ട് സ്പീഷീസുകളിലും ടെർപ്പിനോയിഡുകൾ, സ്റ്റിറോയിഡുകൾ, ആൽക്കലോയിഡുകൾ എന്നിവ നിരീക്ഷിക്കപ്പെട്ടു. ഗ്ലൈക്കോസൈഡുകളും സാപ്പോണിനുകളും മൈക്രോപോറസ് സാന്തോപ്പസിൽ മാത്രമേ റിപ്പോർട്ട് ചെയ്തിട്ടുള്ളൂ. ആൽക്കലോയിഡുകൾ, ടെർപ്പിനോയിഡുകൾ, കാർബോഹൈഡ്രേറ്റുകൾ, പ്രോട്ടീനുകൾ എന്നിവ അളവനുസരിച്ച് നിർണ്ണയിക്കപ്പെട്ടു. ജി. സി./ എം. എസ് സംവിധാനം വഴി 45 ഓളം രാസവസ്തുക്കളുടെ സാന്നിധ്യം നിർണ്ണയിച്ചു. രണ്ട് സ്പീഷീസുകളിലും ഫാറ്റി ആസിഡ് എന്ന വിഭാഗത്തിൽപ്പെട്ട രാസവസ്തുക്കളാണ് മുൻ നിരയിലുള്ളത്. രാസവസ്തുക്കളിൽ ഏറ്റവും കൂടുതൽ സാന്നിധ്യം കാണിക്കുന്നത് ലിനോയിക് അമ്ലം ആണ്. എച്ച്. ആർ - എൽ. സി / എം. എസ് എന്ന സംവിധാനം വഴി മൈക്രോപോറസ് സാന്തോപ്പസിൽ 31 ഉം മൈക്രോപോറസ് അഫിനിസിൽ 9 സംയുക്തങ്ങളും റിപ്പോർട്ട് ചെയ്തു. വൈവിധ്യമാർന്ന ജൈവപ്രവർത്തനങ്ങൾ ഉള്ള വ്യത്യസ്ത തരം ഫംഗസ് സ്റ്റിറോളുകൾ (സ്റ്റിറോയിഡുകളുടെ ഒരു കൂട്ടം) റിപ്പോർട്ട് ചെയ്യപ്പെട്ടിട്ടുണ്ട്.

വൈവിധ്യമാർന്ന രാസ ഘടകങ്ങൾ കാരണം, ആന്റിഓക്സിഡന്റ്, സൈറ്റോടോക്സിക്, കോശ വളർച്ച തടയൽ, നാഡീകോശങ്ങളുടെ സംരക്ഷണം എന്നീ ജൈവപ്രവർത്തനങ്ങളെക്കുറിച്ച് മൈക്രോപോറസിൽ പഠനം നടത്തി. DPPH, Hydroxyl radical, nitric oxide, reducing power എന്നീ free radical scavenging assays നടത്തി.

സൈറ്റോ ടോക്സിക് പരീക്ഷണത്തിൽ കോശത്തിന്റെ കാര്യക്ഷമത മനസ്സിലാക്കുന്നതിനായി mitotic index, chromosomal abnormality ശതമാനം എന്നിവ ഉള്ളി (*Allium cepa*) യിൽ പഠനവിധേയമാക്കി. DLDI എന്ന കാൻസർ കോശത്തിൽ MTT assay, AO/EtBr എന്നീ പഠനങ്ങൾ നടത്തി. മൈക്രോപോറസ് അഫിനിസ് താഴെ LC₅₀ മൂല്യം കാണിച്ചത് അതിന്റെ ഉയർന്ന സൈറ്റോടോക്സിക് സാധ്യതയെ സൂചിപ്പിക്കുന്നു.

മൈക്രോപ്പോറസിലെ ടെർപ്പിനോയിഡുകളുടെ സാന്നിധ്യം ന്യൂറോണുകളുടെ പ്രവർത്തനങ്ങളെ പിന്തുണയ്ക്കുന്നതിനാൽ ഫംഗസ് സത്തുകൾ IMR32 (Neuroblastoma cell lines) ഉപയോഗിച്ച് ലബോറട്ടറി പഠനത്തിന് വിധേയമാക്കി. നാഡീകോശങ്ങളുടെ നാശത്തിന് (Nerodegeneration) ആയി β -amyloid ഉപയോഗിച്ചു.

നാഡീകോശങ്ങളുടെ സംരക്ഷണ സംബന്ധമായ പഠനത്തിനായി MTT, AO/EtBr എന്നിവ ഉപയോഗിച്ചുള്ള പരീക്ഷണങ്ങൾ നടത്തി. ഈ പഠനത്തിൽ മികവ് പുലർത്തിയത് മൈക്രോപ്പോറസ് സാന്റോപ്പസ് ആയതിനാൽ തുടർപഠനത്തിന് ഈ സ്പീഷീസ് മാത്രം ആണ് ഉപയോഗിച്ചത്. നാഡീകോശങ്ങളുടെ സംരക്ഷണം സ്ഥിരീകരിക്കുന്നതിനു വേണ്ടി ഒരു ഫ്ലൂറൈസ്സെൻ്റ് ഡൈ ആയ DCFDA ഉപയോഗിച്ച് ROS അളക്കൽ, ഫ്ലോസൈറ്റോമെടി എന്ന സാങ്കേതിക വിദ്യ ഉപയോഗിച്ചുള്ള അനക്സിൻ V FITC കോശമരണം കണ്ടെത്തൽ, ഓട്ടോഫാഗി, പ്രോട്ടീനുകൾ കണ്ടെത്തുന്നതിനുള്ള ലാബ് രീതി ആയ Indirect ELISA, ജീൻ പ്രകടനം മനസിലാക്കുന്നതിനുള്ള qRT PCR എന്നിവയ്ക്ക് പ്രത്യേക ഊന്നൽ നൽകി. ഈ പഠനങ്ങളിൽ നിന്നും മൈക്രോപ്പോറസ് സാന്റോപ്പസ് ഔഷധമേഖലയിൽ പ്രാധാന്യം അർഹിക്കുന്നതാണ് എന്ന് മനസിലാക്കുന്നു. പ്രത്യേകിച്ച്, നാഡീവ്യൂഹത്തെ ബാധിക്കുന്ന രോഗങ്ങളായ അൽഷിമേഴ്സ്, പാർക്കിൻസൺസ് എന്നിവയുടെ ചികിത്സയ്ക്കായി മൈക്രോപ്പോറസ് സാന്റോപ്പസ് എന്ന ഫംഗസ് തുടർ പരീക്ഷണങ്ങളിലൂടെ വിനിയോഗിക്കാം എന്ന് ശുപാർശ ചെയ്യുന്നു.

സൂചകപദങ്ങൾ: ബസീഡിയോമൈസെറ്റുകൾ, മൈക്രോപോറസ്, മൈക്കോ കെമിക്കൽ, ജൈവപ്രവർത്തനം, ആന്റികാൻസർ, ആന്റിഓക്സിഡന്റ്, നാഡീസംരക്ഷണം

ABBREVIATIONS

%	:	Percentage
µg	:	Microgram
µL	:	Microlitre
µm	:	Micrometre
µM	:	Micromolar
22Rv1	:	Prostate cancer cell line
3LL	:	Lewis lung carcinoma
A2780	:	Ovarian cancer cell line
A549	:	Lung cancer
ABTS	:	2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
Ac	:	Absorbance of control
ACS	:	American Chemical Society
AD	:	Alzheimer's disease
AGS	:	Gastric cancer cell line
AIF	:	Apoptosis-Inducing Factor
Al	:	Aluminium
AO	:	Acridine Orange
APC	:	Adenomatous polyposis coli protein
APP	:	Amyloid precursor protein
As	:	Absorbance of sample
As	:	Arsenic
AsPC1	:	Pancreatic cancer cell line
Au	:	Arbitrary unit
Aβ	:	Amyloid β
Bak	:	Bcl-2 homologous antagonist/ killer (pro apoptotic protein)
Bax	:	Bcl-2 associated X protein
BBB	:	Blood-brain barrier
B-Cap 37	:	Breast cancer cell line
Bcl-2	:	B-cell lymphoma 2
BHA	:	Butylated hydroxyanisole
BHT	:	Butylated hydroxytoluene
Bid	:	BH3 interacting domain death agonist (a protein family of Bcl-2)

BNDF	:	Brain-derived neurotrophic factor
BRM	:	Biological Response Modifiers
BSA	:	Bovine Serum Albumin
BT- 474	:	Breast cancer cell line
C4-2b	:	Prostate cancer cell line
Ca9-22	:	Oral cancer cell line
CAPAN-2	:	Pancreatic cancer cell line
Cd	:	Cadmium
CE	:	Caffeine equivalent
CfPAC1	:	Pancreatic cancer cell line
ChAt	:	Choline acetyltransferase
CJD	:	Creutzfeldt-Jacob Disease
Cl ⁻	:	Chloride ion
cm	:	Centimetre
CNN	:	Convolutional Neuronal Network
CNS	:	Central Nervous System
CO	:	Carbon Monoxide
CO ₂	:	Carbon Dioxide
COLO 320DM	:	Colorectal cancer cell line
Colo-205	:	Colorectal cancer cell line
CRC	:	Colorectal cancer cell line
Cu	:	Copper
DCF	:	Dichlorofluorescein
DCFDA	:	Dichlorodihydrofluorescein diacetate
DIABLO	:	Direct inhibitor of apoptosis binding protein with low pi (pi-isoelectric point)
DLD1	:	Human colorectal cancer cell line
DMEM	:	Dulbecco's Modified Eagle's medium
DMSO	:	Dimethyl Sulphoxide
DNA	:	Deoxyribonucleic acid
DPPH	:	2, 2-Diphenyl-1- Picrylhydrazyl
DSM-5	:	Diagnostic and statistical manual of mental disorder-5
DU-145	:	Prostate cancer cell line
DW	:	Dry weight
EDTA	:	Ethylene diamine Tetra Acetic Acid
EI	:	Electron ionisation
ELISA	:	Enzyme Linked Immunosorbent Assay
EPG85-257RDB	:	Gastric cancer cell line
EPG85-257RNOV	:	Gastric cancer cell line

ER	:	Estrogen Receptor
EtBr	:	Ethidium Bromide
eV	:	Electron Volt
FBS	:	Fetal Bovine Serum
Fe	:	Iron
Fe ²⁺	:	Ferrous ion
Fe ³⁺	:	Ferric ion
FeCl ₃	:	Ferric Chloride
Fig.	:	Figure
FITC	:	Fluorescein isothiocyanate
g	:	Gram
GAPDH	:	Glyceraldehyde-3-phosphate dihydrogenase
GBC-SD	:	Gall bladder cancer cell line
GC/MS	:	Gas Chromatography/ Mass Spectrometry
GE	:	Glucose equivalent
GFP	:	Green fluorescent protein
GOTO	:	Neuroblastoma cell line
h	:	Hour
H157	:	Lung cancer cell line
H187	:	Lung cancer cell line
H226	:	Lung cancer cell line
H ₂ O	:	Dihydrogen Monoxide (Water)
H ₂ O ₂	:	Hydrogen Peroxide
H ₂ SO ₄	:	Sulphuric Acid
H322	:	Lung cancer cell line
H358	:	Lung cancer cell line
H460	:	Lung cancer cell line
H-4-II-E	:	Liver cancer cell line
H661	:	Lung cancer cell line
HAK-1A, HAK-1B, HAK-2 to HAK-6: Liver cancer cell lines		
HCl	:	Hydrochloric Acid
HCT-116	:	Colorectal cancer cell line
HCT-15	:	Colorectal cancer cell line
HeLa	:	Cervical cancer cell line
HEP-2	:	Larynx carcinoma cell line
Hep3B	:	Liver cancer cell line
HepG2	:	Hepatocarcinoma cell line
HER2-18	:	Breast cancer cell line
Hg	:	Mercury

HIV	:	Human Immunodeficiency Virus
HL-60	:	Leukemia cell line
HLF	:	Liver cancer cell line
HO-1-u-1	:	Oral cancer cell line
HO-8910	:	Ovarian cancer cell line
HR-LC/MS	:	High Resolution-Liquid Chromatography/Mass Spectrometry
HRP	:	Horseshoe Peroxidase conjugate protein
HSC-3	:	Oral cancer cell line
HSC-4	:	Oral cancer cell line
HT-29	:	Colorectal cancer cell line
IARC	:	International Agency for Research on Cancer
IC ₅₀	:	Inhibition Concentration 50%
IMR-32	:	Neuroblastoma cell line
Jurkat E 6.1	:	Leukemia cell line
K562	:	Leukemia cell line
KATO III	:	Gastric cancer cell line
KB	:	Oral cancer cell line
KH ₂ PO ₄	:	Potassium dihydrogen phosphate
KOH	:	Potassium Hydroxide
kPa	:	Kilopascal
KYN-1, KYN-2 & KYN-3: Liver cancer cell lines		
L	:	Litre
L1210	:	Lymphocytic leukemia
L929	:	Normal fibroblast cell line
LAPC4	:	Prostate cancer cell line
LC3	:	Light chain 3
LC ₅₀	:	Least Concentration 50%
LE	:	Lupeol equivalent
LM3	:	Human hepatocellular carcinoma cell line
LNCap	:	Prostate cancer cell line
LoVo	:	Colorectal cancer cell line
LPO	:	Lipid peroxidation
m- Hitt	:	Heparin induced thrombocytopenia
MA	:	<i>Microporus affinis</i>
MCAS	:	Ovarian cancer cell line
MCF-7	:	Michigan Cancer Foundation-7 (Human breast cancer cell line)
MDA1986	:	Oral squamous cell carcinoma cell line

MDA-MB 231	:	M. D. Anderson-Metastatic Breast 231 (Human breast cancer cell line)
MDA-MB-435	:	Human breast cancer cell line
mg	:	Milligram
MHCC	:	Human hepatocellular carcinoma
MI	:	Mitotic index
Mia PaCa-2	:	Pancreatic cancer cell line
min	:	Minute
MKN-28	:	Gastric cancer cell line
MKN-45	:	Gastric cancer cell line
mL	:	Millilitre
mm	:	Millimetre
mM	:	Millimolar
Mn	:	Manganese
MS QTOF	:	Quadrupole time-of-flight Mass Spectrometry
MTOC	:	Microtubule Organizing Center
MTT	:	3-(4,5-Dimethylthiazol-2-yl),2-5-diphenyltetrazolium bromide
MX	:	<i>Microporus xanthopus</i>
N	:	Normal
N417	:	Lung cancer cell line
Na ⁺ /K ⁺	:	Sodium-Potassium pump
NADPH	:	Nicotinamide adenine dinucleotide phosphate (reduced form)
NaOH	:	Sodium Hydroxide
NB-1	:	Neuroblastoma cell line
NCCS	:	National Centre for Cell Sciences
NCI	:	National Cancer Institute
ND	:	Neurodegenerative Disease
NGF	:	Nerve Growth Factor
NIST	:	National Institute of Standards and Technology
nm	:	Nanometre
NO ₂	:	Nitrogen Dioxide
Nrf2	:	Nuclear factor erythroid 2-related factor 2
O ₂	:	Oxygen
O ₃	:	Ozone
OAW28	:	Ovarian cancer cell line
OAW41M	:	Ovarian cancer cell line
OAW42	:	Ovarian cancer cell line

OAW59M	:	Ovarian cancer cell line
°C	:	Degree Celsius
OCD	:	Obsessive compulsive disorder
OD	:	Optical density
OH	:	Hydroxyl group
OV-1063	:	Ovarian cancer cell line
PARP	:	Poly (ADP-ribose) polymerase
Pb	:	Lead
PBS	:	Phosphate Buffered Saline
PC-3	:	Human prostate cancer cell line
pH	:	Potential of Hydrogen (concentration of H ⁺ in a solution)
PI	:	Propidium iodide
PPC	:	Polysaccharide Peptide Complexes
ppm	:	Parts Per Million
PRKN	:	Human parkin gene (Parkin RBR E3 ubiquitin protein ligase)
PrP ^{Sc}	:	Prion protein Scarpie
PS	:	Phosphatidylserine
PSK	:	Polysaccharide K
PSPC	:	Polysaccharide Protein Complexes
PTSD	:	Post-traumatic stress disorder
qRT PCR	:	Quantitative Reverse Transcription Polymerase Chain Reaction
QSM	:	Quorum sensing molecule
RFP	:	Red fluorescent protein
RNS	:	Reactive Nitrogen Species
ROS	:	Reactive Oxygen Species
rpm	:	Rotations Per Minute
RT	:	Retention Time
s	:	Seconds
S-180	:	Mouse sarcoma 180 cell line
SCC-4	:	Oral cancer cell line
SE	:	Standard error
SF295	:	Glioblastoma cell line (brain cancer)
SGC-7901	:	Gastric cancer cell line
SiHa	:	Cervical cancer cell line
SKBr-3	:	Breast cancer cell line
SK-HEP-1	:	Liver cancer cell line

SK-N-AS	:	Neuroblastoma cell line
SK-N-SH	:	Human neuroblastoma cell line
SNCA	:	gene that encodes α -synuclein protein
SO ₂	:	Sulphur Dioxide
SW-1990	:	Pancreatic cancer cell line
Tau	:	Tubulin associated protein
TBA	:	Thiobarbituric acid
TCA	:	Trichloroacetic acid
TCM	:	Traditional Chinese Medicine
TD-20	:	Thermal desorption system
TDP-43	:	Transactive response DNA binding protein
TE buffer	:	Tris- EDTA buffer
TSU-Pr1	:	Prostate cancer cell line
TU159	:	Oral cancer cell line
TYK-Nu	:	Ovarian cancer cell line
U937	:	Leukemia cell line
UV	:	Ultraviolet
V	:	Volt
WHO	:	World Health Organization
WiDr	:	Colorectal cancer cell line
w/v	:	Weight per volume
Zn	:	Zinc
α -syn	:	α -synuclein protein

CONTENTS

	Page No.
1. INTRODUCTION	1-13
2. REVIEW OF LITERATURE	15-46
3. MATERIALS AND METHODS	47-69
1. Fungal Materials	47
2. Methodology	49
PHASE I	49
MYCOCHEMICAL CHARACTERIZATION	
a. Qualitative mycochemical screening	49
b. Quantitative mycochemical screening	51
c. High Resolution – Liquid Chromatography/Mass Spectrometry (HR-LC/MS) analysis	54
d. Gas Chromatography/Mass Spectrometry (GC/MS) analysis	54
PHASE II	55
BIOACTIVITY SCREENING	
a. Free radical scavenging assays	55
i. DPPH scavenging assay	55
ii. Hydroxyl radical scavenging assay	56
iii. Nitric oxide radical scavenging activity	56
iv. Reducing power activity	56
b. Cytotoxicity by <i>Allium cepa</i> assay	57
c. Antiproliferative efficacy of <i>Microporus</i>	58
i. <i>In vitro</i> anticancer activity of DLD1 cell line using MTT assay	58
ii. <i>In vitro</i> anticancer activity on L929 cell line	59
iii. Determination of apoptosis using acridine orange and ethidium bromide (AO/EtBr) double staining	61
d. Preliminary neuroprotective study	62
i. <i>In vitro</i> neuroprotective effect determination by MTT assay	62
ii. Apoptosis determination by using acridine orange and ethidium bromide (AO/EtBr) double staining	63
PHASE III	64
DETAILED STUDY ON NEUROPROTECTION IN <i>MICROPORUS XANTHOPUS</i>	
i. <i>In vitro</i> ROS measurement using DCFDA	64
ii. Indirect ELISA	65

iii. Autophagy	65
iv. Annexin V-FITC apoptosis determination by flow cytometry	66
v. Gene expression studies using qRT PCR	67
4. RESULTS	71-109
PHASE I	71
MYCOCHEMICAL CHARACTERIZATION	
a. Qualitative mycochemical screening	71
b. Quantitative mycochemical screening	72
c. High Resolution – Liquid Chromatography/Mass Spectrometry (HR-LC/MS) analysis	76
d. Gas Chromatography/Mass Spectrometry (GC/MS) analysis	77
PHASE II	84
BIOACTIVITY SCREENING	
a. Free radical scavenging assays	84
i. DPPH scavenging assay	84
ii. Hydroxyl radical scavenging assay	84
iii. Nitric oxide radical scavenging activity	85
iv. Reducing power activity	85
b. Cytotoxicity by <i>Allium cepa</i> assay	88
c. Antiproliferative efficacy of <i>Microporus</i>	93
i. <i>In vitro</i> anticancer activity of DLD1 cell line using MTT assay	93
ii. <i>In vitro</i> anticancer activity on L929 cell line	94
iii. Determination of apoptosis using acridine orange and ethidium bromide (AO/EtBr) double staining	95
d. Preliminary neuroprotective study	95
i. <i>In vitro</i> neuroprotective effect determination by MTT assay	96
ii. Apoptosis determination by using acridine orange and ethidium bromide (AO/EtBr) double staining	97
PHASE III	98
DETAILED STUDY ON NEUROPROTECTION IN MICROPORUS XANTHOPUS	
i. <i>In vitro</i> ROS measurement using DCFDA	98
ii. Indirect ELISA	99
iii. Autophagy	102
iv. Annexin V-FITC apoptosis determination by flow cytometry	106
v. Gene expression studies using qRT PCR	108
5. DISCUSSION	111-144
PHASE I	113
MYCOCHEMICAL CHARACTERIZATION	

	PHASE II	121
	BIOACTIVITY SCREENING	
	PHASE III	135
	DETAILED STUDY ON NEUROPROTECTION IN <i>M. XANTHOPUS</i>	
6.	SUMMARY AND CONCLUSION	145-151
	Deliverables	150
	Recommendations	151
7.	REFERENCES	153-192
8.	APPENDICES	193-195
9.	PUBLICATIONS, PRESENTATIONS AND AWARDS	



Introduction

INTRODUCTION

Fungi are generally underexplored diverse group of organisms with commercial significance. The species richness of mushrooms, and their natural beauty have long fascinated mankind. We have explored them for their medicinal properties, delighted in their succulent taste, and marveled at their miraculous and sudden appearance as fruiting bodies. Macro fungi are characterized by wide range distribution and are seasonal. India is one of the top 10 mega biodiversity nations of the world fortunate to have a varied agroclimate, abundance of agrowastes, relatively low-cost labour, and a rich fungal diversity (Borkar et al., 2015). Polypores are a group of tough, leathery mushrooms with number of minute pores, similar to boletes but lacking a distinct stalk. Many polypore species are, however, employed in pharmacology for the treatment of a wide range of ailments including bacterial and viral infections, allergies, neurological problems, and cancer (Lindequist et al., 2005).

Higher basidiomycetes well studied in previous researches for a serious condition of various type of tumors. Wide range of basidiomycetes are reported with this scenario. As these conditions are increasing day by day, there is need for exploring new sources of medicines. Thereby basidiomycetes with wide spectrum of active chemicals play an important role as study material for cancer research. Thus, some researchers have combined the use of mushrooms both for nutritional and medicinal purposes (Wasser & Weis, 1999a).

A large number of mushroom-derived compounds, both cellular components and secondary metabolites have been shown to affect the immune system and could be used to treat a variety of disease states (Chihara, 1992).

Nutritional value and pharmacological properties of edible basidiomycetes have been explored in various studies. Mushrooms are used as food, nutrient rich, and medicinally important. There are different previous records on mushrooms with significant role in pharmacology as it is used for treatment of diseases, maintains homoeostasis and biorhythm. It is being used for recovery of diseases like cancer,

stroke, and cardiological diseases. It is also being confirmed that mushrooms contain effective substances for decreasing blood cholesterol and could have hypolipidemic, antithrombotic, hypotensive, and various therapeutic properties like antifungal, anti-inflammatory, antiviral, antibacterial, blood pressure regulation, cardiovascular regulation, antidiabetic, immunomodulating, kidney tonic, hepatoprotective, nerve tonic, sexual potentiator, cures chronic bronchitis, etc. (Wasser & Weis, 1999b).

The average age of the global population is increasing and nowadays, society is becoming more aware of the potential side effects of prescription medicines and therefore seek alternative therapeutics (Ekor, 2014; Rowe et al., 2016; WHO, 2015). At present the importance for the area of nutraceuticals have given more attention. For a healthy wellbeing, traditional, quality foods are suggested to include in diet. These mainly target the enhancement of well-being, and quality of life, thus reducing the risk of disease (El Sohaimy, 2012). The term “nutraceuticals” is defined as food constituents that provide medical or health benefits, including the prevention and treatment of disease (Kalra, 2003). Nutraceuticals can be food in the form of extracts, single, purely natural compounds, or nutrients that have been turned into a pharmaceutical formulation (eg. pills or tablets) and they can be applied as dietary supplements or as part of a specific diet (Giavasis, 2014; Reis et al., 2017). Therefore, the role of functional foods is mainly related to reducing the risk of disease, while nutraceuticals are usually consumed to promote well-being through the prevention or treatment of diseases and disorders. For healthy and nutritional aspects, edible mushrooms are studied well. Mushrooms are ideal dietary supplements because they are relatively rich in proteins, contain almost no fat, and possess a variety of fibres and vitamins (Valverde et al., 2015). Many studies have been conducted to prove the benefits of functional foods and nutraceuticals derived from fungi in various pathological complications such as diabetes, atherosclerosis, cardiovascular diseases, cancer, infection, renal, gastrointestinal, and neurological disorders.

For thousands of years, human societies have been utilizing fungi as food sources (Siddiq et al., 2018). In particular, edible mushrooms are ideal for vegetarians because they are excellent sources of protein (typically 20–30% crude proteins as a

percentage of dry matter), have a low-fat content, are free of cholesterol, and contain most of the amino acids essential to human and animal nutrition (Kaur et al., 2018). Fungal taxa are utilized in the food industry, taking advantage of their metabolism and metabolites (Gilbert & Robinson, 1957; Moore & Chiu 2001; Adrio & Demain 2003; Ghorai et al., 2009).

Mushrooms are the chief source of both fat soluble (Vitamin D & K) and water soluble (vitamin B & C) vitamins, minerals (P and K) and selenium as a trace element. Fiber content of the mushrooms is also highlighted. Mushrooms are rich in niacin (B3), pantothenic acid (B5) and riboflavin (B2) (Feeney et al., 2014; Murugesan, 2017), but other vitamins, such as thiamine (B1), pyridoxin (B6), cobalamin (B12), biotin (B7) and vitamin D have also been reported (Pankavec et al., 2019; Rózsa et al., 2019). The only non-animal-based food that contains vitamin D is mushrooms (Rathore et al., 2017) and curiously the content of vitamin B12 in mushrooms is similar to that reported for beef and fish (Feeney et al., 2014). Richness of minerals like P, Ca, K and Mg are also reported in various sites. Because of their low sodium and high potassium concentration, they have a Na^+/K^+ ratio of less than 0.6, being thus a good food option amongst other vegetables for hypertense individuals (Rajaratnam et al., 1998). Indeed, mushrooms are an excellent source of selenium and copper, with high bioavailability (Murugesan, 2017). Mushrooms have also interesting content in tocopherols, carotenoids, ascorbic acid, phenolics, and ergosterol, being even most abundant than that found in most vegetables and fruits (Sánchez, 2017a).

The main chemical constituent in the fruiting bodies of mushrooms falls primarily under the class of polysaccharide compounds and also includes substantial amounts of fibers, other saccharides, terpenoids, and proteins (Singdevsachan et al., 2016). The micronutrients present in mushrooms include mineral elements such as copper, iodine, iron, potassium, selenium, and zinc, along with the vitamin groups and amino acids present, which permits unrestricted use, in trace amounts (Genççelep et al., 2009). These constituents regulate growth, fluid balance, and bone health in humans, as well as serve as efficient nutraceuticals to supplement immune responses

(Lakhanpal & Rana, 2005; Okan et al., 2013). Furthermore, surplus levels of the secondary metabolites in mushrooms are responsible for their various medicinal properties such as antioxidant, anti-inflammatory, anticancer, antihyperlipidemic, immunoregulatory, and cardioprotective properties (Yu et al., 2020).

Mushrooms have been used all over the world as century-old traditional medicines (Chang, 1996). Mushrooms that belong to basidiomycetes produce various classes of secondary metabolites with a wide variety of biological activities, particularly in the antimicrobial, antitumor, and antiviral fields (Jong et al., 1989; Wasser et al., 1999).

Phenolic compounds include simple phenols and phenolic acids (gallic acid, benzoic acid, syringic acid, and chlorogenic acid). Flavonoids and tannins are the two important polyphenols. Flavonoids are a group of polyphenolic compounds with known health-beneficial properties, which include free radical scavenging, and inhibition of hydrolytic and oxidative enzymes (Frankel, 1997). Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity (Gryglewski, 1987). Phenolic compounds have significant biological and pharmacological properties. The bioactivity of phenolics may be related to their ability to chelate metals, inhibit lipoxygenase, and scavenge free radicals (Decker, 1997). Different chromatographic methods like GC/MS, LC/MS, HR-LC/MS etc., widely used to detect chemicals constituents. These methods are used to detect the presence of biologically active chemical compounds in a sample. Even though, these are good for chemical characterization, crude extract may contain different chemicals. So, the specific compound with potent bioactivities can be eluted by combination of different assays. High-resolution liquid chromatography with mass spectroscopy (HR-LC/MS) gives information regarding the molecular structures within a complex database. Prohibition of anonymous drug, identification of positive and negative ionization compounds, identification of new metabolites, mass accuracy and resolving power are the highlights of HR-LC/MS technique.

Volatile chemical components in sample can be identify by GC/MS technique. In this technique, two different techniques like gas chromatography and mass

spectrometry are combined. Gas chromatography separates the components of the mixture and mass spectroscopy analyses each of the components separately (Bai et al., 2014).

Antioxidants are valuable compounds that protect cells against oxidative damage and promote human health. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the free radicals produced through metabolic activity of aerobes. Oxidative stress can be avoided by maintaining a rhythm between the formation of free radicals and antioxidant protective mechanism of our body. Endogenous defence mechanism can be boost up by the intake of food items with antioxidant activities which helps to overcome oxidative damage. Studies revealed that mushrooms are chief source antioxidants. These are mainly due to the presence of phenols, flavonoids, tocopherols, carotenoids, and ascorbic. Flavonoids are important to scavenge ROS and RNS. The ability of hydrogen donation, metal chelation, superoxide scavenging and free radicals scavenging are the basis for the categorization of antioxidants. Phenolic compounds are the main category of chemical components that are responsible for the antioxidant activity of mushroom species (Kurutas, 2015).

In recent years, focus on free radicals have given more priority. Free radicals are chemical species possessing an unpaired electron formed by the loss of a single electron from a normal molecule (Ray & Husain, 2002). About 5% or more of the oxygen inhaled is converted to reactive oxygen species (ROS) such as O_2 , H_2O_2 and OH by univalent reduction of O_2 (Harman, 1993). Superoxide anions, hydrogen peroxide, hydroxyl radicals and nitric oxide are the examples of ROS. Formation of these compounds will be a threat to cells in aerobic conditions. Oxidative stress may arise if equilibrium between the formation of ROS and the antioxidant protective mechanism is disturbed. Through a series of events, it deregulates the cellular functions leading to various pathological conditions including cardiovascular dysfunction, neurodegenerative diseases, gastroduodenal pathogenesis, metabolic dysfunction of almost all the vital organs, cancer, and premature aging (Halliwell, 1996). There are number of studies revealed that the wide spectrum of antioxidant

defences to fight for the formation of free radicals. Naturally, cells are characterized by enzymatic and non-enzymatic defence mechanisms. But to protect cells from oxidative damage external supply of antioxidants are promoted. Restriction in the use of synthetic antioxidants paved the way for the increased interest in natural antioxidants (Ames, 1983; Branen, 1975). Natural antioxidants are widely known for their defence role against oxidative damage and are found abundant in vegetables and fruits, which contain phenolic compounds, carotenoids, tocopherol, ascorbic acid, etc. (Elmastasa et al., 2007). In recent years mushrooms also have attracted attention for their natural medicinal properties.

Oxidative stress plays a pivot role in different neurodegenerative diseases. Chronic responses are not developed when the stress is in low level. Mushrooms are a rich source of antioxidants and effectively mitigate the oxidative stress phenomenon in cells and neurons (Salinaro et al., 2018).

Reactive oxygen species (ROS) are highly reactive and short-lived small molecules in the form of free radicals (Sies & Jones, 2020). Normal cellular physiological activities are positively influenced by ROS under optimum conditions. The increased production of ROS has a negative impact on cells and favours oxidative stress. Both endogenous and exogenous sources promote ROS production. Leakage of mitochondrial transport chain, increased metabolic rate etc., are examples of endogenous source. While, exogenous sources include environmental pollutants, radiations, drugs, etc. ROS are responsible for activating many cellular signalling pathways that lead to cell survival or cell death including autophagy, apoptosis, and necrosis (Cosentino et al., 2019).

Oxidation is necessary in living organisms for the production of energy to fuel various biological processes (Aziz et al., 2007). However, numerous physiological processes and external factors including many environmental factors occasionally produce oxygen-centered free radicals and other reactive oxygen species (ROS), which may contribute to diseases including atherosclerosis, diabetes, cancer, and cirrhosis (Halliwell & Gutteridge, 1984). Most organisms have evolved antioxidant defence and repair systems to protect them against oxidative damage; however, these

systems are insufficient to prevent the damage entirely (Simic, 1998). The demand and scope of researches regarding new sources of antioxidants is becoming highly appreciable. DPPH (1,1-Diphenyl-2-picrylhydrazyl) is the one of the most common free radical scavenging assays. In the presence of an antioxidant, the DPPH radical obtains one more electron and the absorbance decreases (Koleva et al., 2002). SOD (superoxide dismutase) plays a key role in protecting organisms from oxygen derived free radicals and toxicity due to oxygen. One of the leading harmful free radical substances in nature is the superoxide, which can be neutralized by SOD.

Ganoderma represents the most studied bracket fungus which shows strong antimicrobial properties. Wang & Ng (2006) isolated 'Ganodermin', an antifungal protein from fruiting bodies of *G. lucidum* (Curtis) Karst. In India, polypores are well studied for their inhibitory actions. Antimicrobial activity of various solvent (aqueous, ethanol, methanol, and acetone) extracts (40 µg/mL) of *Ganoderma lucidum* against six species of bacteria viz., *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Salmonella typhi* and *Pseudomonas aeruginosa* was studied (Quereshi et al., 2010). Search for new antimicrobial agents is a demanding area as new diseases and microbes emerge.

Cytotoxicity is the quality of being toxic to cells. Treating cells with the cytotoxic compound can result in a variety of cell fates. Chemotherapy as a treatment of cancer often relies on the ability of cytotoxic agents to kill or damage the cells that are reproducing; this preferentially targets rapidly dividing cancer cells (Aneesh & Thoppil, 2019).

Cancer is a serious issue in present scenario, which is characterised by abnormal cell division and ultimately results in deaths. It is a multifactorial and stepwise disease. Even though, there are number of synthetic drugs, in effect these are not economical. Thus, there is a constant need for the development of cost-effective, novel drugs (Aneesh & Thoppil, 2020).

Cancer is one of the multifactorial diseases with improper proliferation and cell division. These abnormal malignant cells can evade tumor suppressor factors of the human immune system, then develop into tumors and destroy adjacent tissues

(Saeidnia & Abdollahi, 2013). The mode of cancer treatments depends on the stage. Chemotherapy, radiation therapy, and surgery are the widely used for the treatment of cancer. From previous works, it was reported that number of cytotoxic substances effective for chemotherapy. Among these, few of them specifically target tumor cells and are less toxic to regular, healthy human tissue (Petrelli et al., 2012; Cai et al., 2013; Zugazagoitia et al., 2016). Targeted therapy, usually the conjugated element for cancer treatments, delivers drugs to genes or proteins that are specific to cancer cells or the surrounding tissues that promote the growth of cancer (Padma, 2015). Searching for new antitumor fungal substances became a matter of great significance (Wasser et al., 1999b).

Studies reported that mushrooms can stimulate lymphocytes which shows their anticancer efficacy. Presence of polysaccharides in mushrooms form a key to anticancer properties. Mode of immune stimulation, inhibition of cell proliferation and induction of apoptosis are the different parameters of anticancer properties. For example, in the case of mushrooms, bioactive compounds such as lentinan, polysaccharide K (PSK), and schizophyllan are the main derivatives that are responsible for the anticancer potential (Lemieszek & Rzeski, 2012).

Colorectal cancer (CRC) means abnormal growth of cells in colon. It is mainly due to the development of non cancerous cell growth (polyp) in the mucosal layer of the colon. All polyps are not led to CRC. Only less than 10% led into cancer by 10–20 years. CRC occurs predominantly in adults aged above 50 (ACS, 2020). In 2020, the third most common cancer type worldwide was CRC and it forms the second most common cause of cancer death. Geographically, Asian regions are leading for the report of CRC. The International Agency for Research on Cancer (IARC) estimated that the global burden of CRC will increase by 56% and CRC-related mortality will increase by 69% between 2020 and 2040 (Honegger et al., 2013). Therefore, research on the discovery of novel anticancer therapeutics is more significant.

Apoptosis is the process of programmed cell death and the most common target parameter to study cancer. Dysregulation of apoptotic signals in cancer promotes abnormal cell growth and tumorigenesis (Wong, 2011). The effectiveness

of cancer treatment is measured based on the capacity to recover the apoptotic cells. The initiation of apoptosis in cells can be identified by morphological changes such as nuclear fragmentation, condensation of chromatin, cell shrinkage, and membrane blebbing (Elmore, 2007; Hassan et al., 2014). Apoptosis is categorized as either caspase-dependent or caspase-independent. There are number of anticancer substances which primarily stimulate the caspase-dependent mitochondrial pathway. During the last stages of apoptosis, caspases are stimulated by endoplasmic reticulum pathways and death receptor-mediated. Disruption of the mitochondrial membrane potential followed by translocation of AIF and endonuclease G to the nucleus induces caspase-independent apoptosis (Lee et al., 2006). Caspase-3 is the executioner caspase in all caspase-dependent apoptosis pathways. Caspase-3 can be cleaved and activated by the upstream caspase (caspase-8) in the extrinsic pathway as well as by caspase-9 (an initiating caspase) in the intrinsic pathway (Liu et al., 2017). Cleavage of PARP by caspases is considered to be a hallmark of apoptosis and an indicator that caspase-dependent apoptosis has been accomplished (Chaitanya et al., 2010).

In chemotherapy, substances are able to induce cytotoxicity and the death of cells through apoptosis. Apoptosis or programmed cell death is a cell suicide process that activates when continuous survival is blocked (Gerl & Vaux, 2005). Apoptotic cells are characterized by the presence of morphological changes like shrinkage of cells, chromatin condensation, nuclear fragmentation, and membrane blebbing. Certain biochemical changes like caspase activation, breakdown of DNA and protein are also observed. Conversion of normal cells into malignant cells results in mutations in genetic structure which in turn cause cancer cells to evade natural cell death. Disruption of the balance between pro-apoptotic and anti-apoptotic proteins, reduction in caspase function, and impairment of death receptor signalling cause apoptosis resistance in cancer cells (Wong, 2011). Therefore, restoration of these functions by therapeutics can successfully prevent cancer progression. Furthermore, manipulations that occur in cell cycle regulators at checkpoints cause abnormal cell proliferation in cancers (Kasibhatla & Tseng, 2003).

Autophagy mainly involves the formation of an autophagosome by engulfing damaged organelles, fusion with lysosome, and degradation of cellular debris by lysosomal hydrolase (Yang et al., 2011; Sun et al., 2013). Spread of cancer cells may occur by different reasons and such important one is autophagy. Autophagy suppresses tumor in initial phases of cancer by keeping cell homeostasis. Conversely, autophagy promotes tumor growth in more advanced stages of cancer by increasing stress tolerance (Grácio et al., 2017). Activation of autophagy can either promote or suppress the survival of cancer cell. Therefore, the role of autophagy is crucial in anticancer drug development (Sui et al., 2013). Clear cut idea about the relationship between autophagy and apoptosis is not yet investigated.

Alzheimer's and Parkinson's diseases are the neurodegenerative diseases in man. In such victims, neuronal populations are disrupted. Previous studies reported that there are number of chemical compounds that positively affects the functioning of neurons. Such compounds were reported from mushrooms such as *Antrodia camphorata*, *Ganoderma spp.*, *Hericium erinaceus*, *Lignosus rhinocerotis* and *Pleurotus giganteus* and these are using to enhance the functioning of peripheral nervous system. For maintaining neuronal health in human brain nerve growth factors are required. Scientists have been attempting for over last few years to discover fungi-derived neuroactive components which are able to cross the blood-brain barrier and induce nerve growth factor production. *Hericium erinaceus* was explored more for neurodegenerative diseases. This fungus was able to produce hericenones, and erinacines, common terpenoids developed from its fruiting body and mycelia, which can activate the synthesis of nerve growth factor via the TrkA/Erk1/2 pathway (Thongbai et al., 2015). Additional studies have revealed neuroprotective activities in extracts from medicinal mushrooms that induce nerve growth factors, but the active principles remain to be identified.

Chemical components like hericenones, erinacines, scabronines, and dictyophorines reported from mushrooms were found to favor the formation of nerve growth factors. Studies also revealed that it protects the brain from neurodegeneration by hindering the production of beta-amyloid and the protein tau. Mushroom extracts

and their isolated compounds have many important health benefits, including immune-modulating effects on brain cells (Phan et al., 2017). Numerous studies on culinary and medicinal mushrooms have established their neuroprotective effects with the ability to prevent neuronal death and control neurodegenerative diseases and neurotrauma (Wong et al., 2017). Age-related neurological disorders can be rectified by using wild mushrooms. Basidiomycetes, especially mushrooms exhibit a wide range of health benefits such as immunological, anticancer, neuroprotective, and nephroprotective activities due to the presence of various biologically active ingredients with many pharmaceutical properties (He et al., 2017). Future perspectives of mushrooms and their bioactivities in the treatment of neurodegenerative diseases are more significant as it supplements pharmaceutical ingredients.

Alzheimer's disease is the most common type of neuronal disorder. It is associated with notable degeneration and loss of cholinergic neurons in the basal forebrain, which is correlated with the formation of senile plaques and neurofibrillary tangles (Yankner, 1996). The main component of senile plaques is the amyloid β ($A\beta$) peptide which comprises 39-42 amino acids (Tordera et al., 2007).

Among dementia the most prevalent one is Alzheimer's disease (AD). Studies by Selkoe & Hardy, 2016 reported that there are more than 40 million people worldwide suffering from AD. AD is an age-related physical change, usually occur after the age of 60. But its origin and establishment happen during the initial phases of life (Yang et al., 2013; Selkoe & Hardy, 2016). According to the amyloid cascade hypothesis, AD is caused by the accumulation of amyloid beta peptide ($A\beta$), derived from the plasma membrane-bound amyloid precursor protein (APP), either inside the neurons or outside the neurons. $A\beta$ aggregates cause neuronal cell toxicity, cell function loss, and cell death leading to AD complications (Selkoe & Hardy, 2016). Oxidative stress and an elevated level of blood cholesterol are the most common etiological factors leading to AD. In the plasma membrane bilayer, elevated level of plasma cholesterol, through the formation of "lipid rafts" facilitates the deposition of the neurotoxic $A\beta$ (Stampfer, 2006).

Majority of the neurodegenerative diseases (NDs) are age-related, i.e., their incidence progresses with an increasing age. An expanding body of evidence argues

that different NDs are caused by prions, which are formed from normal proteins (Prusiner, 2012). Prions are emerged when normal proteins acquire an additional conformation. PrP^{Sc}, tau, and α -synuclein are the prion proteins that cause CJD, AD, and Parkinson's disease respectively. There is no any single medication effective for ND at present.

Bracket fungi are a complex group of mushrooms formed by different representatives of Basidiomycetes (Agaricomycetes). Among basidiomycetes, polypores represent the most important bracket fungi with diverse morphology of fruiting bodies (Hibbett et al., 2014). The polypores are wood-inhabiting bracket fungi with potentially useful economic and medicinal properties (Sanchez, 2009; Wasser, 2010).

Polyporaceae is the most striking family of Basidiomycetes explored for the genera *Ganoderma*. *Microporus* is the least explored bracket fungus with 12 genera, which have not yet been studied more to reveal its medicinal impacts. So, the present study is mainly focused on the two species of the least explored *Microporus* (*M. affinis* and *M. xanthopus*).

Microporus xanthopus (Fr.) Kunt., a tropical lignicolous species is found on rotting wood. The fruiting body is dark brown with white margin and a stipe of 2.5 cm long. The upper portion is dark brown in color and the lower surface is white with numerous minute pores. The initial stage of the fruiting body is simply a white fleck on the wood surface. This enlarges into a hemispherical cushion up to a millimetre wide and elongates to develop the stipe. Mature fruiting bodies have funnel-shaped caps that are thin and concentrically zoned in various shades of brown, usually with a pale margin (sometimes wavy). The cap can be up to 150 mm wide. The fertile lower surface of the cap is white to dull yellow and can extend down the stipe. The central or off-centre stipe can be up to 40 mm long and 5 mm wide, expanding at the top. It is included in medicinal mushrooms (Bagyalakshmi et al., 2016). The vernacular name is 'kulat lading' (Torres et al., 2020).

Microporus affinis (Blume T. & Nees) Kunt., is an important polypore that grows on dead trunks of trees. It was earlier known under the genera *Polyporus*. It is a thin, flat, leathery bracket fungus banded with colour varying from light yellowish, brown to black usually darker at the centre and creamy white margin in the upper

surface and white fine pores on the lower surface with an eroded edge and lateral stipe. The vernacular name is ‘kulat bitakan’ (Torres et al., 2020).

Briefly, considering all these aspects, the present study was set with a focus on the exploration of the two species of *Microporus* viz., *M. xanthopus* and *M. affinis*. Based on the aforesaid background, the study emphasized the following objectives:

- ❖ To reveal chemical constituents by qualitative and quantitative analyses
- ❖ To unravel the non-volatile and volatile metabolites of the fungus by HR-LC/MS and GC/MS analysis.
- ❖ To evaluate the free radical scavenging potential of the fungi
- ❖ To screen the cytotoxic potential of two fungal species using *Allium cepa* bioassay
- ❖ To decipher the antiproliferative potential of fungi on the DLD1 (Human Colorectal Adenocarcinoma) cell line.
- ❖ To determine the *in vitro* neuroprotective effect of two species of fungi on IMR 32 (Neuroblastoma cells) cell line by MTT assay
- ❖ To determine apoptosis by acridine orange and ethidium bromide double staining
- ❖ To carry out *in vitro* ROS measurement using DCFDA (2, 7 – Dichlorofluorescein diacetate)
- ❖ To validate apoptosis by flow cytometry
- ❖ To elucidate gene expression analysis by qRT PCR



Review of Literature

REVIEW OF LITERATURE

The term mycology is derived from the Greek words, 'Mykes' which means mushroom and 'Logos' that means discourse or 'study of'. Etymologically, it is the study of mushrooms and indeed that was how mycology began in the dim past. Antonio Von Leeuwenhoek's invention of microscope in 17th century led to the beginning of systematic study of fungi. The man who deserves the honor of being called as the founder of the science of Mycology is Pier' Antonio Michelli, the Italian botanist who in 1729 published *Nova Plantarum Genera* in which his researches on fungi were included (Alexopoulos et al., 1996).

Fungi are the group of organisms with diverse nature. In the past, their quick arrival has stimulated the talk of "spontaneous generation". What we see as the fungus is only a fruiting body, one stage of a complex life history that takes place mostly underground (Hall, 1977).

Nowadays, there are so many scientists, biologists and botanists studying and exploring the world of fungi. At the same time, many people have a very negative concept regarding fungi as they are disease causing organisms. Although this impression is not entirely wrong, importance of fungi are far better than already assumed. Their significant role is also highlighted. The wonder drug "penicillin" is the most important example. Without fungi, we would not have leavened bread, cheeses, beer, wine, and other alcoholic beverages (Idaya, 2001). This kind of focus on fungi have given extra attention in the 1970s and many researches were done to ensure the safety of medicinal impacts and the role of chemical constituents found in these fungi. In the past decade, this research has accelerated and the products of importance have been marketed in larger amounts (Miles & Chang, 1997). In 1991, different products from fungi have marketed at an approximate rate of US\$1.2 billion due to their medicinal and tonic significance. This was based on the sale value of the products from *Coriolus*, *Ganoderma*, *Lentinula*, *Schizophyllum* and few other mushrooms (Chang & Miles., 1993). An estimated value of US\$8.5 billion was there for edible fungi in that year. Thus, one can see that the value of mushroom products

was not an insignificant amount. It has been proved that mushrooms are useful in different ways especially in medicinal aspects that led to the large-scale production of mushrooms. In 1999, world production of mushrooms amounted to US\$18 billion, roughly equal to the value of coffee sales (Wassor, 2002).

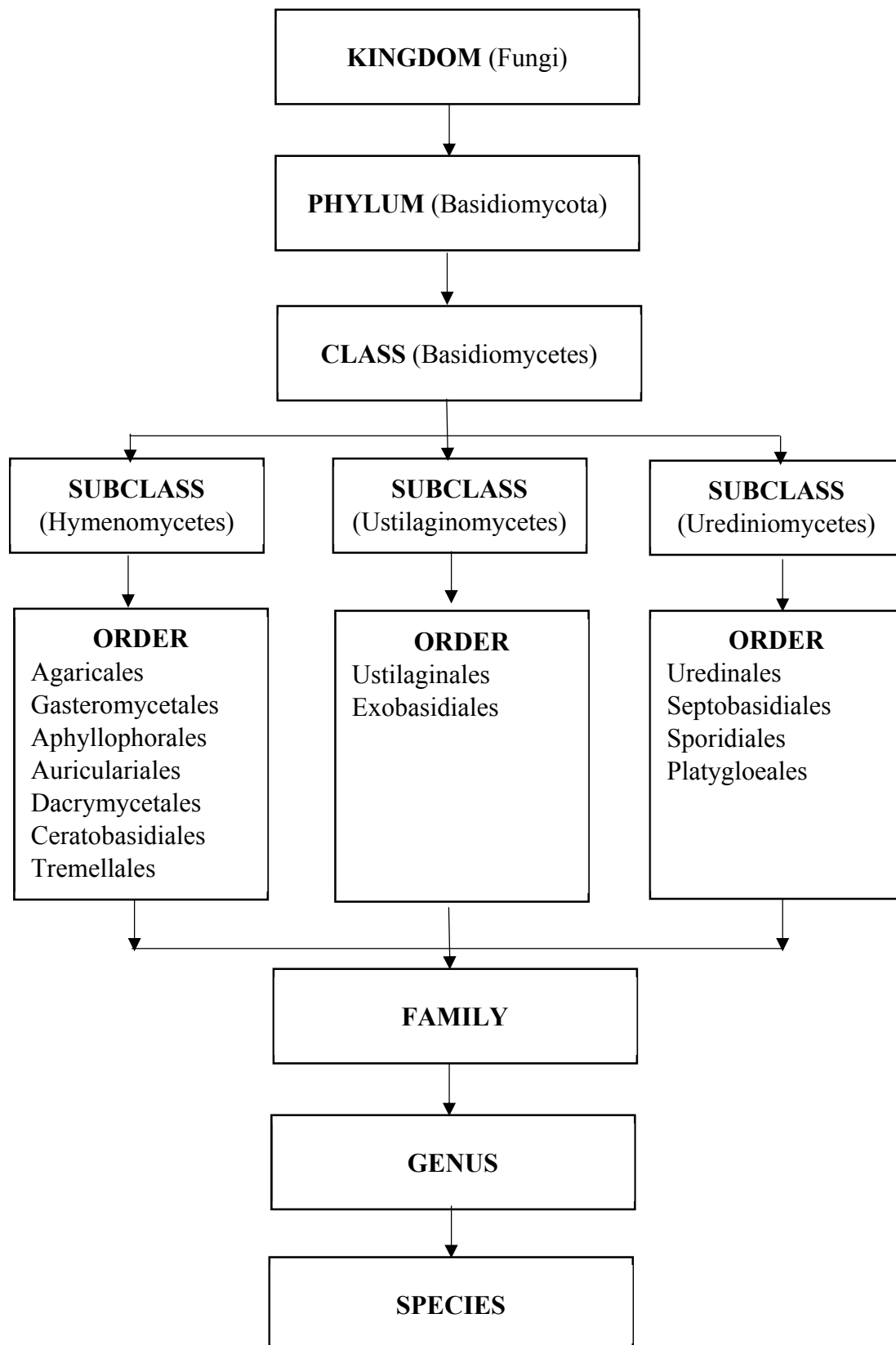
i. Fungal systematics

Fungi represent a heterogenous (polyphyletic) group of organisms. While mycologists have learned a great deal about fungi in the last 30 – 35 years, there was still no agreement as to how the best to classify the fungi nor will there likely be any agreement at a later time (Idaya, 2001). Basidiomycetes are a large and diverse group of fungi and include different forms commonly known as mushrooms, boletes, puffballs, earthstar, stinkhorns, bird's-nest fungi, jelly fungi, bracket or shelf fungi, rust, and smut fungi (Reshetnikov & Tan, 2001).

Basidiomycetes are considered to be a very interesting group of fungi given their exceptional adjustment abilities to accommodate themselves to the detrimental conditions of the environment where they constantly act as natural lignocellulose destroyers (Peralta et al., 2017).

Distribution of macro fungi decreases day by day mainly because of the improper handling of mushrooms in collection site, destruction of forest, and by varying climate. Macro fungi are cosmopolitan in nature and occur seasonally in various habitats all over the world (Ao et al., 2016).

➤ **Systematics of Basidiomycetes:**



(Source: Gams, 2002)

Polyporales represent the most explored group of fungi. According to MycoBank, there have been 577 taxonomic proposals in Polyporales from 2010 to 2017, including 42 new genera and one new family, Fragiliporiaceae (Zhao et al., 2015). Over the same period, 2183 publications with the keyword “Polyporales” are recorded in PubMed. 2183 publications are reported in PubMed with a keyword of “Polyporales” during that period. Because of the role as decomposers, Polyporales are taken into special attention by mycologists.

Bracket fungi are the representatives of Polyporales, a group of morphologically distinct, basidiomycetes. Most of them grow on dead and decayed wood. Shelf fungi or bracket fungi are one among them with a woody and semicircular/ circular fruiting body (conks). Bracket fungi are characterized by the short or absence of stipe. (Fogel & Rogers, 2006). These fungi are reported to possess various medicinal activities. Johnston (2005) reported that *Ganoderma lucidum* is used in TCM (Traditional Chinese Medicine) for the treatment of cancers. Sliva (2006) and Stanley et al. (2005) also mentioned that *G. lucidum* is a popular medicinal mushroom and used in TCM in Asian countries over the past two millennia and preserve human vitality and promote longevity. *G. lucidum* is one of the most used “herbs” in Asia and preclinical studies have established that the polysaccharide fractions have potent effects (Chen et al., 2006). Lin et al. (2006) referred *G. tsugae* Murrill as the Chinese mushroom ‘Songshan lingzhi’, which is cultivated in Taiwan and used extensively to treat diseases. Stanley et al. (2005) have demonstrated that *G. lucidum* induces apoptosis, inhibits cell proliferation and suppress cell migration of highly invasive human prostate cancer cells PC-3.

ii. Medicinal importance of Basidiomycetes

Folk medicines use mushrooms for the treatment of various ailments. Polypores have been of great interest to those looking for novel medicinal compounds, owing to their rich history of medicinal use by various cultures (Ryvarden, 1993). The activities of hypertension, hyperglycemia, hepatitis, chronic bronchitis, bronchial asthma and liver protection have been demonstrated from the fruiting bodies and cultured mycelia of *G. lucidum* (Yuen & Gohel, 2005). Shieh et al., 2001 reported

the superoxide scavenging activity of *G. lucidum* may led to the hepatic and renal protection. According to Lakshmi et al., 2006, fruiting bodies of *G. lucidum* collected from South India is found to possess antimutagenic activity. Antioxidant defense was restored with the extract of *G. lucidum* and reported to prevent hepatic injury. Besides this, several compounds with medicinal properties like triterpenoids and polysaccharides. They have been isolated from *Ganoderma* species. Antifungal and antibacterial activity was also reported. Wang & Ng (2006), isolated ‘Ganodermin’, an antifungal protein from fruiting bodies of *G. lucidum* (Curtis) Karst. Ganodermin was found to possess antifungal activity. Wang et al. (2006) isolated an antifungal polypeptide from fresh fruiting bodies of *Polyporus alveolaris*.

Studies on macrofungi have been an area of importance for scientists in particular and people in general, because of their role in human welfare, in food industry, in medicines and biodegradation (Ozturk et al., 2003). Diversity studies macrofungi have been conducted long years ago, but worldwide distribution is understudied. Mycologists continue to unravel the unexplored, hidden and fascinating fungal biodiversity as many macrofungi are becoming extinct or facing the threat of extinction because of habitat destruction and global climate change (Swapana et al., 2008).

Nowadays, medicinal mushrooms are regarded as functional foods and exist as over-the-counter health supplements used in complementary and alternative medicines (Rathees et al., 2012; Ayeka, 2018).

Traditional medicine was found to use *Inonotus obliquus*, commonly known chaga in Russia. Since the 16th century, it has been used as a folk remedy to treat cancer, diseases of the digestive system, and tuberculosis (Zjawiony, 2004). Recent studies claim its anti-AIDS, anti-aging, blood lipid decreasing, blood pressure lowering and immune-stimulating effects (Zhang et al., 2009).

To treat headache, nausea, and hepatic problems, *Fomitopsis pinicola* was used. Moreover, due to their astringent effects the fruit bodies have been used as haemostatics and anti-inflammatory agents (Leeser, 1987).

Many reviews focused exclusively on polypores, which are considered a major source of pharmacologically active natural products. The secondary metabolites of polypores exhibit a wide range of biological activities such as antimicrobial, antiviral, antifungal, anticancer, cardiovascular, anti-inflammatory, antioxidant, immunostimulating, nematocidal, and other activities (Stametes, 2002).

Table 1: List of some mushrooms with medicinal importance:

Mushroom species	Medicinal uses
<i>Auricularia auricula - judae</i>	Antitumor, to prevent coagulation, hypocholesterolemia
<i>Auricularia polytricha</i>	Anticoagulant, reduction in cholesterol level
<i>Cantharellus cibarius</i>	Antimicrobial
<i>Coprinus disseminatus</i>	Antitumor
<i>Daldinia concentrica</i>	Remedy for allergic problems in skin and wound healing
<i>Fomes fomentarius</i>	Fever
<i>Ganoderma lucidum</i>	Antiviral, to eradicate bacteria and fungi anticancer
<i>Hygrocybe conica</i>	Antitumor
<i>Lactarius piperatus</i>	Antitumor, antibacterial, antioxidant
<i>Lentinus edodes</i>	Antitumor, to treat HIV, natural antitode
<i>Lentinus squarrosulus</i>	Used as nutraceutical
<i>Phellinus linteus</i>	To treat blood circulation disorders, cancer
<i>Pleurotus ostreatus</i>	Antibacterial, hypocholesterolemic, anticancer, antiviral
<i>Pleurotus pulmonarius</i>	Anti-HIV, hyperglycemic
<i>Pycnoporus cinnabarinus</i>	Wound healing
<i>Microporus xanthopus</i>	To prevent children from breastfeeding
<i>Russula cyanoxantha</i>	Antitumor, antioxidant
<i>Schizophyllum commune</i>	Anticancer (drug Schizophyllan)
<i>Trametes gibbosa</i>	Anti inflammatory, antitumor, antiviral
<i>Trametes versicolor</i>	Anticancer (drug Krestin), antiviral, antibacterial, antifungal
<i>Lycoperdon perlatum</i>	Against microbes

Sources: Chang & Miles, 2004; Das, 2009; Deshmukh, 2004; Ao et al., 2016; Khastini et al., 2018

iii. Edible mushrooms

In earlier studies, mushrooms are evaluated for nutritional value and pharmacological importance. Mushrooms form an important source of active pharmaceutical products. Medicinal mushrooms represent an important source of polysaccharides. Higher Basidiomycetes mushrooms contain biologically active polysaccharides in fruit bodies, cultured mycelia and cultured broth (Wasser, 2002; Wasser, 2010; Smith et al., 2003). Moreover, mushrooms are rich in minerals like K, Ca, P, and Mg. Because of low sodium and high potassium concentration, they have a Na^+/K^+ ratio of less than 0.6, being thus a good food option amongst other vegetables for hypertensive individuals (Rajaratnam et al., 1998). Mushrooms are an excellent source of selenium and copper (Feeney et al., 2014; Murugesan, 2017). In comparison to vegetables and fruits, mushrooms contain tocopherols, carotenoids, ascorbic acid, phenolics, and ergosterol being most abundant (Sánchez, 2010).

Due to hard and corky nature, many of the polypores are not edible with some exceptions like young fruiting body of *Laetiporus sulphureus* which is commonly called “chicken polypore” or “chicken of the woods”. In some parts of Germany and North America, it is being used as an alternative for chicken. However, gastrointestinal problems have been reported after eating this fungus as well as the occurrence of severe adverse effects including allergic reactions, vomiting, and fever (Jordan, 1995; Watling, 1997).

Anti- β -glucan antibodies in man has been increased by the intake of fresh mushrooms. There was also a report to provide better defense against pathogenic fungi by the Ohno group from Japan (Ishibashi et al., 2001). Edible mushroom comprises a maximum number of all essential amino acids (Hyes, 1978) with arginine, glutamic acid and aspartic acid being the most abundant ones (Manzi et al., 1999). In 2010, Dembitsky et al., reported that mushrooms can be considered as a great alternative to animal products. Indeed, lysine and tryptophan, which are absent in vegetables, are present at high concentrations when compared to cysteine and methionine (Murugesan, 2017).

Consumption of edible mushrooms can provide up to 25% of the recommended dietary fiber intake since mushrooms are a good source of different dietary fiber including β -glucans, polysaccharide-protein complexes (PSPC), chitin, hemicellulose, mannans and xylans (Rathore et al., 2017).

Many edible mushrooms (mostly Basidiomycetes) are good sources of carbohydrates such as β -glucans; phenolics such as tocopherols; B-vitamins such as niacin, flavin and pyridoxine; organic acids such as ascorbate, shikimate, malate and fumarate; monoterpenoids and diterpenoids; lipids; proteins such as hydrophobins and trace elements such as selenium (Aggarwal et al., 2012; Iwalokun et al., 2007; Yim et al., 2010). These substances are found to be responsible for the antimicrobial, antitumor, anti-aging, and antioxidant potentials of mushrooms (Lindequist et al., 2005).

iv. Nutraceutical values of Basidiomycetes

Mushrooms have got global attention because of their significant role in medicine and for culinary purposes. Some mushrooms are regarded as nutraceuticals (Ribeiro et al., 2007; Yaltirak, 2009). In 2018, Muszyńska et al., reported that low-fat content of mushrooms is the remarkable feature of their low calories. The total energetic value of 1 kg of fresh mushroom is between 250 to 350 calories (Panneerselvam et al., 2009; Sánchez, 2010). The dry matter of mushrooms comprises around 50% - 65% carbohydrates, 19% - 35% proteins, and 2 % - 6% fat (Rathore et al., 2017). Regarding fats, mushrooms are rich in polyunsaturated, monounsaturated, and saturated fatty acids such as palmitic acid, oleic acid, and linoleic acid (Kavishree et al., 2008; Muszyńska et al., 2018). There are some reports on mushroom products for culinary and medicinal purposes. Thereby consumers are interested in these so-called “mushroom nutraceuticals”. The market value of mushroom dietary supplement products from *Ganoderma lucidum* species alone worldwide is estimated to be \$5-6 billion per year, with \$1.6 billion for the United States (Chang & Buswell, 1999; Wasser et al., 2000). Since mushrooms grow in any kind of waste materials such as decayed wood, dried leaves of paddy, sawdust etc., wild edible mushrooms form the major source of nutrient rich food for tribes. In general, mushrooms are the

chief source of nutritional food and are often regarded as “poor man’s meat”. It is very clear that mushrooms have the potential to provide a healthy diet to the rural tribal population (Ao et al., 2016). Mushrooms are called “host defense potentiators” as they are abundant in different nutrients, antioxidant substances and the property of lowering cholesterol level (Wani et al., 2010). Many workers have carried out studies on the nutritional analysis of different species of mushrooms where it is reported that the different species of mushrooms are rich in protein (30% - 48%), carbohydrate (12.5% - 40%), fat (1% - 4%), ash (7% - 17%), fiber (16% - 20%) etc. (Pushpa & Purushothama, 2010; Manikandan, 2011). Reports by Feeney et al. (2014) and Murugesan (2017) revealed the richness of vitamins like niacin (B₃), pantothenic acid (B₅), and riboflavin (B₂). Studies conducted by Pankavec et al. (2019) and Rózsa et al. (2019) have also mentioned the presence of other vitamins such as thiamin (B₁), pyridoxin (B₆), cobalamin (B₁₂), biotin (B₇) and calciferol (Vitamin D). The only non - animal-based food that contains vitamin D are mushrooms (Feeney et al., 2014; Murugesan, 2017).

v. Secondary metabolites

Secondary metabolites are the key for drug development. Mushroom compounds of therapeutic interest are secondary metabolites like lactones, terpenes, alkaloids, antibiotics etc., strengthen the therapeutic value of mushrooms, which favors the functioning of immune system. Different enzymes like laccase, superoxide dismutase, glucose oxidase and peroxidase were reported in various medicinally important mushrooms. It has been shown that enzyme therapy plays an important role in cancer treatment preventing oxidative stress and inhibiting cell growth (Wasser & Weis, 1999b; Zaidman et al., 2005).

For the discovery of drugs, medicinally important mushrooms play a pivot role. “Blockbuster drugs” like antibiotics (penicillin, tetracycline and erythromycin), antiparasitics (ivermectin), antimalarials (quinine, artemisinin), lipid control agents (lovastatin and analogs), immunosuppressant for organ transplants (cyclosporin, rapamycin) and anticancer drugs (taxol, doxorubicin) revolutionized medicine (Li &

Vederas, 2009). Different chemicals present in fungi led to the discovery drugs as listed above.

Several excellent review articles have been published on the subject of biologically active secondary metabolites from Basidiomycetes (Wasser & Weis, 1999c; Reshetnikow & Wasser, 2001). There are a number of publications describing the antimicrobial properties of secondary metabolites isolated from various polypores (Mothana et al., 2000; Smania et al., 1998; Abate & Abraham, 1994). Extracts of fruiting bodies of *Ganoderma lucidum*, *G. pfeifferi* and *G. resinaceum* found to possess antibacterial activity against *Bacillus subtilis*. For example, two secondary metabolites, ganomycin A and ganomycin B isolated from *G. pfeifferi* showed moderate growth inhibition of several bacterial strains, particularly Gram-positive strains such as *B. subtilis*, *Staphylococcus aureus*, and *Micrococcus flavus* (Mothana et al., 2000). In 2007, Ribeiro et al. reported that the mushrooms contain several bioactive compounds such as cinnamic acid, ergosterol and p-coumaric acid, p-hydroxyl benzoic acid, and protocatechuic acids.

Consumption of *Laetiporus sulphureus* has been reported to cause hallucinations. Therefore, it has been assumed that this species might contain alkaloids similar to those found in psychoactive plants (Appleton et al., 1988). Polypore species are mainly noted for this kind of hallucinogenic effects.

Previous studies on crude extract of the European wood-rotting fungus *Trametes versicolor* (as *Polyporus versicolor*) reported the presence of cytotoxic, polyoxygenated ergosterol derivatives (Valisolalao, 1983).

Mushrooms are well known for their nutritional and medicinal value (Acharya et al., 2007; Chatterjee et al., 2011; Biswas et al., 2012; Acharya et al., 2012; Chatterjee et al., 2013). In 2012, Chen et al. reported that mushrooms play a crucial role in treating various diseases. Many edible mushrooms especially Basidiomycetes are good sources of carbohydrates such as β -glucans; phenolics such as tocopherols; B-vitamins such as niacin, flavin and pyridoxine; organic acids such as ascorbate, shikimate, malate and fumarate; monoterpenoids and diterpenoids; lipids and proteins such as hydrophobins (Yim et al., 2010; Aggarwal et al., 2012; Iwalokun et al., 2007).

These substances in mushrooms are found to be responsible for their antimicrobial, antitumor, anti-aging, and antioxidant potentials (Lindequist et al., 2005).

Terpenes are the compounds responsible for the antioxidant, anticancer and anti-inflammatory activities among many other biological activities exerted by mushrooms (Sánchez, 2017b; Ruan & Popovich, 2012).

In a study conducted by Chepkirui et al. (2018), revealed the isolation, structure elucidation, and biological activity of microporenic acid A–G (1–7) as well as seven new diterpenes with an attached isocitric acid moiety from cultures of a *Microporus* species (Polyporales). This forms the first reported metabolites from this group of fungi.

In an earlier study by Liew et al. (2015), *Microporus xanthopus* indicated the absence of triterpene, alkaloid and steroid content and demonstrated weak saponin activities.

The chemical profiling of the methanolic extract of *Hexagonia tenuis* was determined by using GC/MS analysis. Different types of compounds were determined and were categorized into 13 classes of compounds such as esters, alcohols, acyl halides, ketones, quinolines, phenols, carboxylic acids, fatty acid esters, fatty acids, alkenes, sterols, triterpenoids and hydrocarbons (Aneesh & Thoppil, 2019).

Previous works of Takaku et al. (2001) have shown that ergosterol may exhibit some degree of antitumor property. According to Rajakumar et al. (2007), ergosterols are known to act as biological precursors of vitamin D hence they can be grouped as provitamins. GC/MS analysis of *Agrocybe aegerita* conducted by Zhang et al. (2003) revealed the presence of linoleic acids and ergosterol. Ergosterol has shown mild antioxidant activities.

vi. Important bioactivities

a. Antimicrobial activity

Nature is the ultimate source of wide range of medicinally important compounds for many years. Emergence of antibiotic resistant bacteria is a major issue.

Nowadays, the need to discover new and more potent agents as accessories or alternatives to antibiotic therapy is stronger (Butler, 2004; Lam, 2007).

Earlier studies reported the antimicrobial activities of extract of *Ganoderma lucidum*. Wang & Ng (2006) isolated 'Ganodermin', an antifungal protein from fruiting bodies of *G. lucidum*. Ganodermin is reported to hinder the growth of mycelium of *Botrytis cinerea*, *Fusarium oxysporum*, and *Physalasporea piricola*. Wang et al. (2006) isolated an antifungal polypeptide from fresh fruiting bodies of *Polyporus alveolaris*. Growth of mycelium in *B. cinerea*, *F. oxysporum*, *Mycosphaerella arachidicola* and *P. piricola*, can be controlled by antifungal polypeptide called alveolarin.

A study conducted by Parkash & Sharma (2016) revealed the antimicrobial potential of aqueous and ethanolic extracts of bracket fungi such as *Ganoderma lucidum* and *Polyporus officinalis* against four phytopathogenic fungi (*Alternaria solani*, *Curvularia lunata*, *Aspergillus terreus* and *F. oxysporum*) along with bacteria (*E. coli* and *Bacillus subtilis*). Ethanolic extract of *G. lucidum* was found to retard the growth of *A. solani*, *C. lunata*, *A. terreus* and *F. oxysporum* at the concentrations of 500 ppm and 1000 ppm. While the aqueous extract was able to prevent completely the growth of *A. terreus* and *F. oxysporum* at 1000 ppm and 500 ppm concentrations. Anti-fungal activity of ethanolic extract of *P. officinalis*, against *A. solani* and *A. terreus* was reported at 1000 ppm concentration. In case of *C. lunata* and *F. oxysporum*, complete inhibition was reported at the concentration of 1000 ppm and 250 ppm. Ethanolic extract of *G. lucidum* was screened for antibacterial properties and it was found a zone of inhibition of 0.09 mm against *E. coli* at a concentration of 1000 ppm and the inhibitory zone of 0.05 mm against *B. subtilis* at the concentration of 250 ppm. Aqueous extract of *G. lucidum* was not able to show any inhibitory action against *E. coli* and *B. subtilis*. A maximum inhibitory zone of 10 mm was reported for *E. coli* at a concentration of 1000 ppm in the ethanolic extract of *P. officinalis* and for *B. subtilis* a mild inhibitory zone having a diameter of 0.2 mm was reported at 250 ppm concentration. From these studies it was clear that *G. lucidum* and *P. officinalis* are having considerable amount of antimicrobial properties.

According to Hleba et al. (2014) the methanolic extracts of both fungi *Ganoderma lucidum* and *Trametes versicolor* showed the strongest antimicrobial activity against *Saccharomyces cerevisiae*. However, these fungal extracts exhibited a lower antimicrobial activity against Gram positive microorganisms. On the other hand, antibacterial activity has been observed against Gram-positive bacteria using the basidocarp extract of *G. lucidum* and *G. orogonense* Murr. (Kim et al., 1993). Sudirman & Muziyati (1997) observed that seven Indonesian *Ganoderma* species inhibit the growth of *Bacillus subtilis*. Coletto & Mondino (1991) noted that methanolic extract of *G. resinaceum* Boud and *G. lucidum* inhibited *Bacillus subtilis* Cohn. *G. resinaceum* also inhibited *Staphylococcus aureus* Rosen.

Studies conducted on antimicrobial activity by Keypour et al. (2008) showed that the chloroform extract of *G. lucidum* had antibacterial effect against two Gram positive bacteria, *Bacillus subtilis* and *staphylococcus aureus*. However, negative results were observed against the other tested bacteria such as *E. coli* and *Pseuedomonas aeruginosa*.

In a study conducted by Chepkirui et al. (2018), all the compounds (microporenic acids A-G) were tested for their antimicrobial activities against various bacteria (*Bacillus subtilis* DSM 10, *Micrococcus luteus* DSM 1790, *Staphylococcus aureus* DSM 346 and *Escherichia coli* DSM 498) and some fungi (*Mucor plumbeus* MUCL 49355, *Candida tenuis* MUCL 29982 and *Pichia anomala* DSM 6766). Moderate antimicrobial activities were observed against *Bacillus subtilis*, *Micrococcus luteus*, and *Staphylococcus aureus* for compounds 4 and 5. While, compounds 1-5 were observed for moderate activity against *M. luteus*. Compounds 4 and 5 also showed better activity against fungi such as *Candida tenuis* and *M. plumbeus*.

Fomitopsis officinalis exhibits many vital biological activities. Chlorinated coumarin from mycelia has been used for the treatment of pulmonary diseases, especially tuberculosis, and pneumonia, where it showed antibacterial activities toward *M. tuberculosis* and *Bacillus pneumoniae* as well as other microorganisms (Girometta, 2019).

Liew et al. (2015) reported the high antimicrobial activity of *Microporus xanthopus* against *Clostridium difficile*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*.

b. Antioxidant activity

Oxidation is a necessary process for many living organisms for the production of energy (Aziz et al., 2007). However, numerous physiological processes and external factors (**Fig. 1**) occasionally produce oxygen centered free radicals and other reactive oxygen species (ROS), which may contribute to diseases like atherosclerosis, diabetes, cancer, and cirrhosis (Halliwell & Gutteridge, 1984).

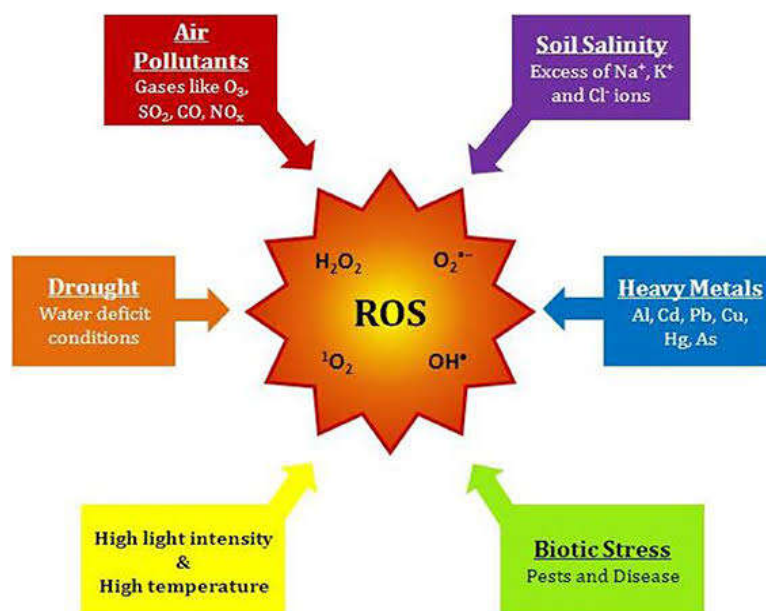


Fig. 1: Factors affecting ROS
[Reference: Das & Roychoudhary, 2014]

Most organisms have evolved antioxidant defense and repair systems to protect them against oxidative damage; however, these systems are insufficient to prevent the damage entirely (Simic, 1998). Thus, the researches on finding antioxidants in normal food items is highly appreciable. Free radical scavenging assay like DPPH assay is the common and reliable method for screening antioxidants. In the presence of an antioxidant, the DPPH radical obtains one more electron, and the absorbance decreases (Koleva et al., 2002).

Antioxidant activity may vary with the nature of the extract and the portion of mushroom used. Alcohol extract of *Tricholoma giganteum* possesses better antioxidant activity than water and ethyl acetate extract (Chatterjee et al., 2011). Antioxidant activity depends on the portion of the mushroom. Methanol extract of the cap portion from *T. portentosum* showed better antioxidant activity than its stipe portion, although both are lower than the entire mushroom (Ferreira et al., 2007).

Studies conducted by Kim et al. (2008) reported that medicinal mushrooms had various phenolic compounds that have shown limitless potential in functional foods and medicinal industries and also confirmed that the phenolic content of mushroom could make a significant contribution to the antioxidant properties. Brief study on different mushroom species such as *Pleurotus tuber-regium*, *Termitomyces robustus*, *Lentinus squarrosulus*, *Pleurotus ostreatus*, *Pleurotus sajor-caju* and *Auricularia auricula* conducted by Obodai et al. (2014) has reported a trend in decreasing order of elements and that was $Fe > Pb > Zn > Cu > Mn$ respectively.

Reactive oxygen species (ROS) are induced by various sources which can be endogenous or exogenous (Fig. 2).

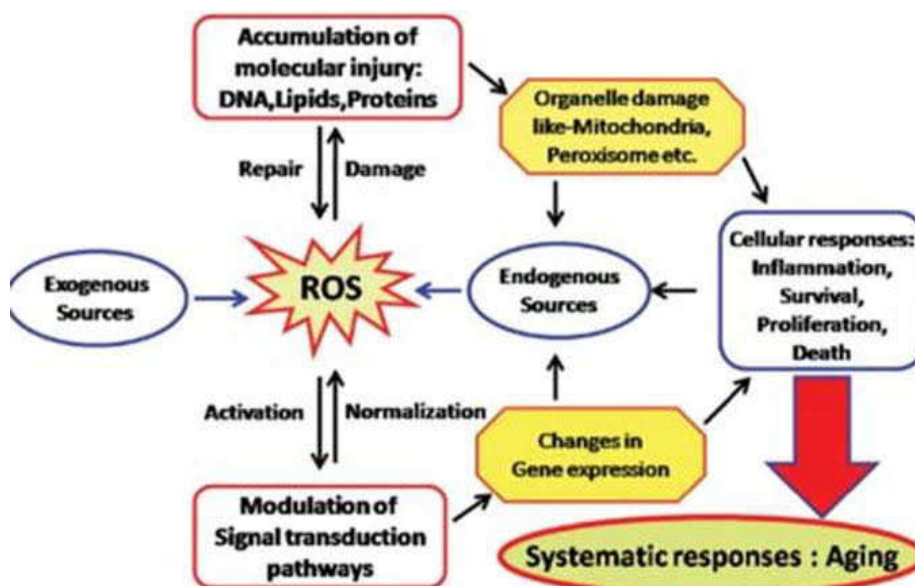


Fig. 2: Endogenous and exogenous sources of ROS

[Reference: Pandey & Rizvi, 2010]

Complete prevention of damages induced by oxidation stress is not possible even when organisms show antioxidant properties. Thus, there is need for external supply of antioxidants to reduce oxidative damage in human beings.

Nowadays, the consumption of mushrooms has increased in large amounts even when it has been used for many years. Phenolic acid, flavonoids, tocopherols, ascorbic acid, and carotenoids are the major components of mushrooms. Polysaccharides are also important biologically active ingredients (Khatua et al., 2013). Free radicals like reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced in aerobic organisms through normal cellular metabolism. Oxidative stress can be avoided by maintaining the balance between antioxidant defense system and free radicals. Natural antioxidants promote the endogenous defense system to reduce damages due to oxidative stress. In general, mushrooms contain oxidative resistant constituents such as phenols, flavonoids, tocopherols, carotenoids and ascorbic acid that act as a rich source of antioxidants (Anusiya et al., 2021). Based on the ability of donating hydrogen ions, chelating metals and scavenging superoxide and free radicals, antioxidant properties of mushroom extracts can be classified. Phenolic compounds are responsible for antioxidant activities in mushroom extracts through acting as decomposers of peroxidase, activators of metals, oxygen scavengers or inhibitors of free radicals (Dziezak, 1986). Phenolic compounds are the main category of chemical components that are responsible for the antioxidant activity of mushroom species (Kurutas, 2015). Phenolic compounds are aromatic hydroxylated compounds, possessing aromatic rings with one or more hydroxyl groups (Apak et al., 2007). Mushrooms contain phenolic acid, which forms the major phenolic compound. Due to the presence of phenolic hydrogen, phenolic acid shows antioxidant properties. Studies by Ferreira et al. (2009) reported that gallic acid, caffeic acid, quercetin, rutin, vanillin etc., are a variety of phenolic compounds detected in wild mushrooms. Flavonoids are actually phenolic compounds having antioxidant properties. Generally, fungi are not able to produce flavonoids. Exceptionally, flavonoids have been reported in some mushrooms such as *Lactarius piperatus* (Barros et al., 2007). Tocopherol is the essential component of biological

membrane. Among the four different types (α , β , γ and δ), α -tocopherol has the highest antioxidant activity (Kamal-Eldin & Appelqvist., 1996).

In an earlier study, ethanolic extract of *Agaricus bisporus* was screened for antioxidant properties. For the antioxidant assay, the extract exhibited greater antioxidant activities against superoxide radicals, hydroxyl radicals and reduction of ferric ions (Liu *et al.*, 2013).

Another study revealed the antioxidant property of *Inonotus obliquus*, which is commonly used for the treatment of therapy. The methanolic extract of the fungus was identified with a strong scavenging effect against DPPH and ABTS radicals (Lee *et al.*, 2007a). The methanolic extract of *Pleurotus florida* was examined for its higher antioxidant activity as established by DPPH, hydroxyl and superoxide radical assays together with nitric oxide, reducing power and metal chelating assays (Menaga *et al.*, 2013).

Fortunately, organisms are able to fight against the damages caused by free radicals. If the equilibrium of this antioxidant defense system is disturbed, deterioration of physiological functions may occur (Mau, 2002). Thus, use of dietary supplements that contain antioxidants is highly promoted. Butylated hydroxyanisole (BHA), butylhydroxytoluene (BHT), propyl gallate and tert-butylhydroxyquinone are the widely used synthetic antioxidants. But these have restricted use in the food industry because of its harmful effects in causing liver damage and carcinogenic. Therefore, there is great interest in using natural additives as potential antioxidants (Sherwin *et al.*, 1990).

Free radicals are molecules or molecular fragments containing one or more unpaired electrons (Halliwell & Gutteridge, 1997). In general, these are unstable and attack easily the nearest stable molecule to acquire the required electron for attaining stability. From the previous study reports, different sources of free radicals are represented in Fig. 3. Free radicals derived from molecular oxygen are usually known as ROS and represent the most important class of radical species generated in living systems (Ferreira *et al.*, 2009).

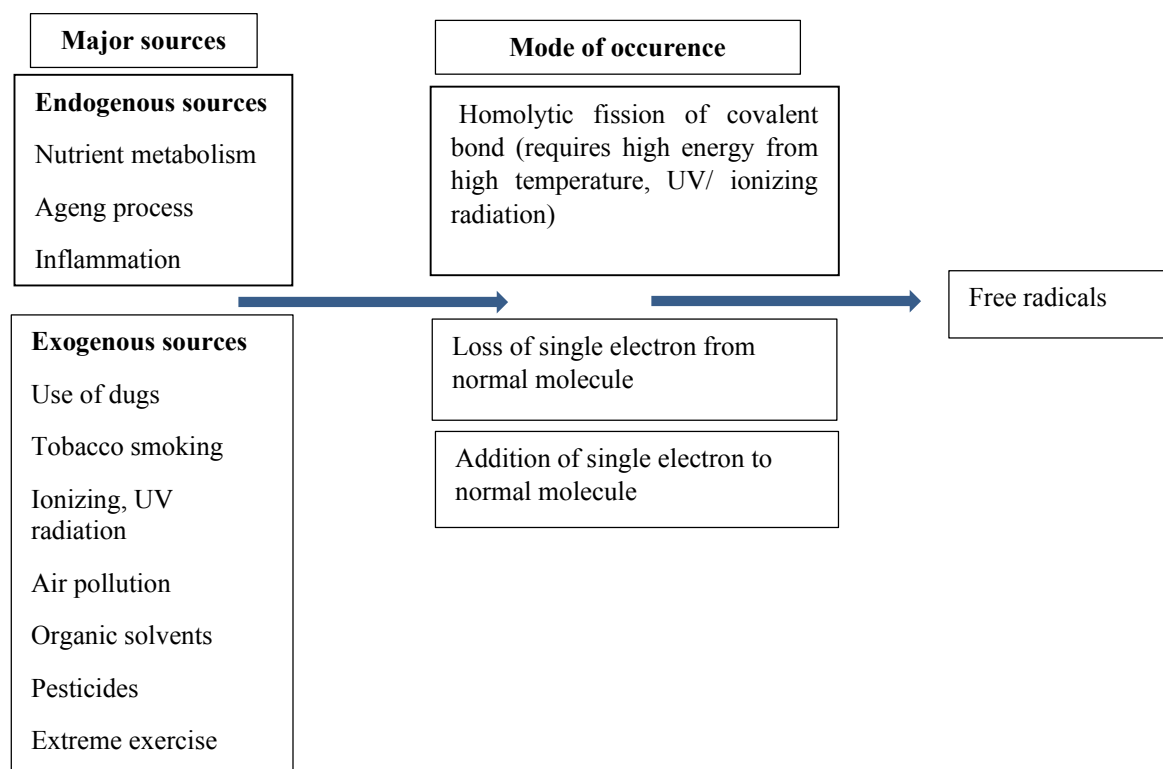


Fig. 3: Major sources of free radicals and chemistry behind the generation of free radicals (Blokhina et al., 2003; Cheeseman & Slater, 1993).

The term ROS includes not only free radicals such as superoxide radical and hydroxyl radical but also molecules such as hydrogen peroxide, singlet oxygen and ozone (Blokhina et al., 2003). Superoxide anion is considered as the primary ROS as it forms by addition of one electron to molecular oxygen.

Over production of free radicals and different conditions like atherosclerosis, cardiovascular diseases, cancer, cirrhosis, diabetes, lung diseases, neurological disorders etc., are correlated. Valko et al. (2007) and Rai et al., 2011 have published excellent reviews on the effect of free radicals on diseases. In 2007, Valko et al. pointed out that there is correlation between O_2 intake and ageing process. *i.e.*, oxygen consumption and the life span are inversely proportional.

Different types of reactive oxygen species of biological interest with their mode of generation are summarized as follows:

Sub classes of reactive species	Generation
Superoxide ($O_2^{\cdot-}$)	By products in electron transfer chain of chloroplast, mitochondria (from both complex I and III), plasma membrane; NADPH oxidases, xanthine oxidase. $O_2 + e^- \rightarrow O_2^{\cdot-}$
Hydroxyl radical ($\cdot OH$)	Fenton's reaction $H_2O_2 + O_2 + Fe^{2+} \rightarrow \cdot OH + OH^- + Fe^{3+}$ Haber-Weiss reaction $O_2^{\cdot-} + H_2O_2 \rightarrow \cdot OH + OH^- + O_2$
Hydrogen peroxide (H_2O_2)	Superoxide radical is dismutated by superoxide dismutase and forms H_2O_2 . $O_2^{\cdot-} \rightarrow H_2O_2$
Peroxyl radical ($ROO\cdot$)	Formed from lipids, proteins, DNA, sugars etc. during oxidative damage.
Hydroxy-peroxy radical/ perhydroxy radical ($HOO\cdot$)	$O_2^{\cdot-} + H^+ \rightarrow HOO\cdot$
Singlet oxygen (1O_2)	Formed during photosensitization and chemical reaction.

(Resource: Blokhina et al., 2003; Valko et al., 2007; Cheeseman & Slater, 1993; Muller et al., 2004; Pastor et al., 2000; Gill & Tuteja, 2010; Devasagayam et al., 2004; Schweitzer & Schmidt, 2003)

The degenerative diseases associated with aging include cancer, cardiovascular disease, immune system decline, brain dysfunction and cataracts (Ames et al., 1993). They are also associated with free radicals because oxidative damage to DNA, proteins and other macromolecules accumulates with age and has been postulated as a major type of endogenous damage leading to aging (Fraga et al., 1990). Earlier studies suggested that tissue damage and cell death are mainly due to O_2 centered free radicals and ROS. **Fig.4** represents the different sources and formation of free radicals.

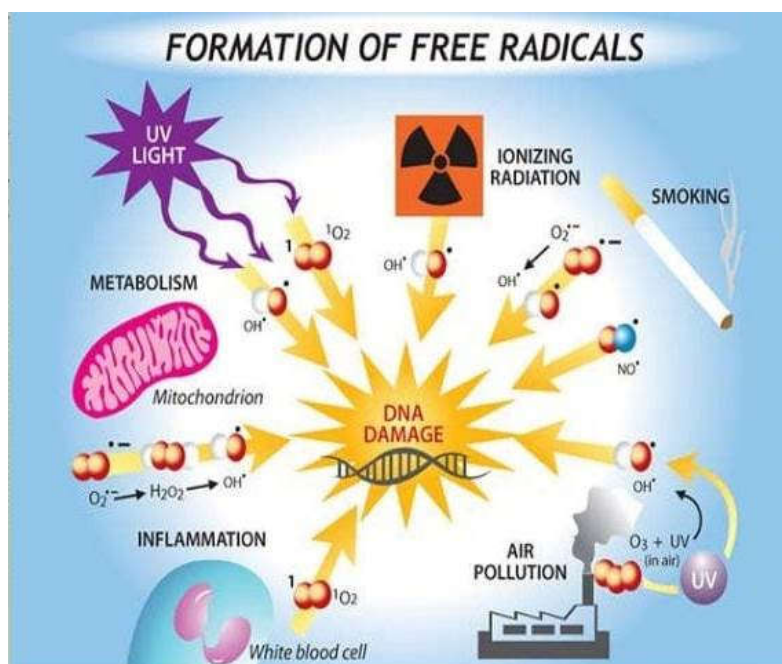


Fig. 4: Formation of free radicals
[Saha & Roy, 2017]

c. Cytotoxicity

The cytotoxicity of aqueous extracts from fruiting bodies of polypore species such as *Fomes fomentarius*, *Fomitopsis pinicola* and *Inonotus obliquus* were reported in earlier studies (Cheng et al., 2008; Chen et al., 2011; Song et al., 2008). The cytotoxic activity of polypore extracts is mostly associated with the presence of polysaccharides, although numerous smaller molecular weight cytotoxic polypore metabolites also are known (Mizuno, 1999; Mizuno et al., 1995). A rare example of a cytotoxic monoterpene is montadial A, which is isolated from the polypore *Bondarzewia montana* (Sontag, 1999). In a previous study, Montadial A is reported for its cytotoxic effects against cancer cell lines L1210 (lymphocytic leukemia cells) in mice at a concentration of 10 $\mu\text{g/mL}$ and also against HL60 (promyelocytic human leukemia cells) at 5 $\mu\text{g/mL}$. Studies conducted by Elbatrawy et al. (2015) reported that *Agaricus bisporus* and *Pleurotus sajor-caju* shows cytotoxicity against both the larynx and breast carcinoma cell lines.

In vitro cytotoxicity (IC_{50}) of compounds 1–5 and 7–9 (microporenic acids A–G from *Microporus* species) was determined by Chepkirui et al. (2018) against

mouse fibroblast L929 and HeLa (KB-3.1) cell lines. Mild cytotoxicity was observed against KB3.1 cell line by compound 1. But no activity was noticed for the remaining compounds against KB3.1 and L929 cell lines.

In a study conducted by Wu et al. (2009) lanostane-type triterpenoids, which was reported in *F. officinalis* extract, showed anticancer activity. It was found to inhibit eukaryotic DNA polymerase, a feature which allows it to be a cytotoxic agent and helpful as a cancer chemotherapeutic agent (Kim et al., 2002).

Earlier studies conducted by Aneesh & Thoppil (2019) reported the cytotoxic impetus of *Hexagonia tenuis* in *Allium cepa* root tip. The studies revealed a number of chromosomal aberrations such as chromatin erosion, bizarre nucleus, chromosome laggards, pulverized chromosomes, binucleate cell, nuclear lesions, chromosome bridge, chromosome rosette, hyperchromasia, coagulated chromosomes, stathmo-anaphase, ghost cell, cytomixis, cytostasis, shift in microtubule organizing centre (MTOC), C-metaphase, macro and micro cell, hypoploid cell, diagonal chromosomes, stickiness, tripolar anaphase and strap cell. This potential of inducing different types of chromosomal aberrations will be a stepping stone towards the antitumor activities of the fungal extract.

In an earlier study by Aneesh & Thoppil (2020), *Micrporus affinis* has been tested for *in vitro* anticancer efficacy by using MTT assay on DLD₁ cell line. Antitumor and antioxidant properties may be due to the presence of sterols and esters. Morphological changes in cells like rounding and shrinkage forms the indicator of cytotoxicity.

In a study conducted by Elbatrawy et al. (2015), the cytotoxic effects of extracts of four different mushrooms such as *Pleurotus ostreatus*, *P. sajor-caju*, *Agaricus campestris* and *A. bisporus* were evaluated using 2 cancer cell lines of larynx carcinoma (HEp-2) and breast carcinoma (MCF-7).

d. Anticancer activity

Cancer is a multifactorial condition which is characterized by the loss of growth factors. These abnormal malignant cells can evade the tumor suppressor

factors of the human immune system, then develop to tumors and destroy adjacent tissues (Saeidnia & Abdollahi, 2014). Chemotherapy, radiation therapy, surgery and immunotherapy are the widely accepted treatments for cancer. However, while many cytotoxic agents are known to science (which could in principle serve as chemotherapeutic agents), only few of them specifically target tumor cells and are less toxic to regular, healthy human tissue (Petrelli et al., 2012; Cai et al., 2013; Zugazagoitia et al., 2016).

Tumor is a malignant neoplasm, an abnormal mass of tissue which results due to the abnormal proliferation of cells (Nowell, 1986; Franks, 1997; Anand et al., 2008; Place et al., 2011). Antitumor agent is the substance that able to act against the malignant tumor. Triterpenes isolated from *Ganoderma* species have been identified as a potent anticancer agent (Paterson, 2006; Cheng et al., 2010; de Silva et al., 2012a; Wu et al., 2012). Medicinal mushrooms are the source of biologically active metabolites which shows anticancer properties at low level of side effects. Previous studies reported that bioactive metabolites isolated from mushrooms provide favorable effects in controlling and preventing development of tumors (de Silva et al., 2012b; Petrova, 2012; Xu et al., 2012a).

Some Basidiomycetes like *Trametes versicolor*, *Ganoderma lucidum*, *Lentinus edodes*, *Grifola frondosa* are clinically used for cancer treatment in Asian countries (Mizuno et al., 1995; Wasser et al., 1997). Activity of these mushrooms is generally related to the presence of polysaccharides which are considered as 'Biological Response Modifiers' (BRM).

In 2004, Thomasi et al., tested the crude methanol extracts of Basidiomycetes fruiting bodies for their ability to inhibit the *in vitro* the growth of two murine cancer cell lines: L1210 (lymphocytic leukemia) and 3LL (Lewis lung carcinoma). IC₅₀ values were compared with the methanol extract of the bark of *Taxus baccata*. Against both the cell lines fungal extract shown to possess less IC₅₀ than that of 100 mg/mL. According to the standards of the National Cancer Institute (NCI), a crude extract may be considered significantly cytotoxic with IC₅₀ ≤ 20 mg/mL (Cordell, 1993). The extracts from *Suillus granulatus* and *Ganoderma lucidum* were more active and exhibited a significant cytotoxic activity against both cell lines, IC₅₀ L1210 = 4.7

mg/mL; IC_{50} 3LL = 6.8 mg/mL and IC_{50} L1210 = 15 mg/mL; IC_{50} 3LL = 10 mg/mL respectively.

There is some promising evidence, indicating that the consumption of some fungal extracts helps in protecting against certain cancers especially breast and gastrointestinal (Kim et al., 2002; Hara et al., 2003). Johnston (2005), reported that *Ganoderma lucidum* is used in TCM (Traditional Chinese Medicine) for the treatment of cancers. Stanley et al., 2005 conducted studies in prostate cancer cells PC-3 and reported that *G. lucidum* induced apoptosis and thereby inhibit the proliferation of cells. Lanostane-type triterpenoids, which was reported in *Fomitopsis officinalis* extract, showed anticancer activity (Wu et al., 2009). It was found to inhibit eukaryotic DNA polymerase, a feature which allows it to be a cytotoxic agent and helpful as a cancer chemotherapeutic agent (Akihisa et al., 2004).

Wu et al. (2014) reported that the ethanol extracts of *F. officinalis* exhibit stronger anticancer activities than that of water extracts toward human breast cancer (MDA-MB-231) cells, hepatoma (HepG2), colon cancer (HCT-116), lung cancer (A549), and mouse sarcoma 180 cells (S-180).

Table 2: mushroom species with effect on different types of cancer

Mushrooms	Cancer type	Reference
<i>Grifola frondosa</i>	Liver cancer Breast cancer	Lin et al., 2016 Soares et al., 2011
<i>Lentinus crinitus</i>	Breast cancer	Arora et al., 2014
<i>Lentinula edodes</i>	Lung cancer	Liu et al., 2012
<i>Pleurotus ferulae</i>	Gastric cancer	Wang et al., 2014
<i>Termitomyces clypeatus</i>	Brain, lung, breast and ovary cancer	Mondal et al., 2016
<i>Ganoderma lucidum</i>	Liver cancer	Yang et al., 2013
<i>Fomes fomentarius</i>	Lung cancer	Kim et al., 2015
<i>Inonatus obliquus</i>	Colorectal cancer	Kang et al., 2015
<i>Phellinus linteus</i>	Colon cancer Liver cancer	Song et al., 2011 Yang et al., 2013
<i>Pleurotus pulmonarius</i>	Liver cancer	Xu et al., 2012b
<i>Fomitopsis officinalis</i>	Breast, colon and lung cancer	Wu et al., 2014

e. Apoptosis

Apoptosis is a type of programmed cell death in which unwanted cells are eliminated to maintain homeostasis. Cell proliferation and apoptosis are two related conditions and are important for regulating tumor growth. Mostly studies are focused on bringing cell death *via* apoptosis so that cellular contents could be eliminated from the system without spillage. The process of apoptosis is represented as follows (**Fig. 5**):

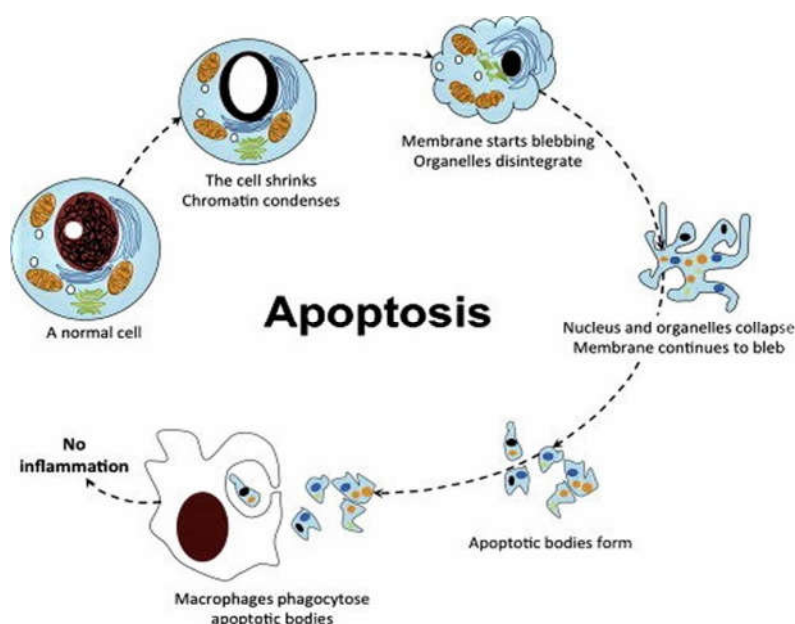


Fig. 5: Mechanism of apoptosis
[Reference: Abou-Ghali & Stiban, 2015]

Suppressed cell death and dysregulated cell proliferation together provide underlying platform for the neoplastic progression (Evan & Vousden, 2001). According to Tu et al. (1996) loss of apoptotic control in favor of cell proliferation is responsible for prostate cancer initiation and progression. Lesions suppress the rate of apoptosis in tumor cells. Thus, targeting these lesions is the major strategy in cancer treatments. It has been found that many cancer chemotherapy drugs exert anticancer effects on malignant cells by inducing apoptosis. In a study by Oh et al. (2008) demonstrated that the extracellular polysaccharide peptide complexes (PPC) obtained by submerged mycelial culture of *Cordyceps sphecocephala* strongly inhibited the growth of human hepatocarcinoma (HepG2) and human neuroblastoma (SK-N-SH)

cells by promoting cell apoptosis involving the expression of apoptosis related proteins.

According to Kobayashi et al. (1998), one of the major genes that regulate apoptosis is the proto oncogene Bcl-2., Bax death promoting molecule which counteracts the anti-apoptotic effects of Bcl-2. The ratio of Bcl-2 to Bax, rather than the levels of individual proteins are considered to be critical in determining the survival or death of cells (Fukamachi et al., 1998; Oltvai et al., 1993).

In cells undergoing apoptosis, there is an activation of proteases known as caspases, which have an obligatory cysteine residue with the active site and cleaves peptides adjacent to an aspartic acid residue (Thornberry, 1997). Caspase cascade has been extensively demonstrated to be involved in apoptotic signal transduction and execution (Budihardjo et al., 1999; Salvesen & Dixit, 1997). Human caspases 1-10 have been described and activation of the caspase cascade is involved in chemical induced apoptosis (Martin & Green, 1995). Inactive proenzymes contain caspases which are activated by proteolytic cleavage.

There are two major pathways involved in apoptosis viz., intrinsic and extrinsic (**Fig. 6**). Each pathway is coordinated by several apoptotic factors including caspases and cytochromes.

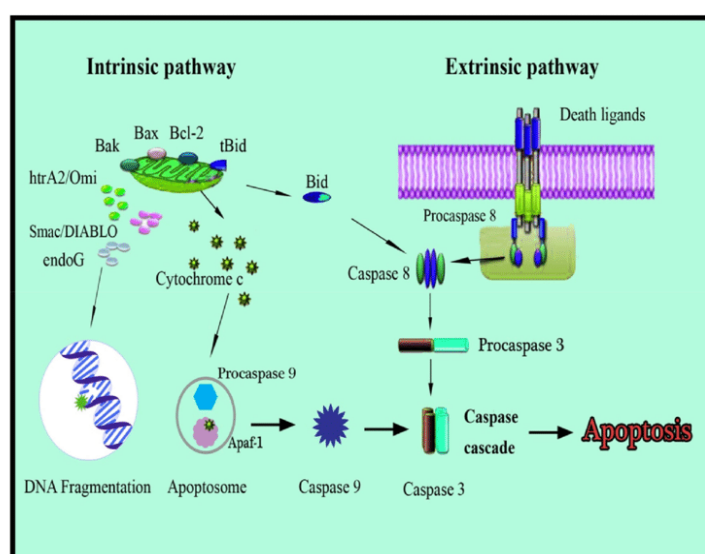


Fig. 6: Intrinsic and extrinsic pathway of apoptosis
[Reference: Badalzadeh et al., 2015]

Cancer cell lines

Normal cells follow a typical cycle growth, division and death. While cancer cells, don't follow this pattern. Cancer cells multiply and continue to reproduce other abnormal cells. Cancer cell lines widely used to study different types of cancer. These cell lines can invade breast, liver, lungs, pancreas etc., of human body.

Table. 3 types of cancer cell lines

Cancer cell lines	Cancer type	References
H157, H226, H358, H661, H460, H322, H187, N417	Lung cancer	Viallet et al., 1996 Kessler et al., 2007
MCF-7, MDA-MB- 435, B-Cap37, MDA-MB231, SKBr-3, BT-474, MCF7/HER2-18	Breast cancer	Liu et al., 1996 Staveren et al., 2009 Kobayashi et al., 1999
PC-3, DU 145, LN CaP, TSU-Pr1, LAPC4, C4-2b, 22Rv1	Prostate cancer	Li et al., 2004 Polkowski et al., 2004 Brussel et al., 1999 Siddique et al., 2011
LoVo, Colo-205, WiDr, DLD1, HCT 116, HT-29, HCT-15, COLO 320DM,	Colorectal cancer	Csernus et al., 1999 Polkowski et al., 2004 Akutsu et al., 1995 Chen et al., 1995 Tarapore et al., 2013 Farah et al., 2003 Awad et al., 1996 Baskar et al., 2010
H-4-II-E, HepG2, SK-HEP-1, Hep3B, MHCC-LM3, HLF, KYN-1, KYN-2, KYN-3, HAK-1A, HAK-1B, HAK-2, HAK-3, HAK-4, HAK-5, HAK-6	Liver cancer	Watjen et al., 2007 Selvendiran et al., 2006 Lee et al., 2011 Oh et al., 2006 Li et al., 2010a
SGC-7901, KATOIII, EPG85-257RDB, EPG85 -257RNOV, AGS, MKN-28, MKN-45	Gastric cancer	Drag et al., 2009 Zhao et al., 2009 Wu et al., 2008 Zhang et al., 2009 Kobayashi et al., 1999
GBC-SD	Gall bladder cancer	Lee et al., 2011

SW-1990, CAPAN-2, CfPAC1, AsPC1, Mia PaCa-2	Pancreatic cancer	Zhou et al., 2010 Miyazawa et al., 2011 Csernus et al., 1999 Morrissey et al., 2005
A2780, OV-1063, OAW28, OAW41M, OAW42, OAW59M, HO-8910, MCAS, TYK-Nu	Ovarian cancer	Król et al., 2015 Turner et al., 1995 Chen et al., 2003 Treeck et al., 2005 Kawakami et al., 2000 Csernus et al., 1999
GOTO, NB-1, SK-N-AS	Neuroblastoma	Rzeski et al., 2009 Król et al., 2015 Mullauer et al., 2009
SF295	Brain cancer	Oliveira et al., 2007
U937, K562, Jurkat E 6.1, HL-60	Leukemia	Król et al., 2015 Rzeski et al., 2009 Li et al., 2010a Park et al., 2007
HeLa, SiHa	Cervical cancer	Liu et al., 2007 Wang et al., 2006
MDA1986, TU159, SCC-4, HSC-3, HSC-4, Ca9-22, KB, Ho-1-u-1	Oral cancer	Lee et al., 2007b Yang et al., 2008 Sato et al., 1997 Otsubo et al., 2007

According to Kishida et al. (1998), DLD1 is the colorectal adenocarcinoma cell line that shows a truncated adenomatous polyposis coli (APC) protein. It has been isolated from the large intestine of a colon adenocarcinoma patient which displayed epithelial properties such as a cobblestone cell shape and can be used in cancer research. These DLD1 cells are adherent, with a doubling time of approximately 20 hours.

MTT assay is widely used to characterize the cell viability. It is used to measure the viability of cells. In this assay, yellow tetrazolium salt is reduced to the violet-blue formazan compounds with the activity of succinate dehydrogenase enzyme present in mitochondria. The absorbance of formazan solution obtained after

solubilizing formazan crystals in an organic solvent like DMSO can be measured spectrophotometrically at 570 nm (Stockert et al., 2018).

f. Neuroprotective effects:

Neurodegenerative conditions are most common in aged people. Most common neurodegenerative disorders include Parkinson's disease, amyotrophic lateral sclerosis and Alzheimer's disease (Brown et al., 2005). Memory loss and a lack of neuronal function are most important symptoms. These disorders are increasing day by day. Developing treatments for these conditions remains challenging with the forthcoming increase in neurodegenerative disorders (Khan et al., 2020).

Genetic risk factors play a role in neurodegeneration's causes, such as the aggregation of toxic proteins and mitochondrial dysfunction (Meek et al., 1998; Khan et al., 2020). The common risk factor is age for these disorders. Other possible components may include oxidative stress, neuroinflammation, vitamin deficiencies, lifestyle behaviors, diabetes, hypertension, stroke and other pre-existing conditions (Brown et al., 2005).

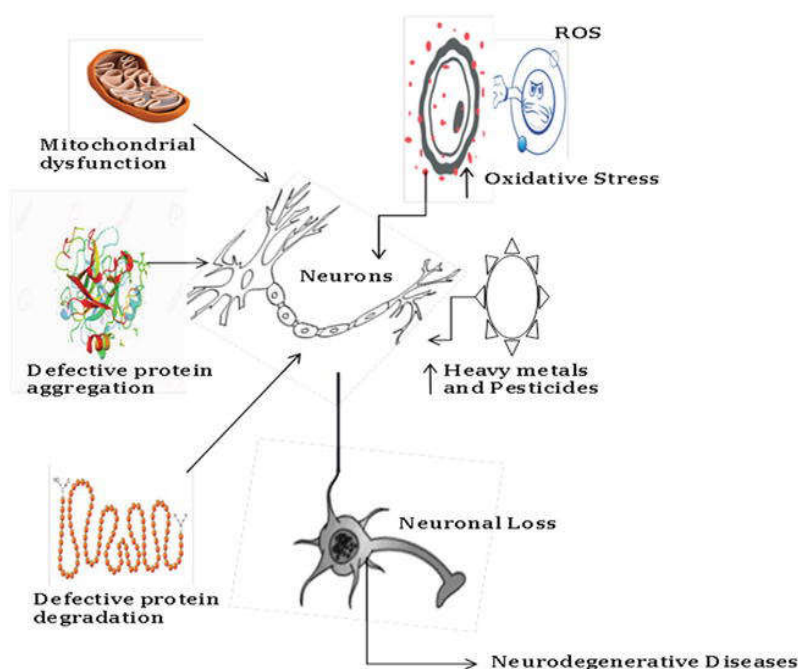


Fig. 7: Different causes of neurodegeneration

[Reference: Patel & Mandal, 2019]

Number of environmental factors are included in the process of neurodegeneration. Cellular homeostatic disruption can happen due to various stress, which ultimately leads to changes in normal cellular function and finally results in cell death.

The mechanism of cell death and dysfunction occurring in neurodegenerative diseases are represented in **Fig. 8**.

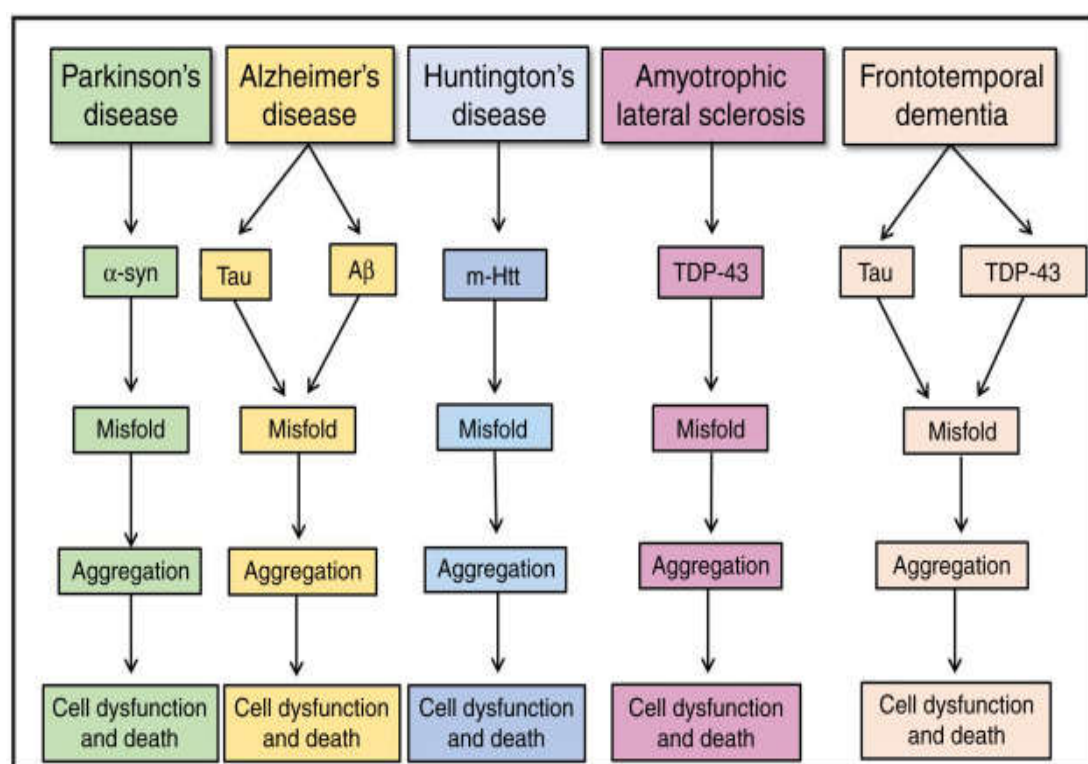


Fig. 8: Mechanism of cell death and cell dysfunctioning

In normal conditions process of autophagy is in low level. According to Adinew et al. (2021), autophagy is controlled by numerous pathways targeting multiple disease states, including viral and bacterial infections, cancer, diabetes and neurodegenerative diseases. Under stressful conditions autophagy is activated to eliminate excess damaged cellular proteins and organelles. It is the report in case of several neurodegenerative diseases. Autophagic dysfunction contributes mainly to these disorders.

Recently, flavonoids have been investigated for their anti-inflammatory and antitumor properties and their connection to autophagy (Zhang et al., 2018; Zhang et al., 2013). Some beneficial effects of flavonoids in oncology and pathology are commonly coupled with their activity on autophagy and non-apoptotic cell death (Li et al., 2008). Multiple articles showed that flavonoids could induce autophagy both, *in vivo* and *in vitro*, resulting from their antioxidant activity in proteosomes and mitochondrial endoplasmic reticulum (Hasima & Ozpolat, 2014). Earlier studies show flavonoids to possess antibacterial and antiviral activities which in turn promotes the activation of autophagy.

Neuroinflammation and oxidative stress can initiate neurodegeneration. Neuroinflammation is an inflammatory response of the central nervous system (CNS) caused by complex immune responses to injury in the brain (Brown et al., 2005; Khan et al., 2020; Meek et al., 1998). These injuries lead to the stimulation of glial cells and the secretion of inflammatory cytokines, which cause complications associated with neurodegenerative disorders (Disabato et al., 2016). Oxidative stress is generated from numerous factors, such as abnormal protein accumulation, high trafficking of calcium ions across the neurons, disturbance in peroxidation, and polyunsaturated fatty acid homeostasis (Cobley et al., 2018). The activation of multiple biochemical pathways induces damage to proteins, DNA and lipids, resulting in the death of neuronal cells (Guo et al., 2014).

Studies have shown that the expression and activity of Nrf2 are reduced in both aged mice and humans (Duan et al., 2010; Suh et al., 2004). Since oxidative stress, mitochondrial dysfunction, and inflammation are characteristics of the aging brain and aging is the leading risk factor of neurodegeneration, Nrf2 is an appealing target for clinical medication (Brandes & Gray, 2020).

According to Chen et al. (2017), one of the most common (60–75%) forms of dementia is Alzheimer's disease (AD). Accumulation of amyloid-beta ($A\beta$) is the characteristic feature of AD. Amyloid-beta is a long polypeptide which contain 37–49 amino acids. It commonly seen in the external part of neurons in the brain. Some metabolic defects such as hyperglycemia, insulin resistance, dyslipidemia, increased

biomarkers of inflammation, chronic neuroinflammation, and oxidative stress can cause AD.

Alpha synuclein is a protein with 140 amino acids. It is encoded by the gene (SNCA gene) that provides instructions for the formation of a small protein called alpha- synuclein. These are mainly seen in neural tissue. It is a member of the protein family of synuclein which includes β -Synuclein and γ -Synuclein. Presynaptic nerve terminals of neurons are characterized by the presence of alpha synuclein. Alpha synuclein is a major constituent of Lewy Bodies. This protein has an important role in dopamine metabolism (Nimmo et al., 2021). According to Nimmo et al. (2021), amyloid beta and alpha-synuclein are the two key proteins involved in the pathogenesis of these neurodegenerative diseases.

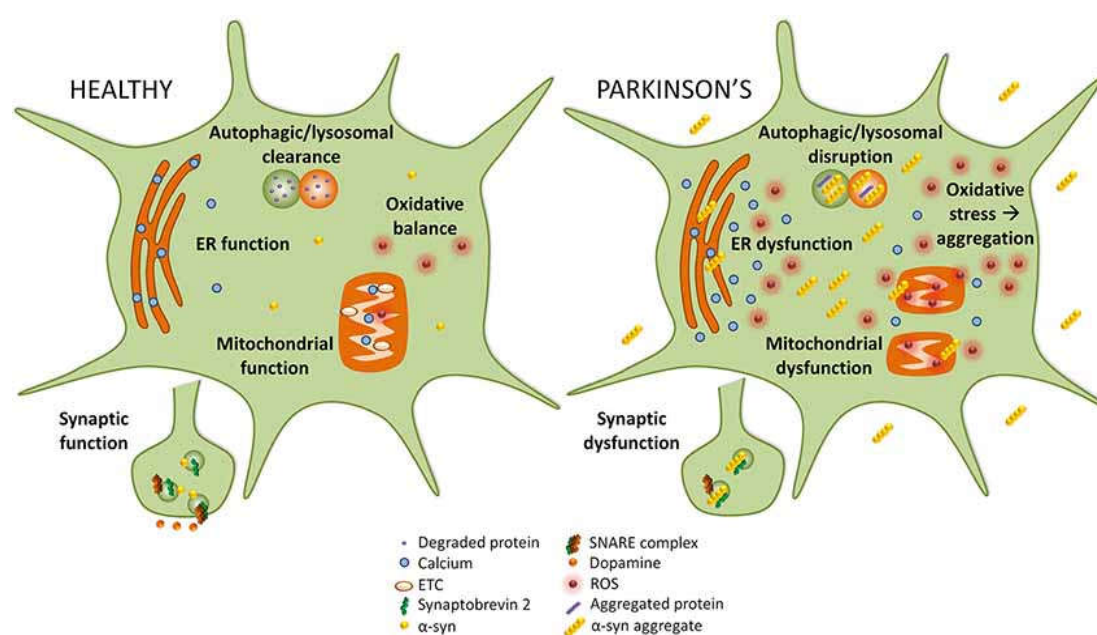


Fig. 9: Comparative changes in a healthy man and a man with Parkinson's disease [Reference: Fields et al., 2019]

The effect of some compounds isolated from mushrooms, such as muscarine, muscimol and ibotenic acid, on the nervous system has been studied (Yavorsky & Lukyanetz, 1997). Neurotrophic effects of extracts of *H. erinaceus* (Grygansky et al., 2001; Moldavan et al., 1999a), *Amanita sp.* (Moldavan et al., 1999b; Moldavan et al., 2000a), *Lentinus edodes* (Moldavan et al., 2001a; Moldavan et al., 2002),

hallucinogenic mushroom *Psilocybe cubensis* (Moldavan et al., 2000b) and other higher Basidiomycetes (Moldavan et al., 1999c; Moldavan et al., 2001b) have also been reported.



Materials and Methods

MATERIALS AND METHODS

The present work has been designed to study mycochemical characterization and bioactive screening of *Microporus xanthopus* (Fr.) Kunt. and *Microporus affinis* (Blume & T. Nees) Kunt. The experimental procedures and protocols followed throughout the entire study are presented in this section. Mycochemical characterization was carried out by qualitative and quantitative estimation as well as with HR-LC/MS and GC/MS techniques. Bioactive screening was mainly focused on the antioxidant, antimicrobial, cytotoxic, antiproliferative and neuroprotective potential of two species of *Microporus*. Special emphasis was given to the neuroprotective effects of *M. xanthopus*.

1. FUNGAL MATERIALS

Two species of *Microporus* (*M. affinis* and *M. xanthopus*) were selected for the present study. Fruiting bodies of both the species were collected from the premises of Pookode lake, Wayanad; Thusharagiri wild life sanctuary, Kozhikode; Iringole kavu (near Perumbavur), Ernakulam; Kava (behind Malampuzha), Palakad; Paithalmala, Kannur and Sky walk area (premises of Calicut University campus), Malappuram, Kerala. Collected specimens were authenticated by Dr P. Manimohanan, Professor (Retd), Department of Botany, University of Calicut and Dr Arun Kumar T. K., Assistant Professor, Department of Botany, Zamorin's Guruvayurappan College, Kozhikode.

Description of fungal specimens collected

Among the 12 species of *Microporus* (accepted by Index fungorum as on 2017), two species were selected.

Division	:	Basidiomycota
Class	:	Basidiomycetes
Order	:	Polyporales
Family	:	Polyporaceae
Genus	:	<i>Microporus</i>

a). *Microporus xanthopus* (Fr.) Kunt.

M. xanthopus (**Plate 1**) is commonly called yellow footed polypore.

It is mainly distributed in moist deciduous to evergreen forests and occurs solitary or in large clusters on decaying wood, branches and twigs. The term xanthopus is derived from two Greek words and literally means ‘yellow foot’. The mature fruiting bodies have thin, funnel shaped caps that are concentrically zoned in various shades of brown. Mushroom cap 4-9 cm wide, dark brown, violet brown at disc, brownish orange towards margin, shiny, concentrically sulcate, radially fibrillose with concentric zonation. Margin of cap is thin and wavy. The lower surface is white to pale brown in colour and is covered with numerous minute pores (6-8 pores per mm), hence the general name polypore. Pore surface off white becomes brownish orange. Pores are round, smooth and shiny. Stipe is central to excentric, yellowish white, round, slightly expanded upwards and expanded to a disc like base. Stipe is 4-10 cm long and 2-6 cm wide.

b). *Microporus affinis* (Blume & T. Nees) Kunt.

In 1826, German botanists Carl Ludwig Blume and Theodor Nees described first it as a species of *Polyporus*. Otto Kuntze transferred it to *Microporus* in 1898. It is a widespread polypore that is common in tropical and subtropical regions of both the Northern and Southern hemispheres. Usually members grow in groups on fallen wood.

M. affinis (**Plate 2**) looks like a tiny *Ganoderma* species, but it has thinner context than *Ganoderma*. It is found to be growing gregariously and sometimes the caps are fused with each other.

Fruiting body is thin and leathery. Cap is 1-5 cm in size. Upper surface is dry with a velvety texture due to an irregular covering of fine hairs. It is characterized by the presence of approximately parallel bands of purple, brown, cream or black of varying widths with the outer margin white. Lower surface is white to fawn and finely pored (3-5 pores per mm). Stipe is up to 2 cm long, hard and brown to reddish often black coloured. It has lateral stipe. *M. affinis* is distinguished from *M. xanthopus* by

PLATE 1



Microporus xanthopus (Fr.) Kunt.: a & b. habitat; c & e. dorsal view; d. yellow foot; f. ventral view showing micropores

PLATE 2



Microporus affinis (Blume T. Nees) Kunt.: a. habitat; b. dorsal view; c. ventral view showing micropores

its lateral stipe which never has the yellow disc. Pores are thick walled, squarish to oval shaped, white or pale pink in colour. Spore print is white. The literature suggests that spores are colourless and do not stain in iodine solution. Flesh is tough and leathery white. From literature, *M. affinis* is found to be edible.

2. METHODOLOGY

For the convenience, work has been divided into three phases.

Phase I: Mycochemical characterization

Phase II: Bioactivity screening

Phase III: Detailed study on neuroprotection in *M. xanthopus*

PHASE I: MYCOCHEMICAL CHARACTERIZATION

To detect the presence of metabolites in the methanolic extract, different standard qualitative tests were used. For the determination of non-volatile and volatile compounds in the fungal extract, chromatographic techniques like HR-LC/MS and GC/MS were used.

a). Qualitative mycochemical screening

The entire fruiting body was used for the present study. For the preparation of extract, collected specimens were shade dried and powdered using blender and stored in moist free sealed containers for further use. For preliminary mycochemical screening, 3 different extracts were prepared using the solvents like hexane, methanol and distilled water. Extracts were prepared by mixing 10 g of powder of fungal material with 100 mL of each solvent separately and were allowed to stand overnight. Then these extracts were filtered using Whatman filter paper No.1 and the filtrates thus obtained were used for qualitative mycochemical analyses.

The 3 different extracts of fungal materials were subjected to preliminary mycochemical analyses to detect the presence of bioactive secondary metabolites using standard protocols (Sofowara, 1993; 2005; Harborne, 1998).

i. Tests for alkaloids

- **Wagner's test**

A few drops of Wagner's reagent (Appendix 1) are added to the test tube containing 1 mL of the extract. The formation of reddish-brown precipitate indicates the presence of alkaloids

- **Hager's test**

To 1 mL of the extract, Hager's reagent (saturated picric acid solution) is added. The formation of an orange-yellow coloured precipitate indicates a positive test.

ii. Tests for flavonoids

- **Alkaline reagent test**

One mL of the extract was treated with a few drops of NaOH solution. Formation of an intense yellow colour which disappears on the addition of dilute HCl indicates the presence of flavonoids.

- **Lead acetate test**

A few drops of lead acetate solution were added to 1 mL of the extract. Precipitate with yellow colour indicates the presence of flavonoids.

iii. Test for phenolic compounds

- **FeCl₃ test**

2 mL of the extract was treated with a few drops of Ferric chloride solution. Formation of blue-black colour indicates the presence of phenols.

iv. Test for glycosides

- **Keller Killiani test**

Five mL of the extract was treated with 2 mL of glacial acetic acid and a drop of FeCl₃ was added to it. It was underlaid by 2-3 drops of concentrated H₂SO₄. Glacial

acetic acid containing 1% (w/v) FeCl_3 gives a brown ring in the presence of 2-deoxysugar in the glycone portion the mycochemical.

v. Test for saponins

- **Foam test**

About 2 mL of the extract is diluted with 2 mL of distilled water. The suspension was shaken well and observed for a stable persistent froth. Formation of a persistent layer of foam indicates the presence of saponins.

vi. Test for steroids

- **Leibermann- Burchard test**

The extract was mixed with 1 mL of chloroform and 3 mL of acetic anhydride. Concentrated H_2SO_4 was added to the mixture along the sides of test tube to form a layer underneath. Formation of blue-green colour indicates the presence of steroids.

vii. Test for tannins

- **Braymer's test**

About 2 mL of the extract is treated with 10% alcoholic FeCl_3 solution. Development of blue green colour indicates the presence of tannins.

viii. Test for terpenoids

- **Salkowski test**

Three mL of chloroform was added to 5 mL of the extract and mixed well. Concentrated H_2SO_4 was added to this mixture along the sides of the test tube to form a layer.

b). Quantitative mycochemical screening

✓ **Preparation of extract:**

Methanolic extract showed higher amount of precipitation as well as colour intensity in most preliminary mycochemical tests among the three different fungal

extracts prepared. It indicates the greater quantity of secondary metabolites and also exhibited maximum number of chemical constituents. Thus, methanol was selected as the solvent for the preparation of fungal extracts for all further studies.

For a duration of 10 hours, 30 g of powdered samples were subjected to Soxhlet extraction using methanol as a solvent. The methanolic extract thus obtained was cooled, filtered through a Whatman No. 1 filter paper and concentrated by removing entire solvent. For further experiments, final extract was stored in amber coloured glass bottles at 4°C.

Among the major chemical constituents revealed from qualitative mycochemical screening, alkaloids, terpenoids, total carbohydrate and proteins were estimated using standard protocols as described below.

i. Estimation of alkaloids:

The total alkaloid estimation was performed by spectrophotometric method of Dragendorff's reagent (Appendix 2) as it was described by Sreevidya & Mehrotra (2003). Caffeine was used as the standard. For stock preparation, 1 mg of each extract was accurately weighed and made up to 1 mL with DMSO. 0.5 mL of samples were mixed with 1 mL of 0.5 N HCl. Then 0.25 mL of Dragendorff's reagent was added to this mixture for precipitation and the precipitate was centrifuged over 5 minutes at 3000 rpm. This precipitate was further washed with 0.25 mL of ethanol. The filtrate was discarded and the residue was then treated with 0.25 mL of disodium solution (1%). The brownish black precipitate formed was then centrifuged for 5 minutes at 3000 rpm. This residue was dissolved in 0.2 mL of concentrated nitric acid and 0.1 mL was then pipetted out and mixed with 0.5 mL of thiourea solution (3%). The absorbance of this solution was measured at 435 nm using UV-visible spectrophotometer (Agilent, Cary 60) against a blank containing 0.1 mL of concentrated nitric acid and 0.25 mL of thiourea solution (3%) and the total alkaloid content of the extracts were calculated and expressed in milligram caffeine equivalent per gram of dry weight (mg CE/g DW). All the samples were analysed in triplicates.

ii. Estimation of terpenoids:

The total terpenoid content was determined by spectrophotometric method using the following procedure (Biswas et al., 2021). Lupeol was used as the standard.

One mg of extract was dissolved in 1 mL of methanol. Then, 100 μ L of each of these solutions was mixed with 150 μ L of vanillin – glacial acetic acid solution (Appendix 3) and 500 μ L of perchloric acid solution (Appendix 4). The sample solutions were heated for 45 min at 60°C and then cooled in an ice – water bath to ambient temperature. After the addition of glacial acetic acid (2.25 mL), each sample solution's absorbance was measured at 548 nm using a UV-Visible spectrophotometer. The calibration curve was plotted with the concentrations of lupeol as 100, 200, 400, 800 and 1000 μ g/mL. The total terpenoid content of the extract was expressed in milligram lupeol equivalent per gram of dry weight (mg LE/g DW). All the samples were analysed in triplicates.

iii. Estimation of total protein:

The total protein estimation was done by Lowry's method. Bovine Serum Albumin (BSA) was used as the standard. Protein solution was prepared by dissolving 50 mg of BSA in distilled water and made up to 50 mL. 0.1 mL of the sample was taken and made up to the volume of 1 mL. Then 5 mL of alkaline copper solution (Appendix 5) was added to each test tube including the blank (water), mixed well and incubated at 37°C for 3 minutes. Then 0.5 ml of Folin-Ciocalteu reagent (Appendix 6) was added, mixed well and incubated at 37°C for 3 minutes. Absorbance was measured at 660 nm. A standard graph was plotted and calculated the amount of protein in the sample.

iv. Estimation of total carbohydrate:

The estimation of total carbohydrates is done by phenol sulphuric acid method. Glucose was used as the standard. To 500 μ L of the sample, 1 mL of phenol solution was added. To this solution, 5 mL of 96% sulphuric acid was added and shaken well. After 10 minutes, it was placed in water bath at 25-30°C for 20 minutes. After cooling,

the absorbance was taken at 490 nm. The amount of total carbohydrate present in the sample was calculated using the standard graph of glucose.

c). High Resolution - Liquid Chromatography/ Mass Spectrometry (HR-LC/MS) analysis

Identification of non-volatile components in the methanol extract of selected species of *Microporus* was done with HR-LC/MS analysis. Chromatographic separations were performed using HR-LC/MS Q-TOF (Agilent, USA) equipped with an electrospray ionization source maintained at 40⁰ C. Column employed for separation is G4226A, 4.6 mm × 250 mm dimensions with a particle size of 5 μm. The gradient flow was at the rate of 0.3 mL/min. The elution was performed using a mobile phase consisting of 95% water and 5% acetonitrile. Injection volume was 3 μL and the total run time was 30 min. The eluted compounds were detected with MS Q-TOF equipped with an electrospray ion source in positive ion modes using nebulizer gas as nitrogen 13 L/min; gas temperature at 250⁰ C and nozzle voltage as 1000 V.

d). Gas Chromatography/ Mass Spectrometry (GC/MS) analysis

The volatile compounds present in the methanolic extracts were mainly evaluated by using GC/MS (Shimadzu QP-2010 plus) analysis with a thermal desorption system TD 20, fitted with 60 m × 0.25 mm × 0.25 m WCOT column coated with diethylene glycol (AB-Innowax 703142, Japan). Helium was used as the carrier gas at a flow rate of 1.2 mL/min at a column pressure of 77.6 kPa. The temperature of injector and detector were maintained at 260⁰ C. Samples (8 μL) were injected into column with a split ratio of 10:0. Separation of the components was achieved with a linear temperature program of 70-260⁰ C at 3⁰ C/min and then held at 260⁰ C for 6 min. The total run time was 40-45 min. The parameters used for Mass Spectrometry were electron ionisation (EI) voltage 70 eV, peak width 2 s and detector voltage 1.5 V. The constituents were identified by comparison of their linear retention indices. The MS pattern was checked with pure compounds of known composition and compared with MS fragmentation patterns of National Institute of Standards and Technology (NIST) libraries and quantification was done by using percentage peak area calculation.

PHASE II: BIOACTIVITY SCREENING

a) Free radical scavenging assays

Extract stock preparation

To prepare stock solution for antioxidant activity of the methanolic extracts, 10 mg of extract was dissolved in 1 mL of DMSO. From the stock, different concentrations like 125, 250, 500, 1000 and 2000 µg/mL were prepared for the assays such as nitric oxide free radical scavenging activity, hydroxyl radical scavenging assay and reducing power activity. The concentrations like 12.5, 25, 50, 100 and 200 µg/mL were taken for DPPH assay. In reducing power assay, the absorbance itself is considered as a percentage of inhibition. While the inhibition percentage in all the three assays were calculated as follows:

$$\text{Inhibition (\%)} = \frac{Ac - As}{Ac} \times 100$$

Where 'Ac' and 'As' are the absorbance values of control and sample respectively. Each extract was tested in triplicates and values expressed in mean ± SE.

i. DPPH scavenging assay

The radical scavenging activity of different extracts was determined by using DPPH assay according to Chang et al. (2001). 0.1 mM DPPH solution was prepared by dissolving 4 mg of DPPH in 100 mL of methanol. Ascorbic acid (10 mg/mL DMSO) was used as standard. Different concentrations of sample such as 12.5 µg/mL - 200 µg/mL from stock solution of 10 mg/mL were made up to a final volume of 20 µL with DMSO and 1.48 mL DPPH (0.1 mM) solution was added. A control without the test compound, but an equivalent amount of distilled water was taken. The reaction mixture was incubated in dark at room temperature for 20 minutes. Then the absorbance of mixture was taken at 517 nm. 3 mL of DPPH was taken as control. The percentage of inhibition was calculated using the above-mentioned formula.

ii. Hydroxyl radical scavenging assay

For this assay, gallic acid was taken as the standard. Different concentrations of sample such as 125 – 2000 μL were mixed with 500 μL reaction mixture [2-Deoxy 2 ribose (2.8 mM), FeCl_3 (100 μM), EDTA (100 μM), H_2O_2 (1.0 mM), ascorbic acid (100 μM) in KH_2PO_4 - KOH buffer (20 mM of pH 7.4)] and was made up to a final volume of 1 mL (Elizabeth & Rao, 1990). Distilled water without test compound was taken as the control. After incubation for 1 hour at 37°C , added 1 mL of 2.8% TCA, then 1 mL 1% aqueous TBA was added and the mixture was incubated at 90°C for 15 minutes to develop the colour. After cooling the absorbance was measured at 532 nm against an appropriate blank solution. The percentage of inhibition was calculated using the above-mentioned formula.

iii. Nitric oxide radical scavenging activity

The determination of nitric oxide free radical scavenging activity was done by using the method of Kumaran & Karunakaran (2006). Sodium nitroprusside (5 mmolL^{-1}) in phosphate buffered saline (Appendix 7) with pH 7.4, was mixed with different concentrations of sample such as 125 $\mu\text{g/mL}$ - 2000 $\mu\text{g/mL}$ and incubated at 25°C for 30 minutes. Distilled water without test compound was taken as the control. After 30 minutes, 1.5 mL of the incubated solution was removed and diluted with 1.5 mL of Griess reagent (Appendix 8). Absorbance was measured at 546 nm and the percentage of scavenging activity was measured with reference to the standard as gallic acid.

iv. Reducing power activity

The reducing power of the extract was determined by the method of Yen & Duh (1993). Quercetin (10 mg/mL of DMSO) was used as a standard. Different concentrations of sample such as 125 $\mu\text{g/mL}$ -2000 $\mu\text{g/mL}$ were mixed with 2.5 mL of phosphate buffer (200 mM) with pH 6.6 and 2.5 mL of 1% potassium ferric cyanide was added, then boiled for 20 minutes at 50°C . Distilled water without test compound was taken as the control. After incubation, 2.5 mL of 10% TCA were added to the mixtures followed by centrifugation at 3000 rpm for 10 minutes. The upper layer (5

mL) was mixed with 5 mL of distilled water and 1 mL of 0.1% ferric chloride was added and absorbance was taken at 700 nm.

b). Cytotoxicity by *Allium cepa* assay

Germinated bulbs with roots having 1-2 cm length were collected at the peak mitotic period (09 - 09.30 am) and washed thoroughly with distilled water. Roots of germinated bulbs were treated with varying concentrations (5 µg/mL, 10 µg/mL, 50 µg/mL and 100 µg/mL) of the extracts taken in bottles, in such a way that only the roots were immersed in the solution. Root tips were cut from bulbs placed in the samples of each concentration at different time intervals such as ½ h, 1 h, 2 h, 3 h and 24 h. Hydrogen peroxide and distilled water were taken as the positive and negative control respectively.

The treated root tips were washed thoroughly with distilled water and immediately fixed in modified Carnoy's fluid (Appendix 9) for a period of 1 h. With the help of improved techniques (Sharma & Sharma, 1990), mitotic squash preparation was done. After 1 h of fixation, root tips were washed with distilled water and hydrolysed using 1N HCl for 5 min to separate the cells during squashing. Then it was washed and stained with 2% acetocarmine (Appendix 10) for 3 h. Destaining was done by using 45% acetic acid. Finally, it was squashed and mounted on clean glass slides.

Mitotic index and percentage of abnormality were calculated using the following formulas:

$$\text{Mitotic index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$$

$$\text{Abnormality percentage} = \frac{\text{Number of aberrant cells}}{\text{Total number of cells}} \times 100$$

c). Antiproliferative efficacy of *Microporus*

For antiproliferative studies, two cell lines were selected:

- Human colorectal adenocarcinoma cell line (DLD1)
- Normal fibroblast cells (L929)

i. *In vitro* anticancer activity on DLD1 cell line using MTT assay

DLD1 (Human Colorectal Adenocarcinoma) cells were initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained in DMEM medium (Appendix 11). The cell line was cultured in 25 cm² tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate (Merck, Germany) and 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). The viability of cells was evaluated by direct observation of cells using Inverted phase contrast microscope and followed by MTT assay.

Cells seeding in 96 well plate:

Two days old confluent monolayer of cells was trypsinized and the cells were suspended in 10% growth medium. The 100 µL cell suspension was seeded in culture plate with 96 well and incubated at 37°C in a humidified 5 % CO₂ incubator.

Preparation of the extract and anticancer evaluation:

One mg of sample was weighed and dissolved in 1 mL DMEM using a cyclomixer. The sample solution was filtered through Millipore syringe (0.22 µm) filter to ensure the sterility.

After 24 hours, the growth medium was removed and different concentrations (100 µg, 50 µg, 25 µg, 12.5 µg and 6.25 µg in 500 µL of 5% DMEM) were prepared. From each concentration, 100 µL were added in triplicates to the respective wells and incubated at 37°C in a humidified 5% CO₂ incubator. Non-treated control cells were also maintained.

Anticancer assay by direct microscopic observation:

Entire plate was observed after 24 hours of treatment in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observations were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of cells were considered as indicators of cytotoxicity.

Anticancer assay by MTT method:

Fifteen mg of MTT (Sigma, M-5655) was dissolved in 3 mL PBS and sterilized by filter sterilization. After 24 hours of incubation period, the sample content in wells were removed and 30 μ L of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37°C in a humidified 5% CO₂ incubator for 4 hours. After the incubation period, the supernatant was removed and 100 μ L of MTT solubilization solution (Dimethyl sulphoxide, DMSO - Sigma Aldrich, USA) was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance was measured by using microplate reader at a wavelength of 540 nm (Talarico et al., 2004).

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ of viability} = \frac{\text{Mean OD of sample}}{\text{Mean OD of control}} \times 100$$

ii. *In vitro* anticancer activity on L929 cell lines

L929 (Fibroblast) cells were initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained in Dulbecco's modified Eagles medium, DMEM (Sigma Aldrich, USA). The cell line was cultured in 25 cm² culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate (Merck, Germany) and antibiotic solution containing: Penicillin (100 μ g/mL), Streptomycin (100 μ g/mL), and Amphotericin B (2.5 μ g/mL). Cultured cell lines were kept at 37°C in a humidified 5% CO₂ incubator (NBS Eppendorf, Germany).

The viability of cells was evaluated by direct observation of cells by inverted phase contrast microscope and followed by MTT assay method.

Cells seeding in 96 well plate:

Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100 μ L cell suspension was seeded in 96 well tissue culture plate and incubated at 37°C in a humidified 5% CO₂ incubator.

Preparation of compound stock:

One mg of sample was weighed and dissolved in 1 mL DMEM using a cyclomixer. The sample solution was filtered through 0.22 μ m Millipore syringe filter to ensure the sterility.

Cytotoxicity evaluation:

After 24 hours, the growth medium was removed. The freshly prepared compound in 5% DMEM were five times serially diluted by two-fold dilution (100 μ g, 50 μ g, 25 μ g, 12.5 μ g, 6.25 μ g in 500 μ L of 5% DMEM) and each concentration of 100 μ L were added in triplicates to the respective wells and incubated at 37°C in a humidified 5% CO₂ incubator. Non-treated control cells were also maintained.

Cytotoxicity assay by direct microscopic observation:

Entire plate was observed after 24 hours of treatment in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observations were recorded as images. Any detectable changes in the morphology of cells such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of cells were considered as indicators of cytotoxicity.

Cytotoxicity assay by MTT method:

Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 mL PBS until completely dissolved and sterilized by filter sterilization.

After 24 hours of incubation period, the sample contents in wells were removed and 30 µL of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well and incubated at 37°C in a humidified 5% CO₂ incubator for 4 hours. After the incubation period, the supernatant was removed and 100 µL of MTT solubilization solution (Dimethyl sulphoxide: DMSO, Sigma Aldrich, USA) was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance was measured by using microplate reader at a wavelength of 540 nm (Talarico et al., 2004).

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ of viability} = \frac{\text{Mean OD of sample}}{\text{Mean OD of control}} \times 100$$

iii. Determination of apoptosis using acridine orange and ethidium bromide (AO- EtBr) double staining

Acridine Orange (AO) and Ethidium Bromide (EtBr) are the DNA binding fluorescent dyes (Sigma, USA) and were used for the morphological detection of apoptotic and necrotic cells (Zhang et al, 1998). AO is taken up by both viable and non-viable cells and emits green fluorescence if intercalated into DNA. EtBr is taken up only by non-viable cells and emits red fluorescence by intercalation into DNA.

After attaining sufficient confluency of DLD1 cell lines, LC₅₀ concentration of samples were added. The LC₅₀ concentrations were 171.759 µg/mL and 193.243 µg/mL for *M. affinis* and *M. xanthopus* respectively. After treatment with the samples, the cells were washed by cold PBS and then stained with a mixture of AO (100 µg/mL) and EtBr (100 µg/mL) at room temperature for 10 min. The stained cells were washed twice with 1X PBS and observed in blue filter of fluorescent microscope (Olympus CKX 41 with Optika Pro5 camera).

The cells were divided into four categories as follows: living cells (normal green nucleus), early apoptotic (bright green nucleus with condensed or fragmented chromatin), late apoptotic (orange stained nuclei with chromatin condensation or fragmentation) and necrotic cells (uniformly orange stained cell nuclei).

d. Preliminary neuroprotective study:

Neuroprotective studies were carried out by using neuroblastoma cells (IMR 32 cell line), purchased from NCCS (National Centre for Cell Sciences), Pune and was maintained in Dulbecco's modified eagles media (HIMEDIA).

i. *In vitro* neuroprotective effect determination by MTT assay

The cell line was cultured in 25 cm² tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and 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).

The viability of cells was evaluated by direct observation of cells by an inverted phase contrast microscope and followed by MTT assay method.

Cells seeding in 96 well plate:

Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100 µL of cell suspension (5×10^4 cells/well) was seeded in 96 well tissue culture plate and incubated at 37°C in a humidified 5% CO₂ incubator.

Preparation of compound stock:

One mg of the sample was dissolved in 1 mL of DMEM using a cyclomixer. The extract solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility. Amyloid (10 µM) was added to induce toxicity.

Cytotoxicity evaluation:

The prepared extracts in 5% DMEM were five times serially diluted by two-fold dilution (100 µg, 50 µg, 25 µg, 12.5 µg and 6.25 µg in 500 µL of 5% DMEM) and each concentration of 100 µL were added in triplicates to the respective wells and incubated at 37°C in a humidified 5 % CO₂ incubator.

Cytotoxicity assay by direct microscopic observation:

Entire plate was observed at an interval of each 24 hours for 3 days in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observations were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in cytoplasm of the cells were considered as indicators of cytotoxicity.

Cytotoxicity assay by MTT method:

Fifteen mg of MTT (Sigma, M-5655) was dissolved in 3 mL PBS and sterilized by filter sterilization.

After 24 hours of incubation period, the sample contents in wells were removed and 30 μ L of reconstituted MTT solution was added to all the test and control wells. The plate was gently shaken well and incubated at 37°C in a humidified 5% CO₂ incubator for 4 hours. After the incubation period, the supernatant was removed and 100 μ L of MTT Solubilization Solution (DMSO) was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance was measured by using microplate reader at a wavelength of 540 nm (Talarico et al., 2004).

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ of viability} = \frac{\text{Mean OD of sample}}{\text{Mean OD of control}} \times 100$$

ii. Apoptosis determination by using acridine orange and ethidium bromide (AO- EtBr) double staining

DNA-binding dyes Acridine Orange (AO) and Ethidium Bromide (EtBr) (Sigma, USA) were used for the morphological detection of apoptotic and necrotic cells (Zhang et al, 1998). AO is taken up by both viable and non-viable cells and emits green fluorescence if intercalated into DNA. EtBr is taken up only by non-viable cells and emits red fluorescence by intercalation into DNA.

After treatment with samples (*M. affinis* and *M. xanthopus*) for 24 hours, the cells were washed by cold PBS and then stained with a mixture of AO (100 µg/mL) and EtBr (100 µg/mL) at room temperature for 10 min. The stained cells were washed twice with 1X PBS and observed by a fluorescence microscope in blue filter of fluorescent microscope and images were taken (Olympus CKX41 with Optika Pro5 camera).

The cells were divided into four categories as follows: living cells (normal green nucleus), early apoptotic (bright green nucleus with condensed or fragmented chromatin), late apoptotic (orange stained nuclei with chromatin condensation or fragmentation) and necrotic cells (uniformly orange stained cell nuclei).

PHASE III: DETAILED STUDY ON NEUROPROTECTION IN *M. XANTHOPUS*

Since *M. xanthopus* is found to be more potent than *M. affinis* in neuroprotective studies, further detailed studies are carried only in *M. xanthopus*. The potential of *M. xanthopus* in neuroprotective studies was evaluated by using *in vitro* ROS measurement using DCFDA, indirect ELISA, autophagy, Annexin-V FITC apoptosis determination using flow cytometry and gene expression study using qRT PCR.

i. *In vitro* ROS measurement using DCFDA

DCFDA (2,7 – Dichloro fluorescein diacetate; Sigma Aldrich) is a fluorogenic dye that measures hydroxyl, peroxy and the other ROS activity within the cell. After diffusion in to the cell, DCFDA is deacetylated by cellular esterases to a non-fluorescent compound, which is later oxidized by ROS into 2, 7 – dichlorofluorescein (DCF) which emits green fluorescence upon excitation with blue filter. Carboxy H₂DCFDA is non-fluorescent but in the presence of ROS, when this reagent is oxidized, it becomes green fluorescent in colour.

The cells were washed with PBS and then added with 50 µL of DCFDA and incubated for 30 minutes. After incubation, excess dye was washed with PBS and fluorescence was imaged in a fluorescent microscope (Olympus CKX41 with Optika

Pro5 CCD camera) and fluorescence was measured using a fluorimeter at 470 nm excitation and emission at 635 nm (Qubit 3.0, Life technologies, USA) and expressed in arbitrary units (AU).

ii. Indirect ELISA

Indirect ELISA is a two-step ELISA which involves a double binding process of primary antibody. The primary antibody is incubated with the antigen followed by the incubation with the secondary antibody.

After attaining sufficient confluency, cells were exposed with sample (*M. xanthopus*) from a stock of 1 mg/mL and incubated for 24 hours at 37°C in a humidified 5% CO₂ incubator. Non-treated control cells and Amyloid treated cells were also maintained. After incubation, the supernatant was collected and 100 µL of each was added to the 96 well plate and kept in incubation at 37°C overnight. The following day, the wells were drained and washed with PBS for 2 times. 200 µL of freshly prepared blocking buffer (Appendix 12) in PBS was added and incubated for 1 hour at room temperature. It was washed twice with PBS Tween. 100 µL of primary antibodies (ChAt, Glutamate Receptor 2, Alpha Synuclein and Tau) were added and left for 2 hours at room temperature. After incubation it was washed 2 times with PBS Tween. 100 µL of secondary antibody (HRP conjugate, Santacruz, USA) was added and left it for 1 hour at room temperature. It was then washed with PBS Tween for two times. 200 µL of O-dianisidine hydrochloride (Appendix 13) was added and left for 30 minutes at room temperature. The reaction was stopped by adding 5N HCl (50 µL). OD value was taken at 415 nm in an ELISA reader. Concentration of protein was also estimated.

iii. Autophagy

Under physiological conditions, autophagy plays a variety of important roles including maintenance of the amino acid pool during starvation, damaged protein and organelle turnover, prevention of neurodegeneration, tumor suppression, cellular differentiation, clearance of intracellular microbes and regulation of innate and

adaptive immunity. Autophagy is considered to be a dynamic, multi-step process which can be regulated at several steps, in both positive and negative manner.

For the analysis of live cells by flow cytometry, cells should be maintained via standard tissue culture practice. Cells were grown overnight to log phase at 37°C in a humidified 5% CO₂ incubator. At the end of the treatment of cells with compound of interest, cells were trypsinized and centrifuged at 1000 rpm for 5 min to pellet the cells.

Cells were washed with 1X assay buffer by suspending the cell pellet in cell culture medium. 250 µL of diluted cyto-ID® Green stain solution (Appendix 14) was added to the sample and mixed well. It was then incubated for 30 minutes at room temperature in the dark, gently pipetting up and down repeatedly. After treatment, collect the cells by centrifugation and wash with 1X assay buffer. Resuspend the cell pellets in 500 µL of fresh 1X assay buffer. Incubate for 20 min with 4% formaldehyde. Wash 3 times with 1X assay buffer. Samples were analysed with dual fluorescence in green (FL1) or orange (FL2) channel of a flow cytometer.

iv. Annexin V- FITC apoptosis determination by flow cytometry

Fluorescein isothiocyanate (FITC) is a derivative of fluorescein used in flow cytometry. Annexin V staining is used to detect apoptotic cells, which can only be done on live cells and tissue.

IMR32 (neuroblastoma cell line) cells were cultured as per standard procedures described earlier. Cells were treated with LC₅₀ concentration of the extract and incubated for 24 hours. The cells were trypsinized after incubation and 100 µL of cells in suspension was transferred to separate tubes. 100 µL of the Muse™ Annexin V & Dead Cell Reagent were added to each tube. The tubes were mixed thoroughly by pipetting up and down or vortexing at medium speed for 3 to 5 s followed by incubation for 20 min at room temperature in the dark. The cells were analysed in a flow cytometer using Muse flow cytometry software. Cells were gated against untreated control cells and analysed for apoptosis using Muse FCS 3.0 software.

v. Gene expression studies using qRT PCR

Regulation of the gene Parkin (PRKN gene) in the human neuroblastoma cell line IMR-32 by effective extract was analysed as follows:

a. Isolation of total RNA (TRIzol method)

Total RNA was isolated using the total RNA isolation kit (Invitrogen, USA). Addition of TRIzol solution (Appendix 15) causes the disruption of cells and the release of RNA. On mixing with isopropanol, RNA gets precipitated as a white pellet on the side and the bottom of the tube.

After attaining 70% confluency of cells in 6 well plate, the cells were treated with IC₅₀ concentration of sample and incubated for 24 hours. A set of untreated control cells was also incubated at 37°C for 24 hours in a CO₂ incubator. After incubation, DMEM medium was removed aseptically and 200 µL of TRIzol reagent was added to culture plate and incubated for 5 minutes. The contents were then transferred to a fresh sterile eppendorf tube. 200 µL of chloroform was added and vigorously shaken 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 min at room temperature and then centrifuged at 14000 rpm for 15 min at 4°C. The supernatant was discarded and pellet thus obtained was washed with 200 µL of 75 % ethanol. It was then centrifuged at 14000 rpm for 5 min at 4°C in a cooling centrifuge (Remi CM12). The RNA pellet was dried and suspended in TE buffer (Appendix 16).

b. Synthesis of cDNA

Template cDNA was synthesized using the cDNA preparation kit (Thermo scientific, Verso cDNA Synthesis kit). About 4 µL of 5X cDNA synthesis buffer, 2 µL of dNTP mix, 1 µL of oligo dT, 1 µL of RT Enhancer, 1 µL of verso enzyme mix and 5 µL of RNA template (0.5 µg of total RNA) were added to an RNase free tube. Then the total reaction volume was made up to 20 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 cDNA synthesis. The following cycling conditions were employed for 30 min at 42°C and 2 min at 95°C.

Step	Temperature (°C)	Time (min)	Number of cycles
cDNA synthesis	42	30	1
Inactivation	95	2	1

c. Gene expression analysis by qRT PCR

Real-Time qRT PCR analysis was carried out using SYBR Green Master Mix (Applied Biosystem, Life technologies). All reactions were performed in triplicates and data were analysed according to $\Delta\Delta C_t$ method using Light Cycler 96 SW 1.1 Software.

The time and temperature for each step involved in the process are represented below.

Steps	Time required	Temperature
Initial activation step	2 min	95°C
3 step cycling:		
Denaturation	10 s	94°C
Annealing	1 min	55°C
Extension	1 min/kb	72°C
Number of cycles	40 cycles	68°C
End of PCR cycling	indefinite	4°C

The primer sequences used for the genes B-actin and Parkin are summarised as follows:

Oligo name	Forward		Reverse	
	Sequence (5' ->3')	Tm	Sequence (5' ->3')	Tm
Human-B actin	TCACCCACACTGTGCCCATCTACGA (25)	66.3	CAGCGGAACCGCTCATTGCCAATGG (25)	67.9
Human-Parkin	GGCTGGCTGTCATTCTGCACAC	64.0	TCCCGGCTGCACTCTTTGAC	61.4

d. Agarose gel electrophoresis

Agarose gel electrophoresis is a method for separating and visualizing DNA fragments. The separation of fragments is based on charge and size. When an electric field is applied, DNA moves through a matrix called agarose gel. The electric field is generated by applying potential across an electrolyte solution (buffer). When boiled in an aqueous buffer, agar dissolves and upon cooling solidifies to a gel. 1.5% of agarose gel was prepared in 1X TE buffer and melted in hot water bath at 90°C. Then the melted agarose was cooled down to 45°C.

6 µL of 10 mg/mL of ethidium bromide was added and poured into a gel casting apparatus with the gel comb. After setting, the comb was removed from the gel. The electrophoresis buffer was poured in the gel tank and the platform with the gel was placed in it so as to immerse the gel. The gel was loaded with the samples and run at 50 V for 30 min. The stained gel was visualized using a gel documentation system (E-gel imager, Invitrogen).



Results

RESULTS

PHASE I: MYCOCHEMICAL CHARACTERIZATION

Fungi are the treasure house of potential drugs and in the recent years, there has been an increasing awareness about the importance of mushrooms for the diverse spectra of chemicals. Chemical characterization in the two species of *Microporus* was carried out in four sections. Preliminary qualitative mycochemical screening, quantitative estimation, identification of non-volatile chemicals through HR-LC/MS analysis and determination of volatile chemical constituents through GC/MS analysis.

a) Qualitative mycochemical screening

To determine the presence or absence of various classes of chemicals, the standard colour reactions and precipitation protocols were adopted in three different extracts of two species of *Microporus*. Among these, methanol extract was found to be more effective having different classes of chemicals. Methanolic extracts in both the species of *Microporus* revealed the presence of major chemical compounds such as terpenoids, steroids and alkaloids. Glycosides and saponins were also reported in *M. xanthopus*. Results of preliminary mycochemical analyses are summarized in Table 4.

Table 4: Qualitative mycochemical analysis of *Microporus affinis* and *M. xanthopus*

Sl No.	Class of compounds	Tests	MA			MX		
			H	M	DW	H	M	DW
1	Alkaloids	Wagner's test	-	+	+	-	+	+
		Hager's test	-	+	+	-	+	+
2	Flavonoids	Alkaline reagent test	-	-	-	-	-	-
		Lead acetate test	-	-	-	-	-	-
3	Phenols	FeCl ₃ test	-	-	-	-	-	-
4	Glycosides	Keller Killiani test	-	-	-	-	+	-
5	Saponins	Foam test	-	-	-	-	+	-
6	Steroids	Leibermann Burchard test	-	+	-	-	+	-
7	Tannins	Braymer's test	-	-	-	-	-	-
8	Terpenoids	Salkowski test	+	+	+	+	+	+

MA - *Microporus affinis*; MX - *M. xanthopus*; H - Hexane; M - Methanol; DW -Distilled water; (+) - Presence; (-) - Absence

b) Quantitative mycochemical screening

Quantitative analyses of four different classes of compounds like alkaloids, terpenoids, carbohydrates and proteins were conducted. The methanolic fraction of fungal extract was used for the assays. The alkaloid content was quantified using the standard compound caffeine. *M. xanthopus* showed the maximum value (189 ± 0.316 mg CE /g of DW extract) than *M. affinis* (169.3 ± 0.158 mg CE /g of DW extract) (**Fig. 10a, 10b**). The total terpenoids were quantified by using the standard compound lupeol. Both the species showed comparatively high amount of terpenoids. *M. xanthopus* showed the maximum concentration (282.3 ± 0.52 mg LE/g of DW extract) than *M. affinis* (237.3 ± 0.34 mg LE/g of DW extract) (**Fig. 11a, 11b**). The total protein estimation was done by Lowry's method. Bovine Serum Albumin (BSA) was used as the standard. Both the species showed a minimum amount of protein. *M. affinis* showed a higher amount (0.211 ± 0.0003 mg/g DW) than *M. xanthopus* (0.153 ± 0.0002 mg/g DW) (**Fig. 12a, 12b**). Total carbohydrate was estimated by using the standard glucose. Both the species showed high amount of carbohydrates. Among these, *M. affinis* showed a higher amount (442.83 ± 0.79 mg GE /g DW) than *M. xanthopus* (392 ± 0.79 mg GE /g DW) (**Fig. 13a, 13b**).

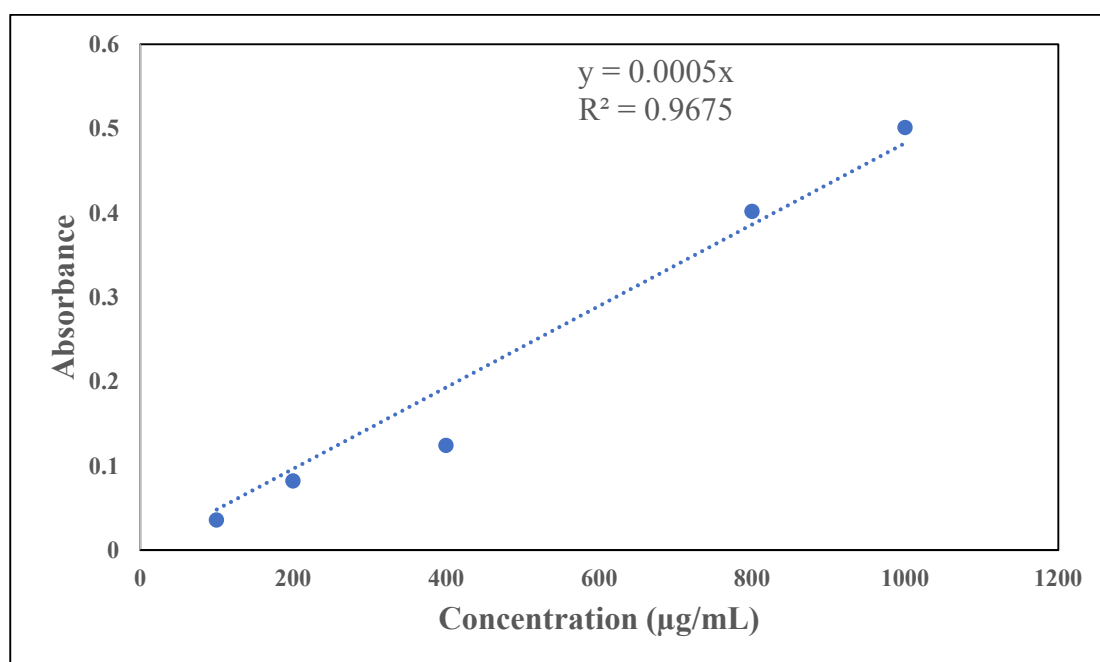


Fig. 10a: Standard calibration graph of caffeine to determine the alkaloid content

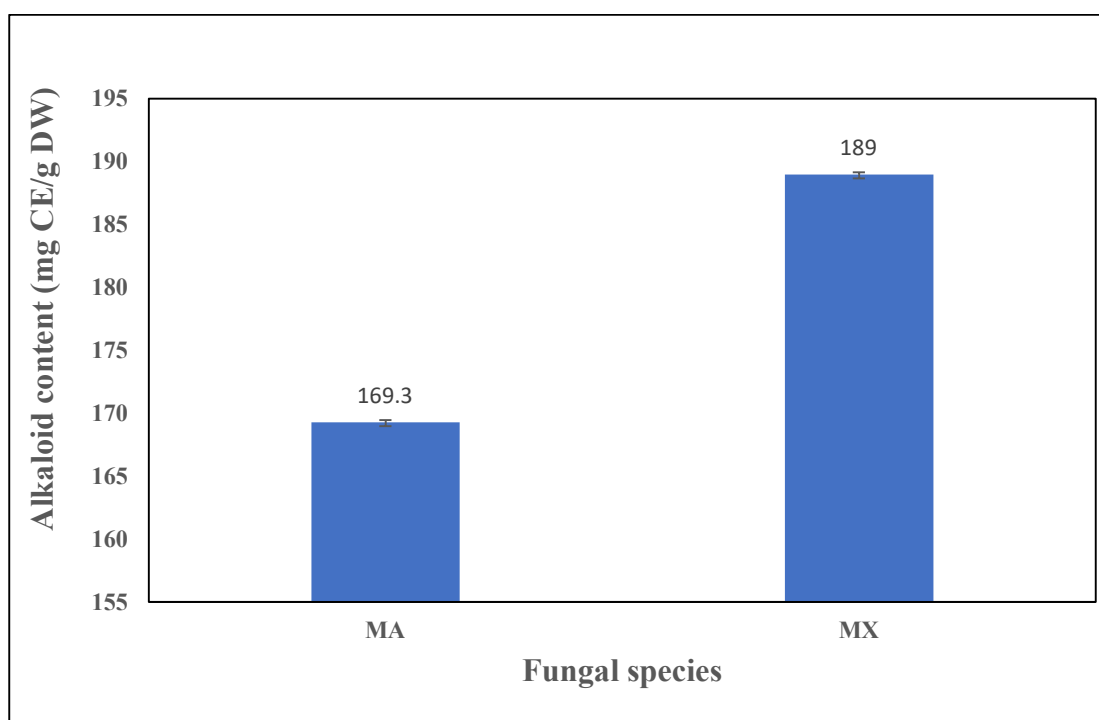


Fig. 10b: Quantitative estimation of alkaloids in the methanolic extract of *M. affinis* (MA) and *M. xanthopus* (MX)

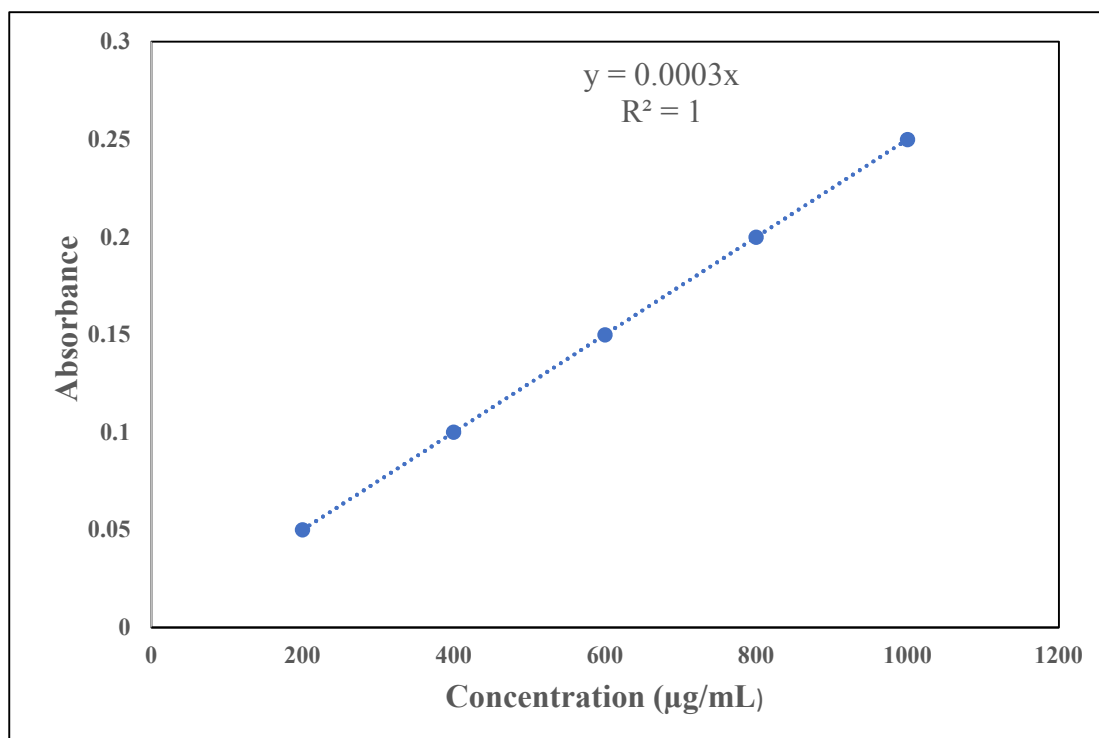


Fig. 11a: Standard calibration graph of lupeol for the quantification of terpenoid content

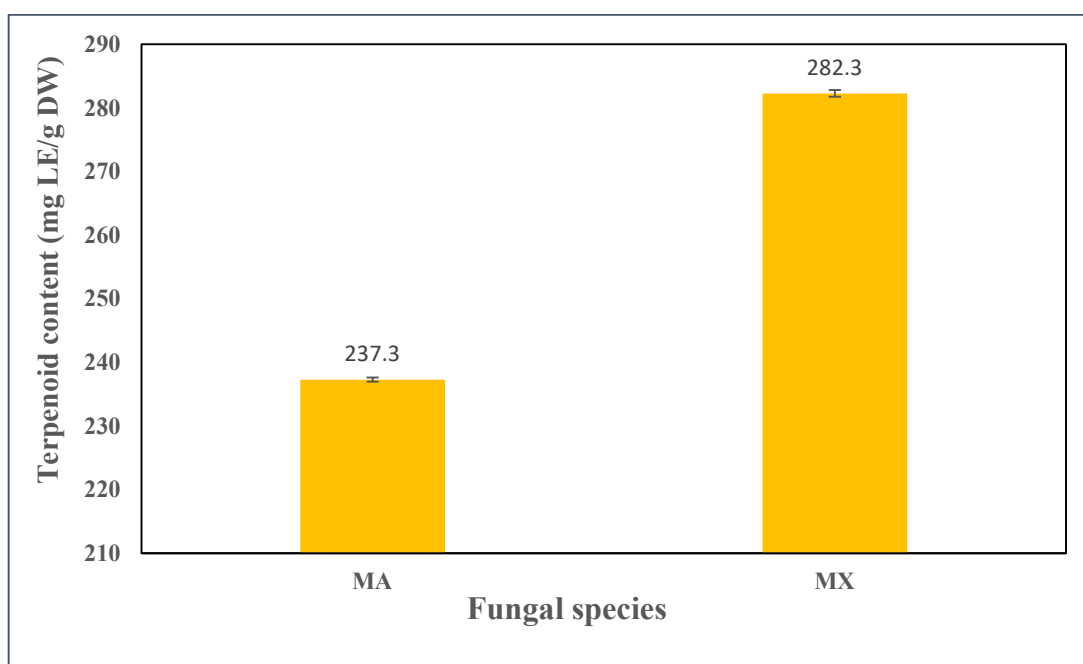


Fig. 11b: Quantitative estimation of terpenoids in the methanolic extract of *M. affinis* (MA) and *M. xanthopus* (MX)

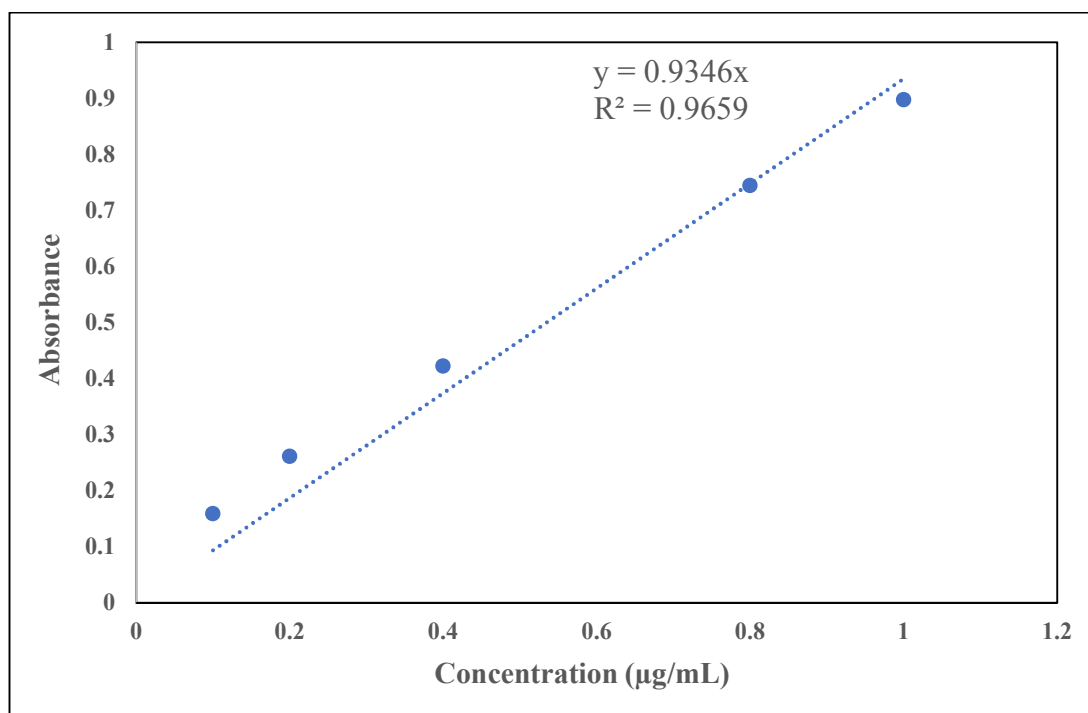


Fig. 12a: Standard calibration graph of Bovine Serum Albumin (BSA) for the quantification of protein content

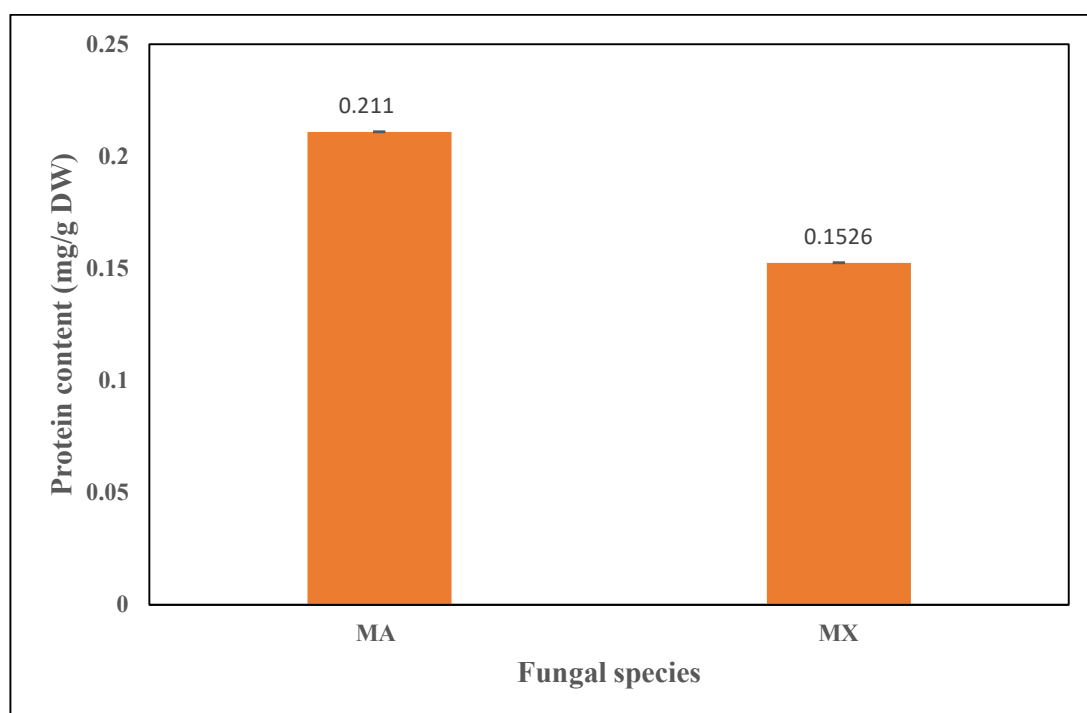


Fig. 12b: Quantitative estimation of protein in the methanolic extract of *M. affinis* (MA) and *M. xanthopus* (MX)

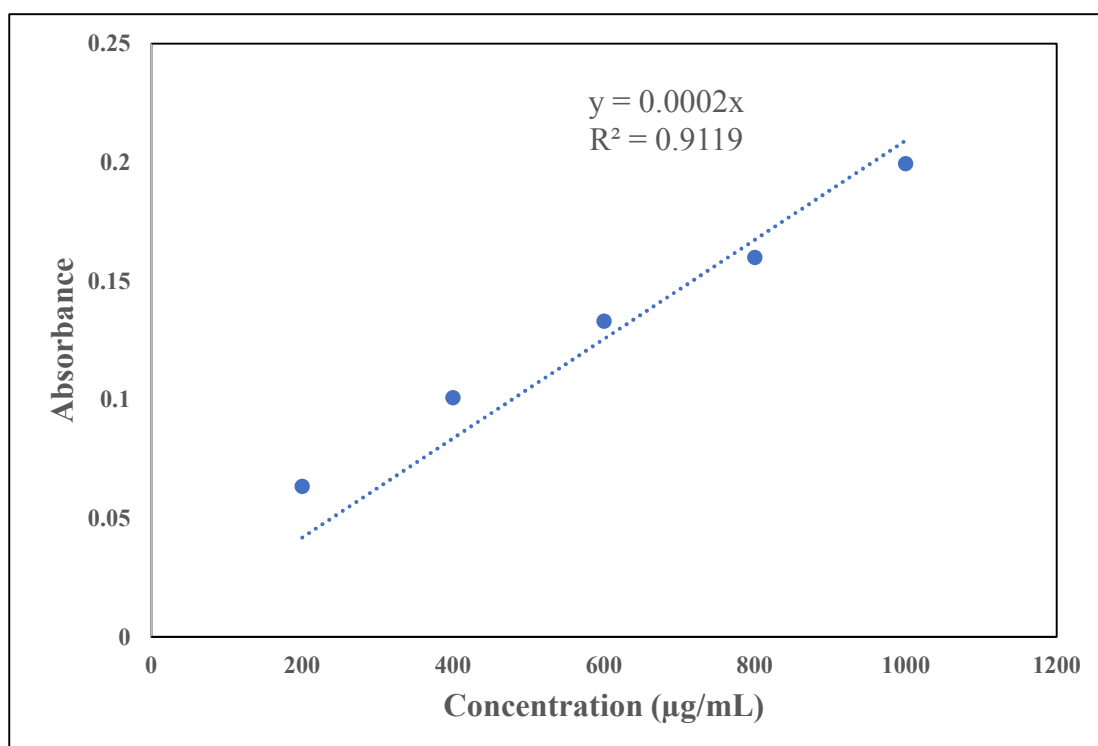


Fig. 13a: Standard calibration graph of glucose for the quantification of carbohydrate content

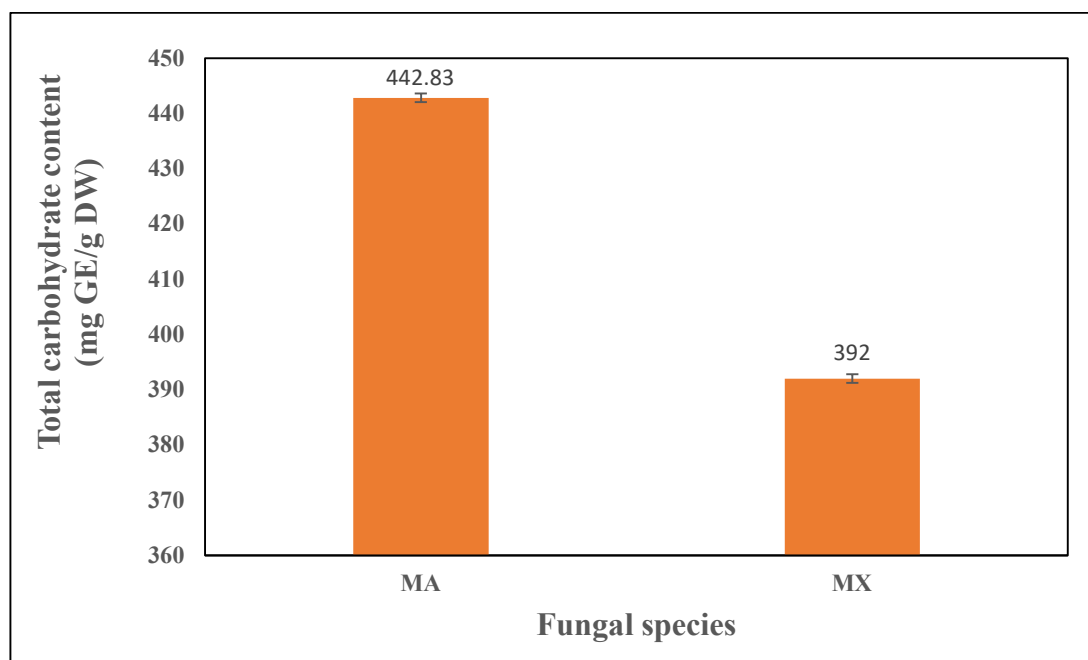


Fig. 13b: Quantitative estimation of carbohydrate in the methanolic extract of *M. affinis* (MA) and *M. xanthopus* (MX)

c) High Resolution - Liquid Chromatography/Mass Spectrometry (HR-LC/MS) analysis

The non-volatile chemical constituents in the two species of *Microporus* were revealed by HR-LC/MS analysis. The identified compounds, their retention time, molecular mass and class of compounds are listed in **Table 5 & 6**. The liquid chromatograms (**Fig. 14a-b; 15a-b**) and mass spectrums (**Fig. 14 i-vi; 15 i-ii**) are represented.

In *M. xanthopus*, a total of 31 compounds were revealed through HR-LC/MS analysis. The compounds obtained are grouped into 16 classes (**Table 5**). These are alkyl amine, amine, amino glycoside, dipeptide, ether, fatty acid, glycoside, monoamine neurotransmitter, organic compound, peptide, phospholipid, sphingolipid, steroid, terpene, diterpene and vitamin. Among these, 6 compounds were fatty acids. A monoamine neurotransmitter called serotonin was also obtained.

In case of *M. affinis*, a total of 9 compounds were revealed. These are belonging to 6 classes. Carboxylic acid, fatty acid, fatty acid ester, organic compound, steroid and terpene are the various classes (**Table 6**). Among these, fatty acid represents the predominant class. Out of the 9 compounds, 4 compounds were found to be fatty acids. HR-LC/MS provided a vivid picture of various mycoconstituents that will not be revealed by GC/MS.

d) Gas chromatography/ Mass spectrometry (GC/MS) analysis

In order to determine the volatile components of the fungal extracts, two taxa of *Microporus* were subjected to GC/MS analysis. Compounds were resolved according to their retention time and those which are present in trace amount were also detected. The structural identification was done by using the NIST libraries. Chromatograms of two taxa of *Microporus* and mass spectra of each compounds obtained were also plotted. Each peak in the chromatogram specify the respective compounds and they had a peak area denoting their abundance in the sample (**Fig. 16a**: GC of *M. xanthopus* and **16b**: GC of *M. affinis*). The compounds thus obtained were analyzed using PubChem.

In *M. xanthopus*, a total of 45 compounds were obtained. The compounds had retention indices ranging from 7.242 to 41.043. The compounds are grouped into 11 classes such as alkane, alkene, aldehyde, alcohol, ester, ether, fatty acid, fatty acid ester, ketone, organic compound and sterol (**Table 7**). Among these, leading classes of compounds are fatty acids (72.66 %), sterols (15.21 %) and fatty acid esters (10.05 %). Linoleic acid represents the compound with highest peak area (26.86 %). Other leading compounds are oleic acid (17.97 %), palmitic acid (15.81%), stellasterol (8.43 %), pentadecanoic acid (6.68 %), monoolein (6.45 %), ergosterol (5.6 %) etc. Mass spectra of each compounds are shown in **Fig. 17 (i-vii)**.

In case of *M. affinis*, a total of 46 compounds were detected through GC/MS analysis. As in *M. xanthopus*, here also compounds are grouped into 11 classes. Majority of classes are similar in both the species. Aldehyde, alkane, alkene, alcohol, ester, fatty acid, fatty acid ester, hexose, ketone, sterol and terpenoid represent various classes of compounds (**Table 8**). Fatty acids are found to be the leading class (48.70

%). Other leading classes are fatty acid esters (28.38 %), sterols (13.07 %), alcohols (3.23 %), ketones (2.80 %) esters (1.59 %) etc. Linoleic acid was found to be with highest peak area (26.35 %) which is followed by palmitic acid (13.73 %), 2-monopalmitin (11.73 %), stellasterol (9.61 %), linoleic acid methyl ester (8.53 %), monoolein (5.93 %), pentadecanoic acid (2.67 %) and methyl palmitate (2.17 %). Mass spectra of all the compounds are shown in **Fig. 18 (i-viii)**.

Table 5: Mycochemical constituents detected from the methanolic extracts of *M. xanthopus* using HR-LC/MS analysis

Sl No.	RT (min)	Compound	Class	Molecular mass
1	1.060	Nefopam	Alkylamine	253.15
2	1.066	Muramic acid	Ether	251.09
3	1.107	3,7-Epoxycaryophyllan-6-one	Organic compound	236.17
4	1.107	11-Aminoundecanoic acid	Fatty acid	201.17
5	1.108	Pro leu	Dipeptide	228.15
6	1.568	Arg Pro	Peptide	271.16
7	3.677	Serotonin	Monoamine neurotransmitter	176.09
8	4.348	Ile Leu Leu	Tripeptide	357.26
9	5.872	Lys Met Lys	Tripeptide	405.23
10	7.168	10-Nitrolinoleic acid	Organic compound	325.22
11	8.251	Phosphatidyl myoinositol-3-phosphate	Phospholipid	470.25
12	8.998	Desoxymycin	Glycoside	567.28
13	10.074	Sphinganine	Organic compound	273.26
14	10.075	Chlorpromazine	Organic compound	256.10
15	10.625	Tetradecylamine	Amine	213.45
16	11.487	Phytosphingosine	Sphingolipid	317.29
17	11.891	6b,11b,16a,17a, 21-Pentahydroxypregna-1,4-diene-3,20-dione 16,17-acetonide	Terpene	432.21
18	12.162	Anandamide	Fatty acid	317.28
19	12.910	Naproxen glucuronide	Organic compound	406.13
20	13.291	Kanamycin	Aminoglycoside	484.28

21	13.430	8-Oxo 9, 11-octadecadienoic acid	Fatty acid	290.18
22	13.684	13-Hydroxy 9, 12-octadecadienoic acid	Fatty acid	296.23
23	13.927	Tilamulin	Diterpene	493.37
24	14.173	9,14-Dihydroxy10,12-octadecadienoic acid	Fatty acid	312.23
25	15.089	2- Tetracos15-enamidoethanesulfonic acid	Organic compound	473.35
26	15.164	Ambroxol	Amine	375.98
27	15.973	1,24,25-Trihydroxy vitamin D2	Vitamin	444.32
28	18.206	17 α -Hydroxypregnenolone	Steroid	323.23
29	18.382	1 α ,25-Dihydroxyvitamin D2	Vitamin	428.33
30	19.701	Calciferol	Vitamin	410.32
31	26.655	14-Hydroxy 5-tetradecenoic acid	Fatty acid	242.18

Table 6: Mycochemical constituents detected from the methanolic extracts of *M. affinis* using HR-LC/MS analysis

Sl No.	RT (min)	Compound	Class	Molecular mass
1	1.149	Glucaric acid	Organic compound	210.25
2	9.163	Sativic acid	Fatty acid	348.25
3	14.234	Methyl 9,12-dihydroxy-13 oxo10-octadecenoate	Fatty acid ester	342.24
4	16.865	Jalapinolic acid	Fatty acid	272.23
5	17.691	9-Hydroxy 12-octadecenoic acid	Fatty acid	298.25
6	18.421	3,4-Dihydroxy- 9,10 secoandrosta 1,3,5 (10) triene-9,17-dione	Steroid	316.17
7	18.577	Dihydroartemisinin	Sesquiterpene	284.16
8	18.640	10-Hydroxystearic acid	Fatty acid	316.17
9	27.061	16-Hydroxy – 4- oxoretinoic acid	Carboxylic acid	330.18

Table 7: Mycochemical constituents detected from the methanolic extracts of *M. xanthopus* using GC/MS analysis

SI No.	RT (min)	Compound	Class	Peak area %
1	7.242	Caprylic acid	Fatty acid	0.300
2	8.554	2,6-Dihydro-2H-pyran -3-one	Ester	0.110
3	8.749	Phenylacetaldehyde	Aldehyde	0.131
4	9.490	Maltol	Ketone	0.186
5	10.939	3,5-Dihydroxy-6-methyl-2,3-dihdropyran-4-one	Ketone	0.951
6	11.610	Glutamine	Amine	1.080
7	15.570	Tetradecene	Alkene	0.235
8	15.704	Tetradecane	Alkane	0.144
9	18.727	Hexadecene	Alkene	0.034
10	18.839	Hexadecane	Alkane	0.023
11	19.722	Dodecylcyclohexane	Alkane	0.148
12	19.862	8-Pentadecanone	Ketone	0.301
13	21.054	Myristic acid	Fatty acid	0.344
14	21.542	Octadecene	Alkene	1.028
15	21.634	Heneicosane	Alkane	0.195
16	22.385	Pentadecanoic acid	Fatty acid	6.685
17	22.582	8-Octadecanone	Ketone	0.231
18	23.226	Methyl palmitate	Fatty acid ester	0.458
19	23.299	Benzenepropanoic acid 3,5-bis (1,1-dimethylethyl)-4-hydroxy methyl ester	Fatty acid ester	0.139
20	23.420	Palmitoleic acid	Fatty acid	0.762
21	23.739	Palmitic acid	Fatty acid	15.810
22	24.094	Nonadecene	Alkene	0.558
23	24.613	Cis-10-heptadecenoic acid	Fatty acid	0.418
24	24.864	Margaric acid (Heptadecenoic acid)	Fatty acid	0.273
25	25.256	Linoleic acid methyl ester	Fatty acid ester	0.648
26	25.336	Oleic acid methyl ester	Fatty acid ester	0.623
27	25.804	Linoleic acid	Fatty acid	26.863

28	25.874	Oleic acid	Fatty acid	17.970
29	26.077	Stearic acid (Octadecanoic acid)	Fatty acid	2.791
30	26.419	Behenic alcohol (Docosanol)	Alcohol	0.128
31	26.497	Dodecyl 4-nitrophenyl ether	Ether	0.086
32	27.371	Fumaric acid 2-dimethylaminoethyl octadecyl ester	Ester	0.119
33	27.764	9,12-Octadecadienal dimethyl acetal	Organic compound	0.328
34	27.890	13,16- Docosadienoic acid	Fatty acid	0.102
35	27.966	9-Octadecenal	Aldehyde	0.493
36	29.334	3-Cyclopentylpropionic acid 2-dimethylamino ethyl ester	Ester	0.270
37	30.137	2- Monopalmitin	Fatty acid ester	1.732
38	30.769	Behenic acid (Docosanoic acid)	Fatty acid	0.108
39	31.155	Tetracontane	Alkane	0.294
40	32.013	Monoolein	Fatty acid ester	6.454
41	32.764	Lignoceric acid (Tetracosanoic acid)	Fatty acid	0.234
42	35.124	Dehydroergosterol	Sterol	0.502
43	39.220	Ergosterol	Sterol	5.600
44	39.595	Stellasterol	Sterol	8.431
45	41.043	Fungisterol	Sterol	0.680

Table 8: Mycochemical constituents detected from the methanolic extracts of *M. affinis* using GC/MS analysis

SI No.	RT (min)	Compound	Class	Peak area %
1	7.239	2,4-Dihydroxy-2,5-dimethylfuran-3-one	Ketone	0.408
2	9.537	Maltol	Pyranone (Ketone)	0.396
3	10.946	3,5-Dihydroxy-6-methyl-2,3-dihydropyran-4-one	Pyranone (Ketone)	0.980
4	12.344	1,4:3,6- Dianhydro-alpha-d-glucopyranose	Hexose	0.408
5	12.579	5-Hydroxymethylfurfural	Aldehyde	0.336

6	14.803	Neric acid	Acyclic monoterpene	0.125
7	15.393	5-Oxo-DL- proline methyl ester	Ester	1.399
8	15.565	1-Tetradecene	Alkene	0.081
9	15.702	Tetradecane	Alkane	0.090
10	17.139	Hydroxycitronellal	Aldehyde	0.094
11	17.194	2,6- Difluorobenzoic acid tridec-2-ynyl ester	Ester	0.068
12	18.723	1-Heptadecene	Alkene	0.192
13	18.835	Nonadecane	Alkane	0.191
14	20.580	Ribitol	Alcohol	1.995
15	21.057	Myristic acid (Tetradecanoic acid)	Fatty acid	0.316
16	21.633	Octadecane	Alkane	0.115
17	21.936	Pentadecanoic acid methyl ester	Fatty acid ester	0.408
18	22.412	Pentadecanoic acid	Fatty acid	2.675
19	22.959	Methyl palmitoleate	Fatty acid ester	0.094
20	23.227	Methyl palmitate	Fatty acid	2.179
21	23.299	Benzene propanoic acid 3,5-bis (1,1 dimethyl) -4-hydroxy ester	Fatty acid ester	0.203
22	23.417	Palmitoleic acid	Fatty acid	0.915
23	23.742	Palmitic acid	Fatty acid	13.73
24	24.358	2-Hydroxycyclopentadecanone	Ketone	1.019
25	24.611	10-Heptadecenoic acid	Fatty acid	0.375
26	24.866	Margaric acid (Heptadecanoic acid)	Fatty acid	0.336
27	25.268	Linoleic acid methyl ester	Fatty acid ester	8.537
28	25.336	Oleic acid methyl ester	Fatty acid ester	1.349
29	25.844	Linoleic acid	Fatty acid	26.356
30	25.879	Oleic acid	Fatty acid	0.478
31	26.070	Stearic acid	Fatty acid	1.017
32	26.306	Geranyl geraniol	Diterpenoid alcohol	0.953
33	26.976	Farnesol	Sesquiterpene alcohol	0.155

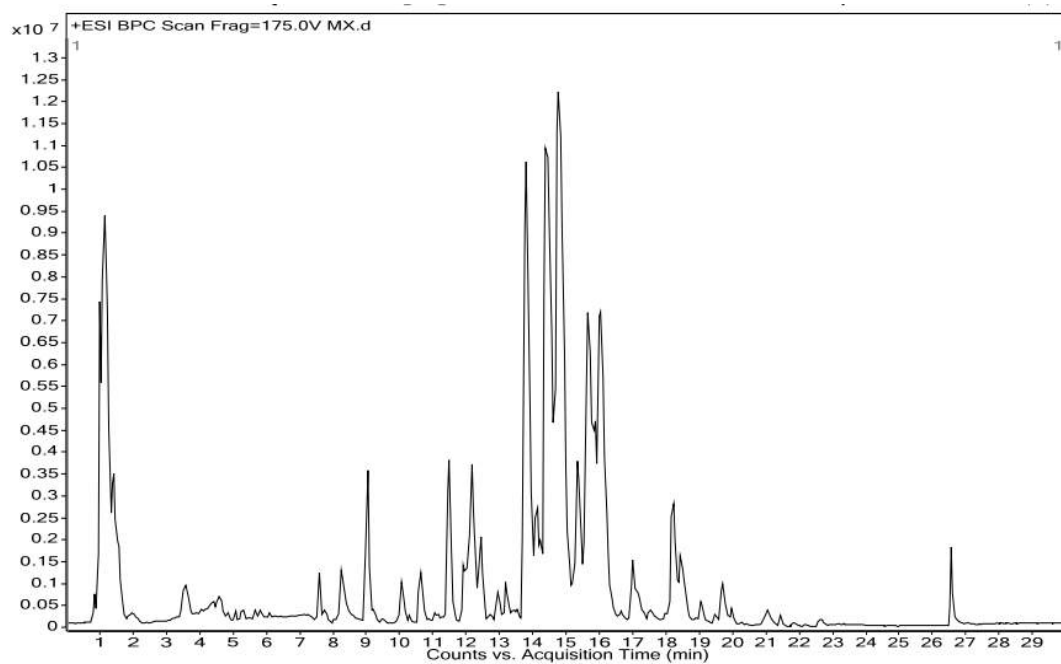


Fig. 14a Liquid chromatogram for positive ionization compounds in the methanolic extract of *M. xanthopus*

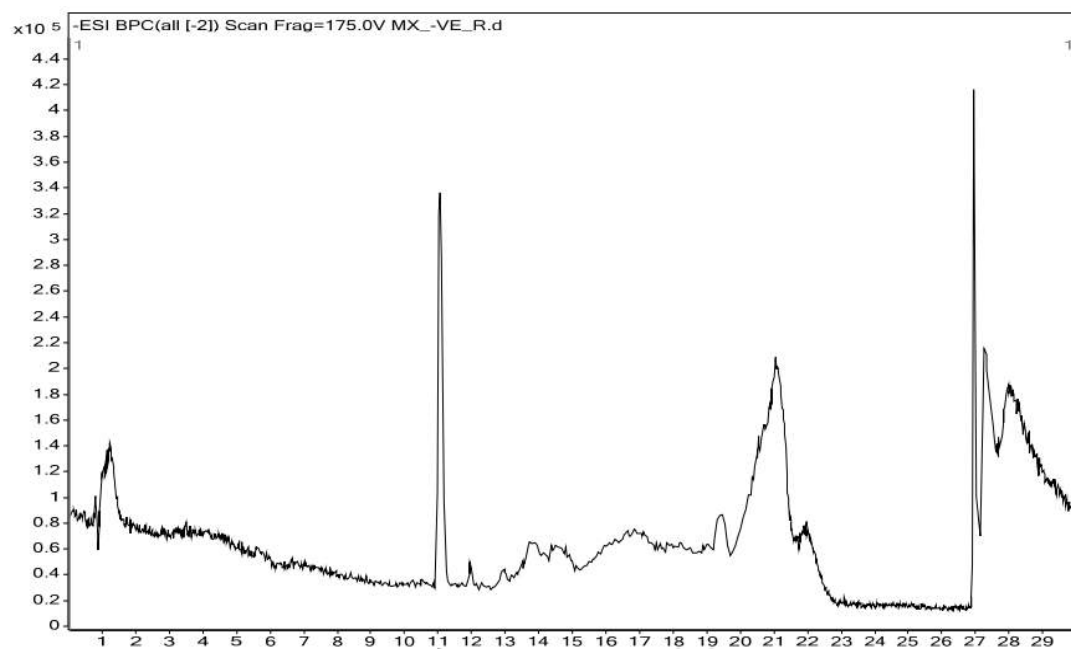
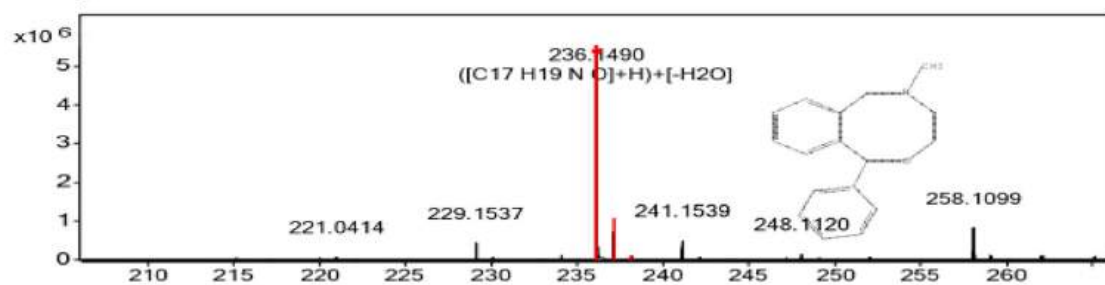
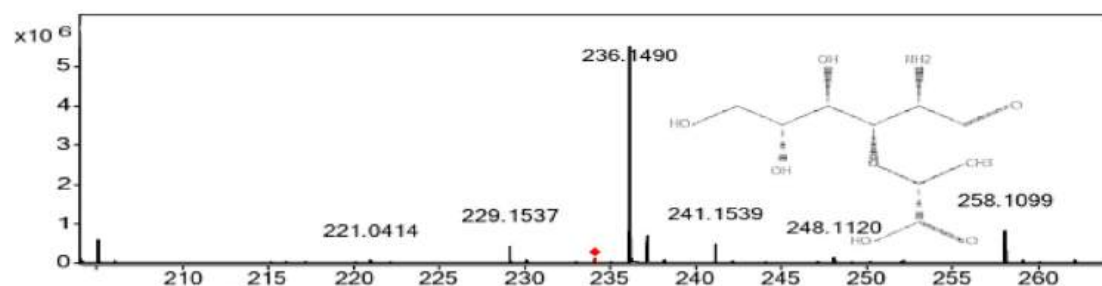


Fig. 14b Liquid chromatogram for negative ionization compounds in the methanolic extract of *M. xanthopus*

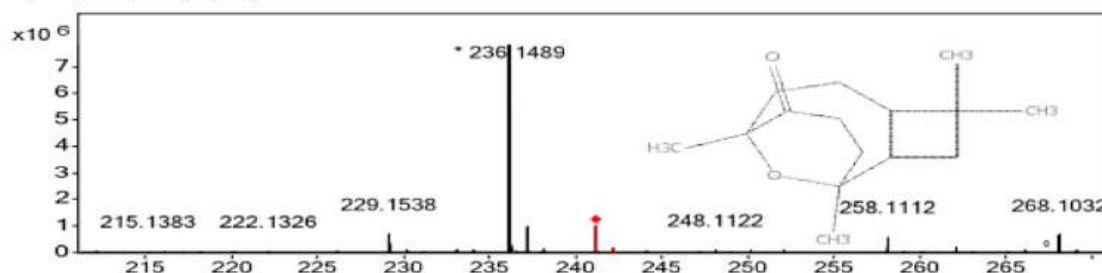
Nefopam



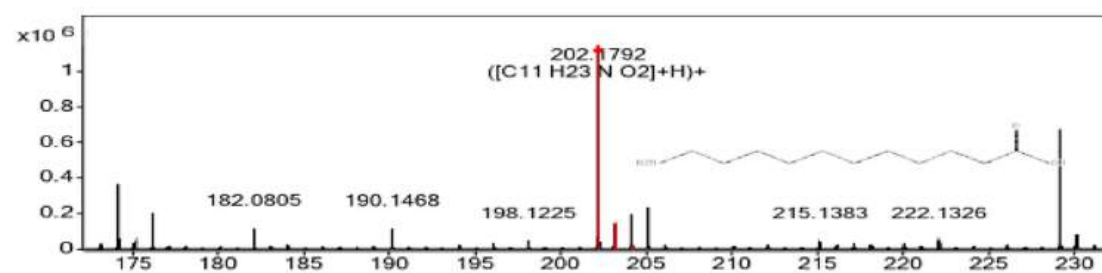
Muramic acid



3,7-Epoxycaryophyllan-6-one



11-Aminoundecanoic acid



Pro Leu

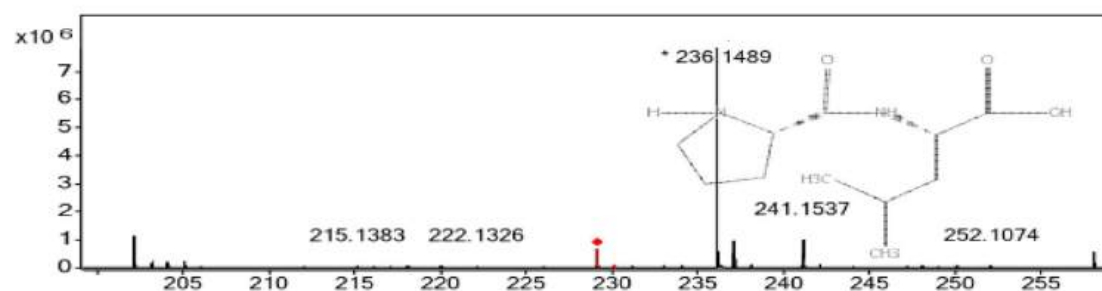
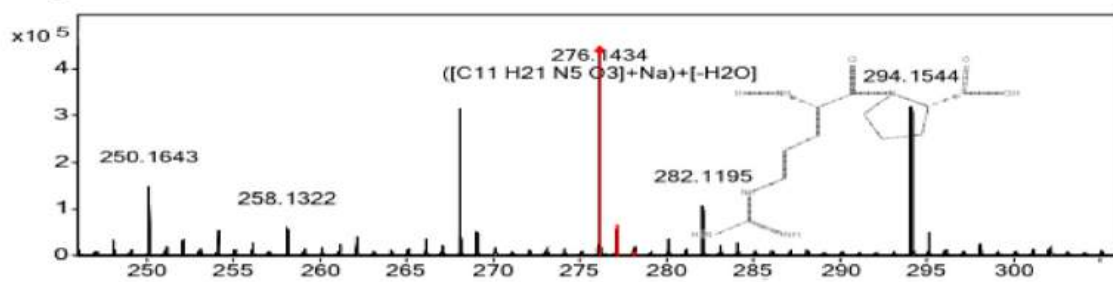
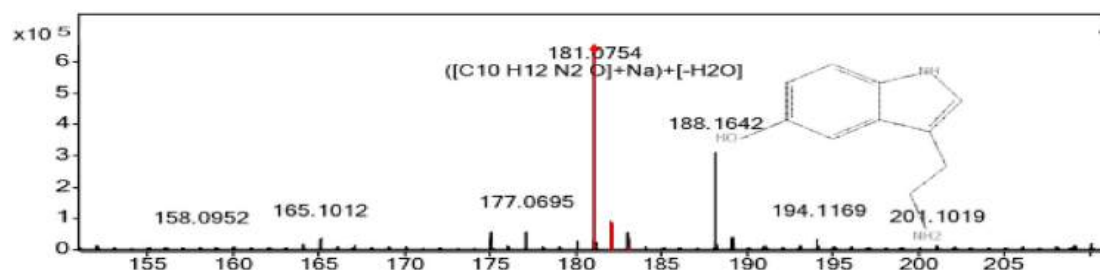


Fig. 14 (i): Mass spectra of compounds detected by HR-LC/MS analysis of methanolic extract of *M. xanthopus*

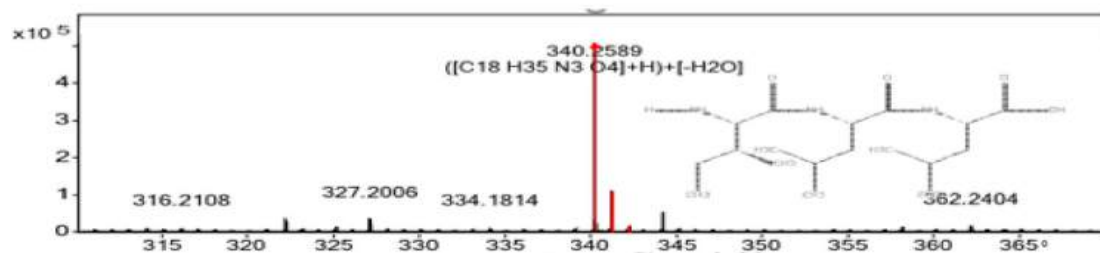
Arg Pro



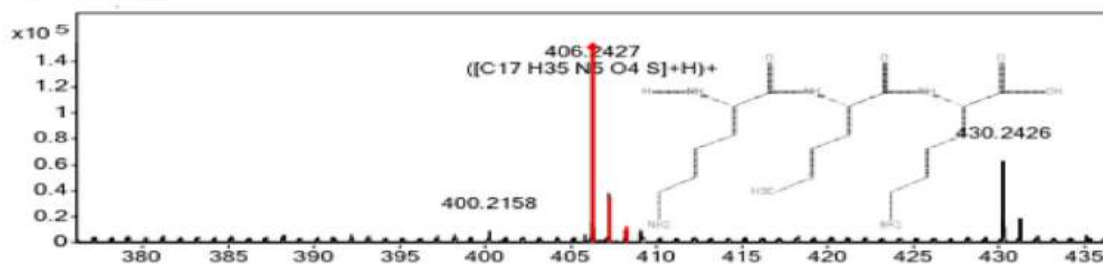
Serotonin



Ile Leu Leu



Lys Met Lys



10-Nitrolinoleic acid

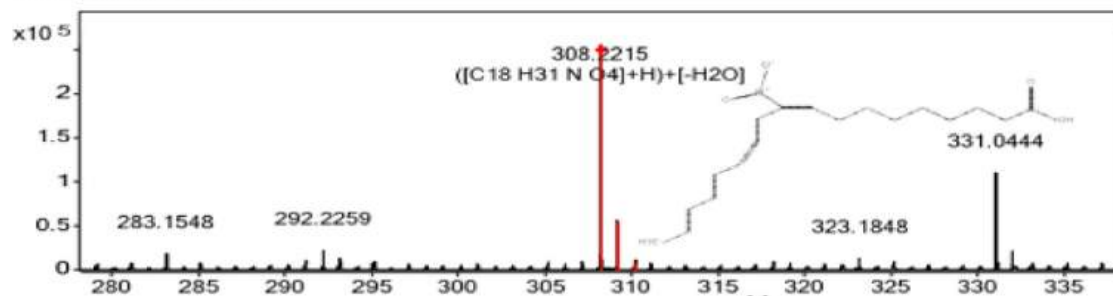
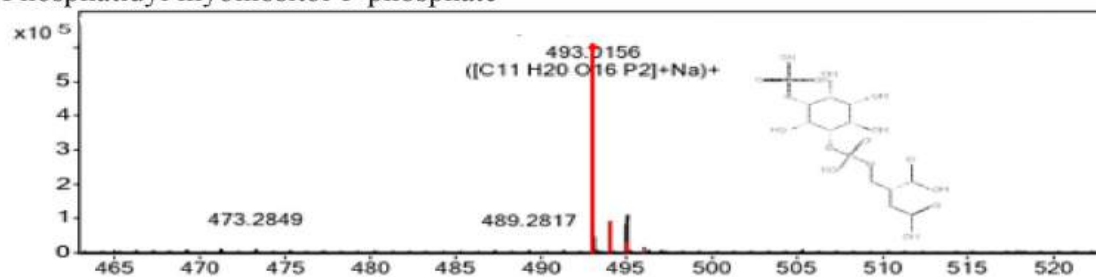
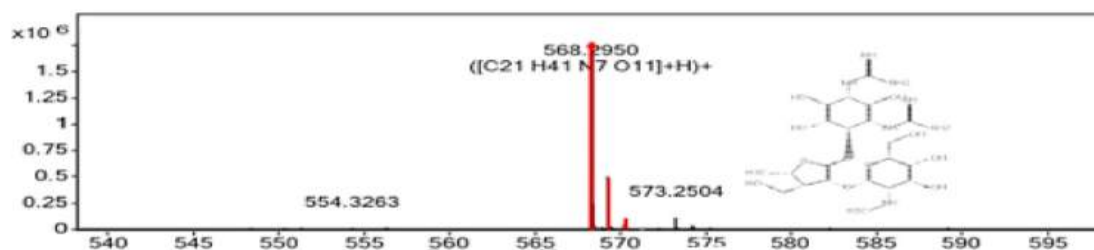


Fig. 14 (ii): Mass spectra of compounds detected by HR-LC/MS analysis of methanolic extract of *M. xanthopus*

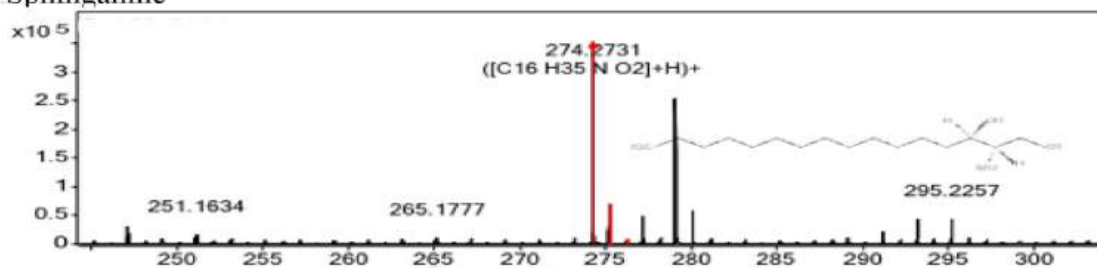
Phosphatidyl myoinositol-3-phosphate



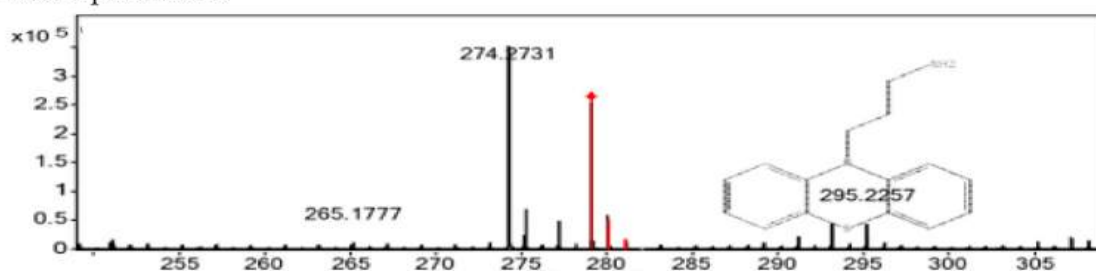
Desoxymycin



Sphinganine



Chlorpromazine



Tetradecylamine

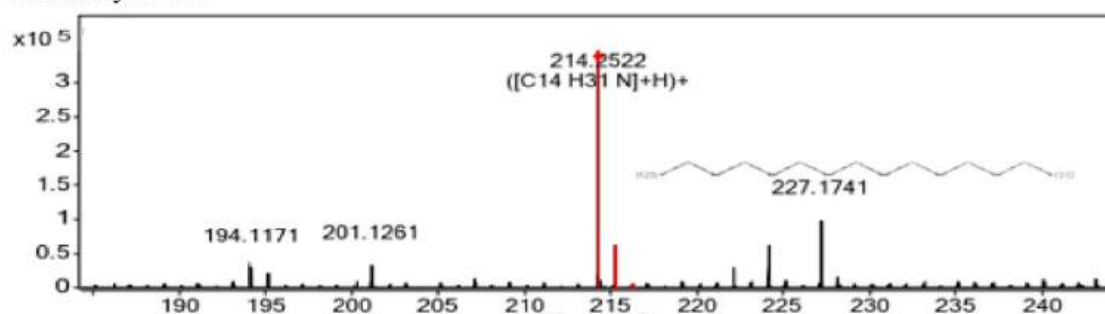
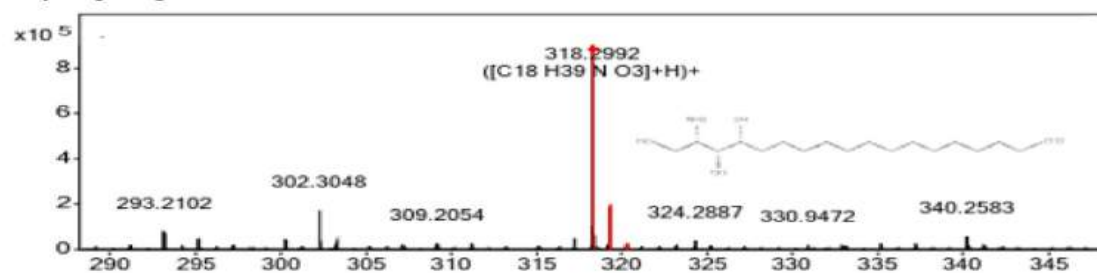
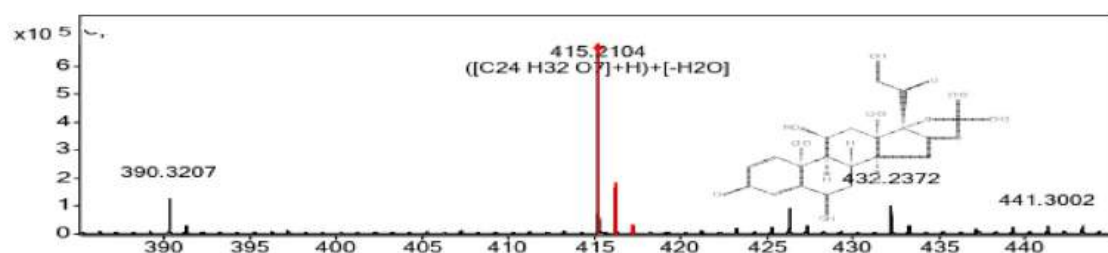


Fig. 14 (iii): Mass spectra of compounds detected by HR-LC/MS analysis of methanolic extract of *M. xanthopus*

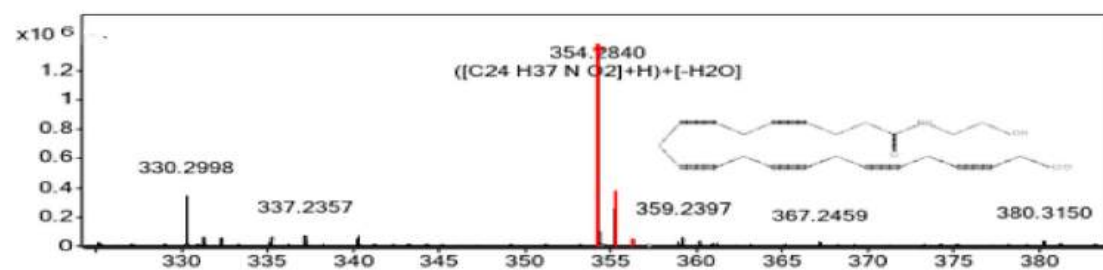
Phytosphingosine



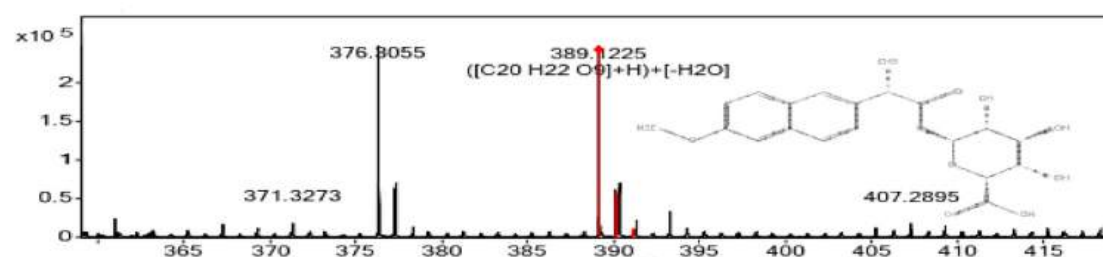
6b,11b,16a,17a, 21- Pentahydroxypregna-1,4-diene-3,20-dione 16,17-acetonide



Anandamide



Naproxen glucuronide



Kanamycin

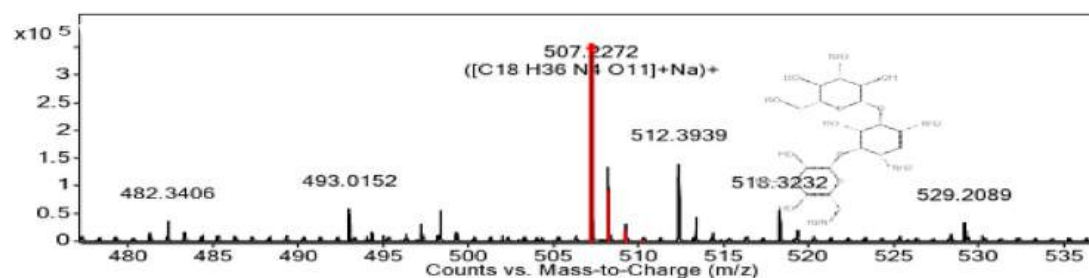
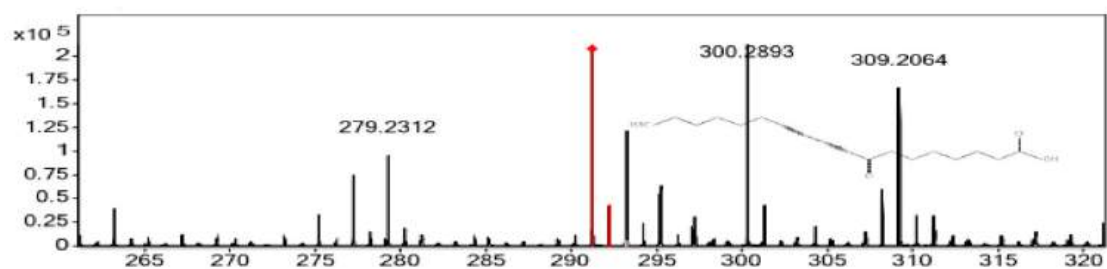
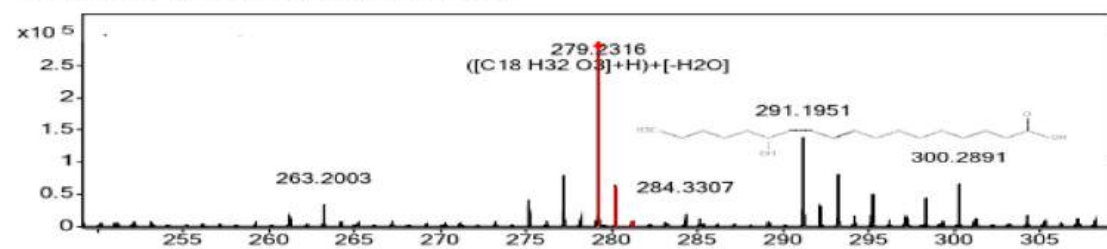


Fig. 14 (iv): Mass spectra of compounds detected by HR-LC/MS analysis of methanolic extract of *M. xanthopus*

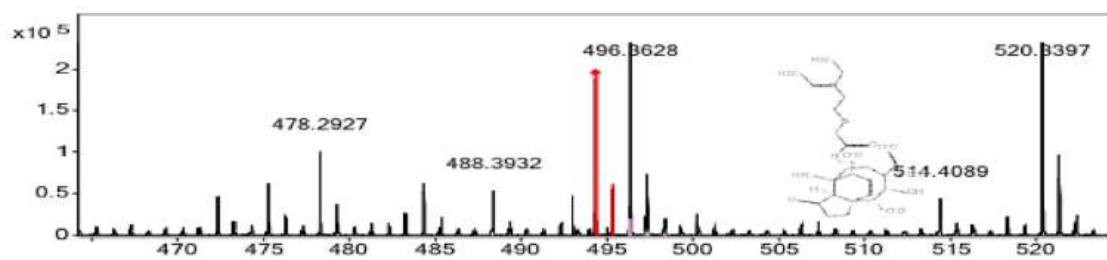
8-Oxo 9, 11-octadecadienoic acid



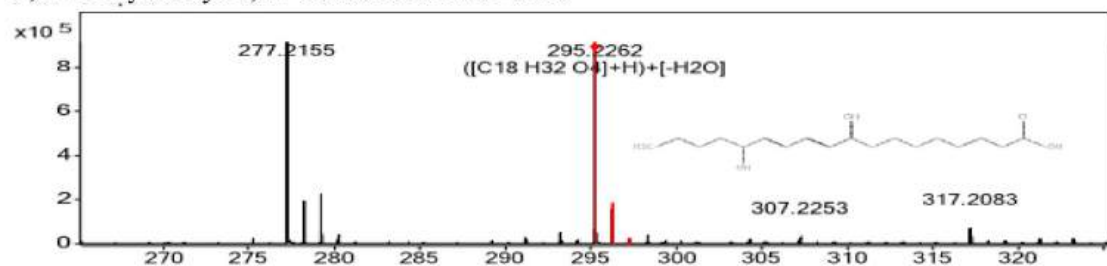
13-Hydroxy 9, 12-octadecadienoic acid



Tilamulin



9,14-Dihydroxy10,12-octadecadienoic acid



2- Tetracos15-enamidoethanesulfonic acid

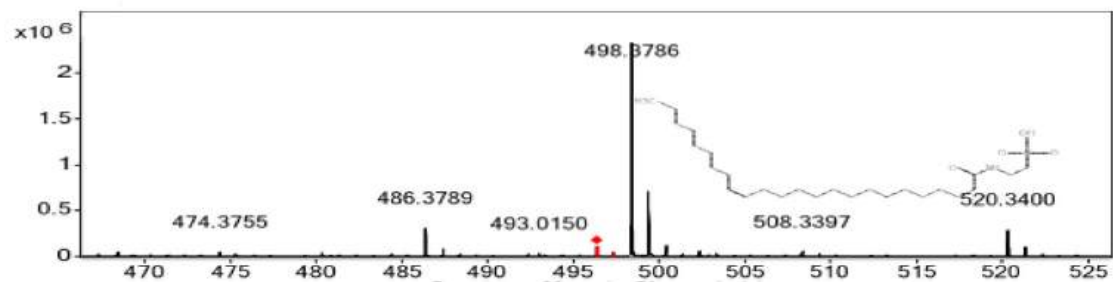
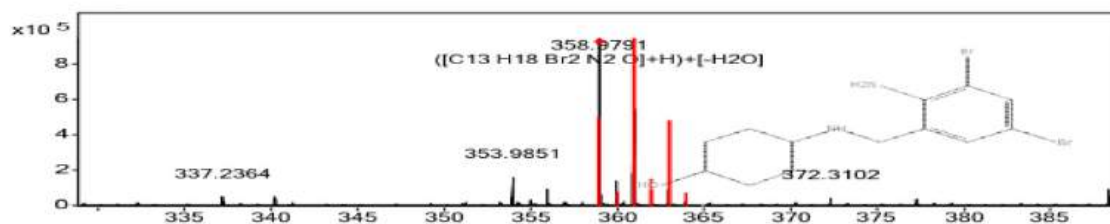
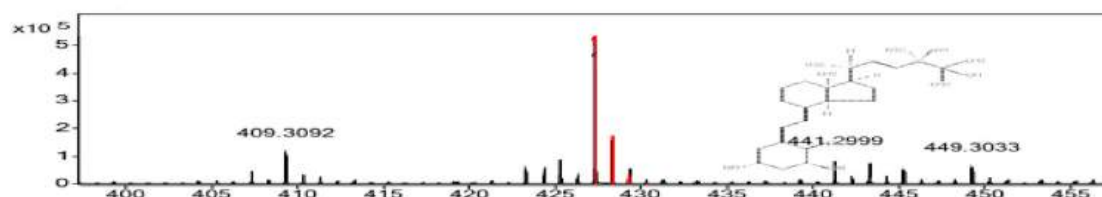


Fig. 14 (v): Mass spectra of compounds detected by HR-LC/MS analysis of methanolic extract of *M. xanthopus*

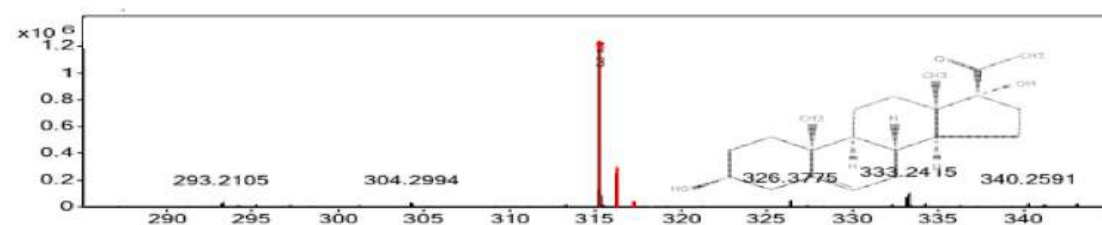
Ambroxol



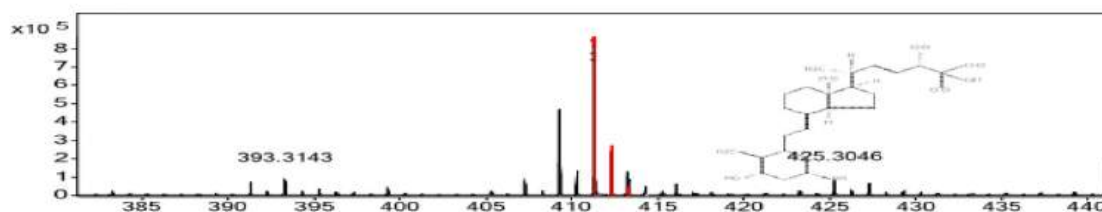
1,24,25-Trihydroxy vitamin D2



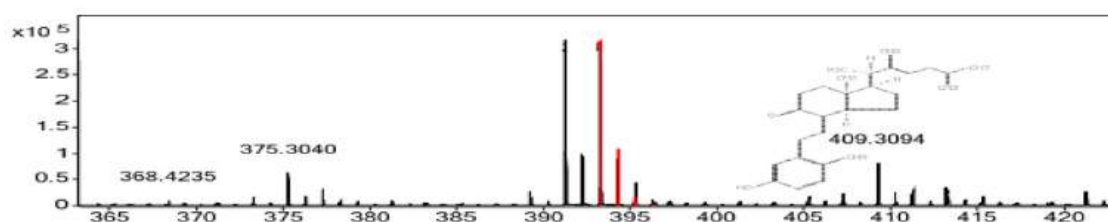
17 α -Hydroxypregnenolone



1 α ,25-Dihydroxyvitamin D2



Calciferol



14-Hydroxy 5-tetradecenoic acid

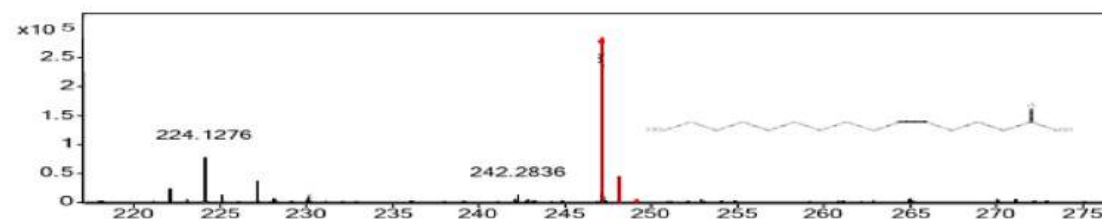


Fig. 14 (vi): Mass spectra of compounds detected by HR-LC/MS analysis of methanolic extract of *M. xanthopus*

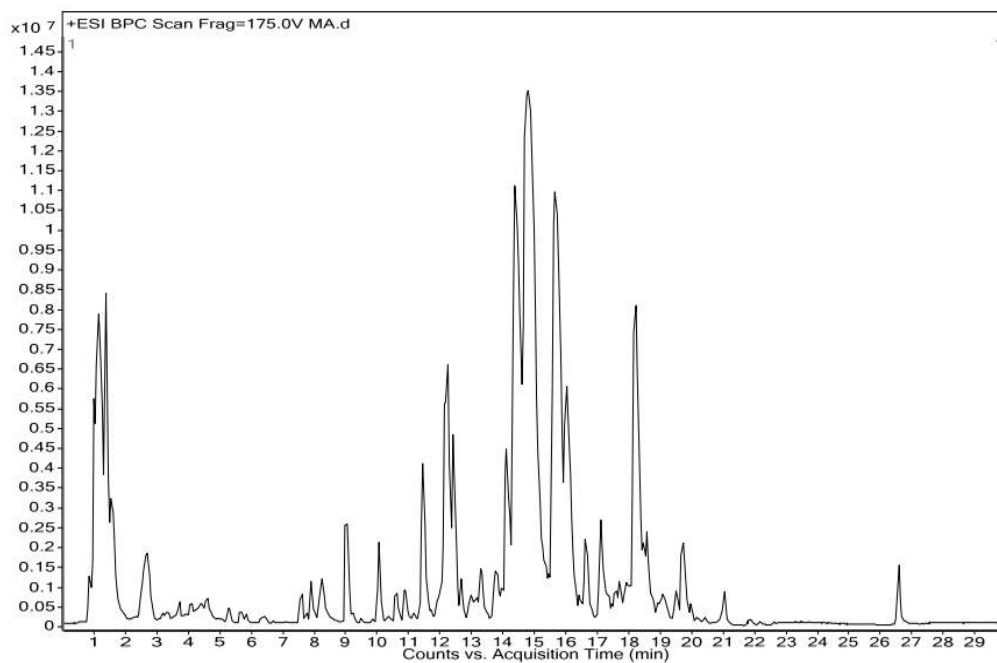


Fig. 15a Liquid chromatogram for positive ionization compounds in the methanolic extract of *M. affinis*

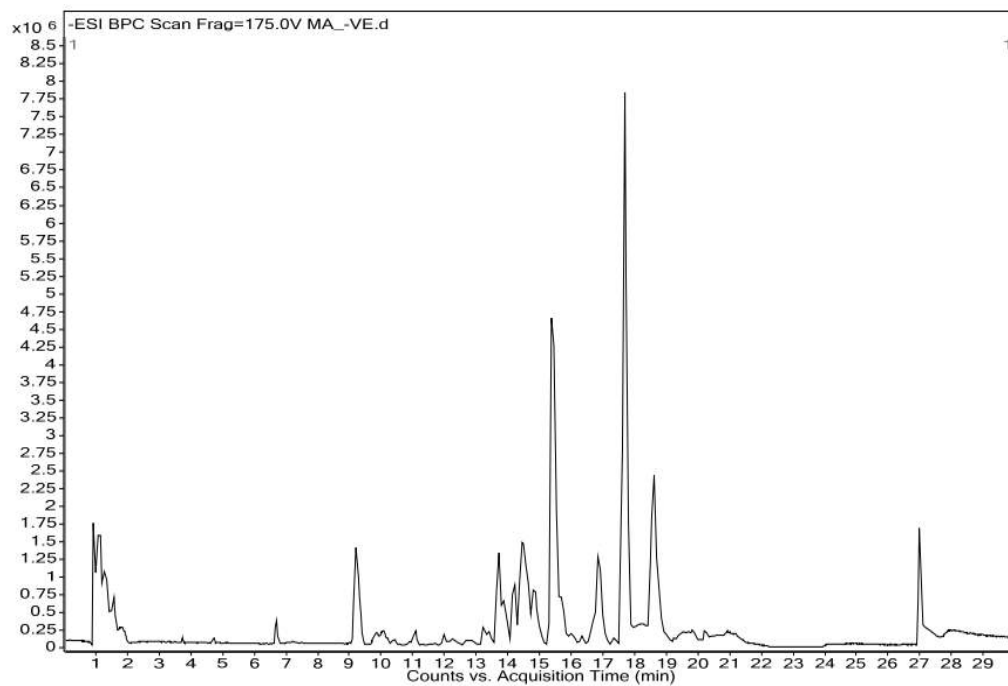
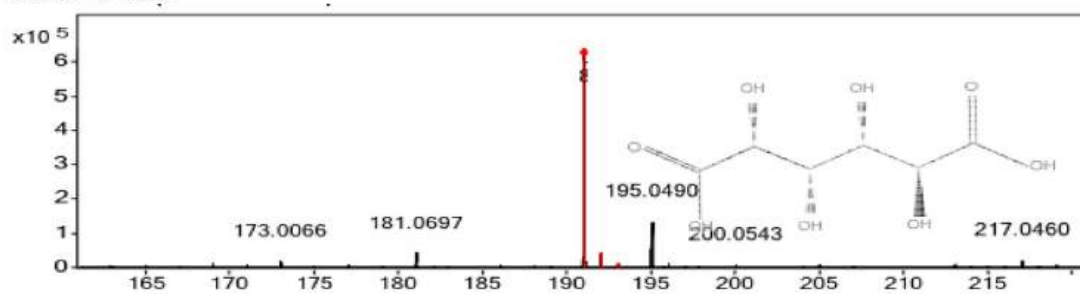
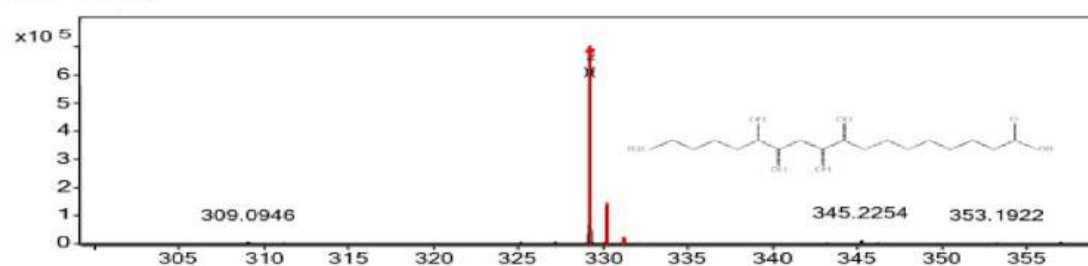


Fig. 15b Liquid chromatogram for negative ionization compounds in the methanolic extract of *M. affinis*

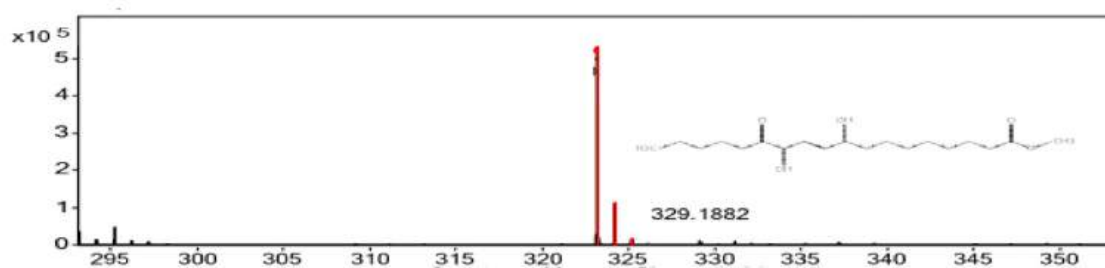
Glucaric acid



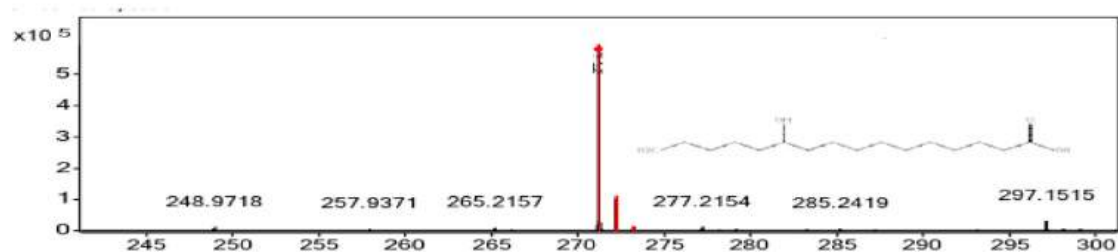
Sativic acid



Methyl 9,12-dihydroxy-13 oxo10-octadecenoate



Jalapinolic acid



9-Hydroxy 12-octadecenoic acid

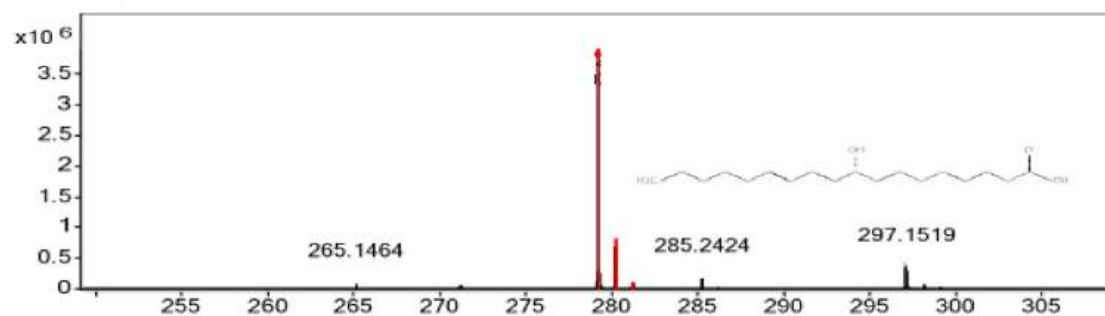
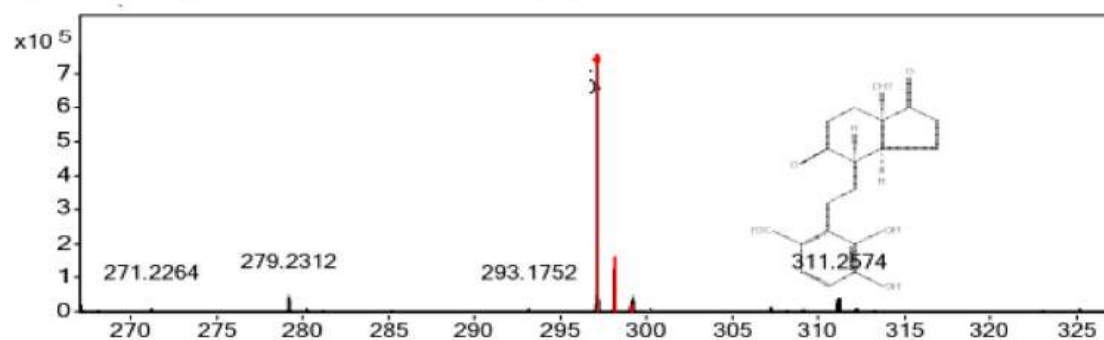
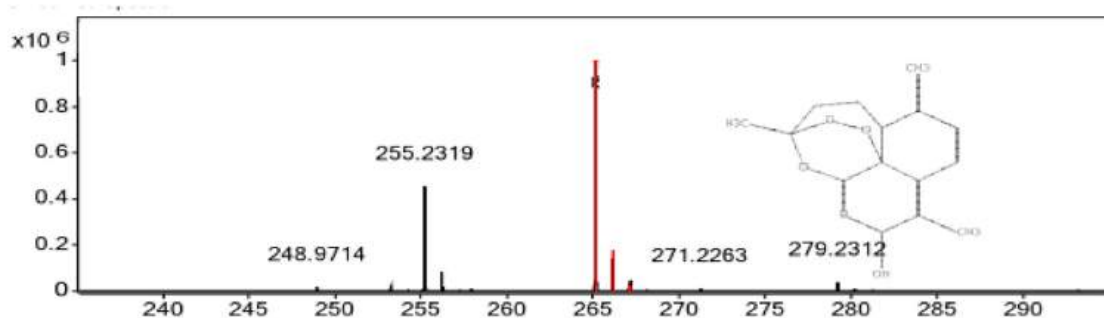


Fig. 15 (i): Mass spectra of compounds detected by HR-LC/MS analysis of methanolic extract of *M. affinis*

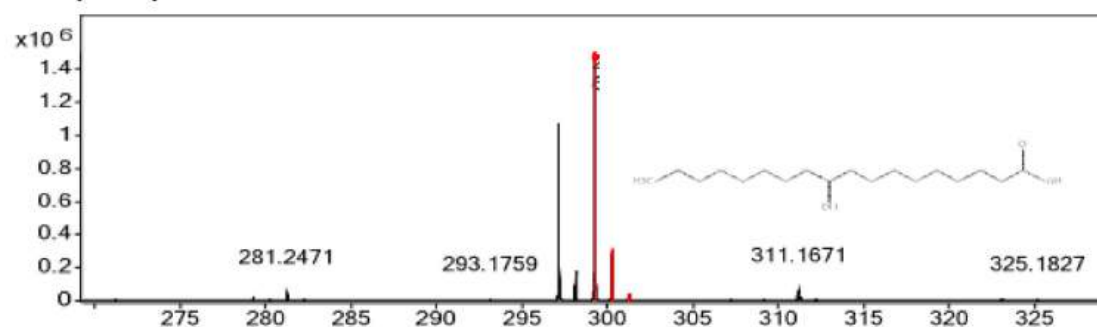
3,4-Dihydroxy- 9,10 secoandrosta 1,3,5 (10) triene-9,17-dione



Dihydroartemisinin



10-Hydroxystearic acid



16-Hydroxy – 4- oxoretinoic acid

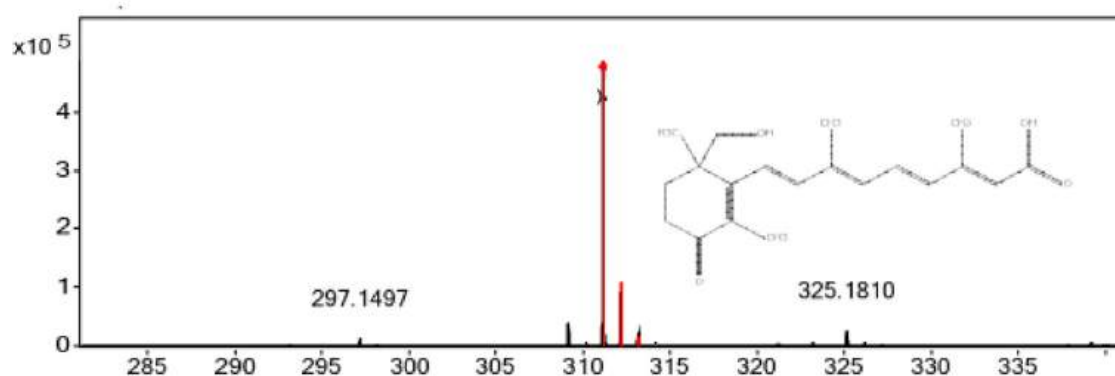


Fig. 15 (ii): Mass spectra of compounds detected by HR-LC/MS analysis of methanolic extract of *M. affinis*

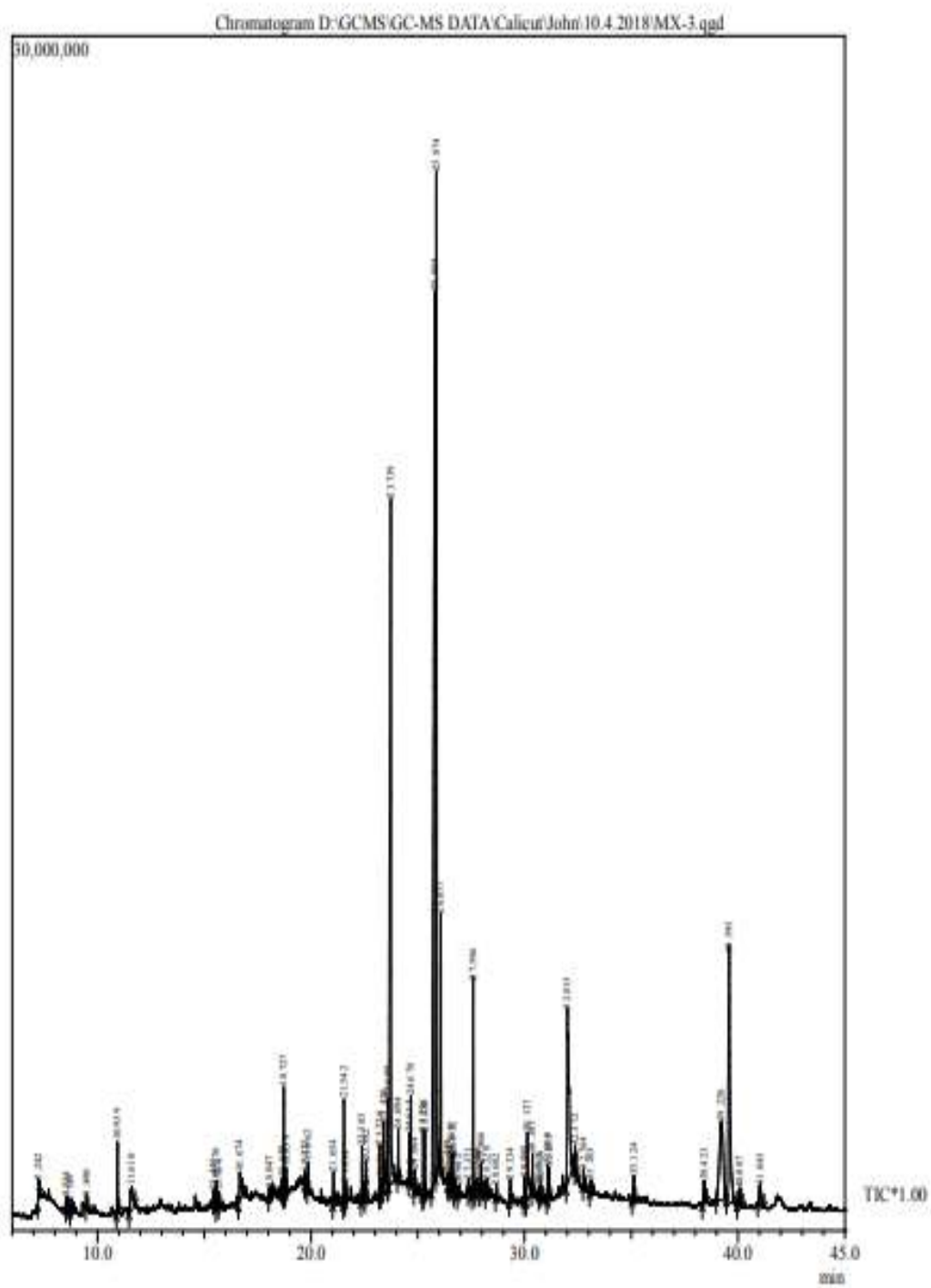
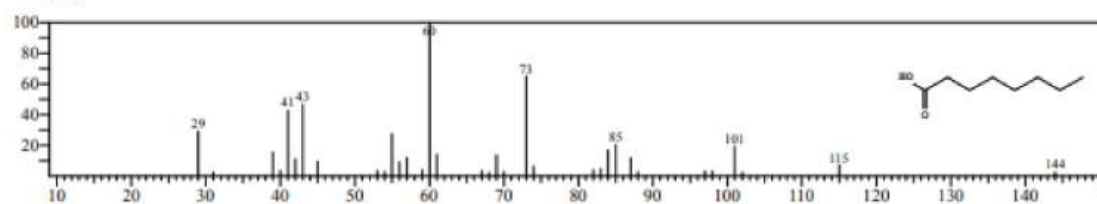
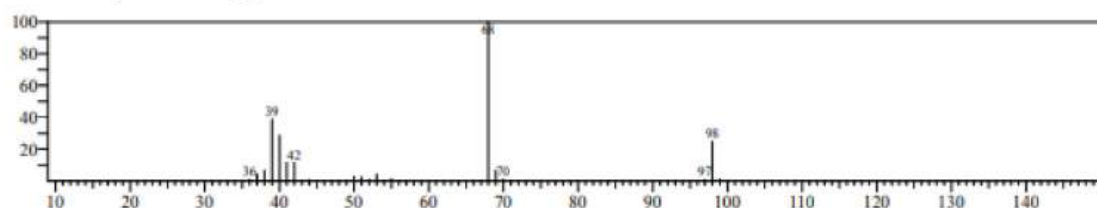


Fig. 16a: Gas chromatogram of methanolic extract of *M. xanthopus*

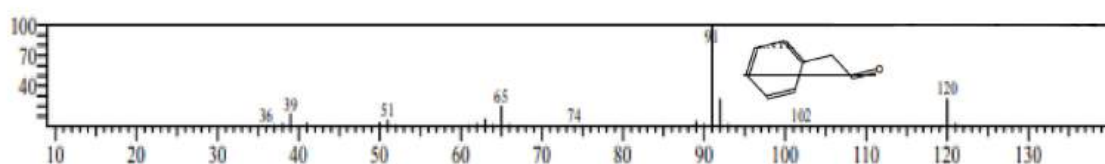
Caprylic acid



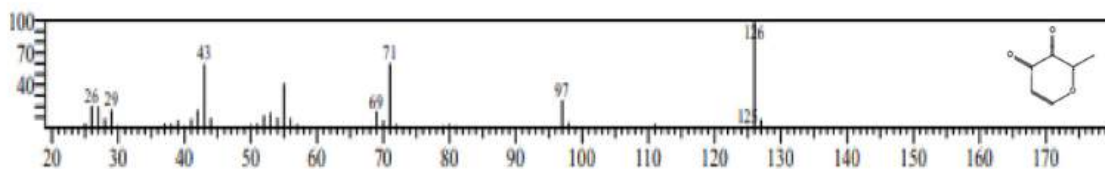
2,6-Dihydro-2H-pyran -3-one



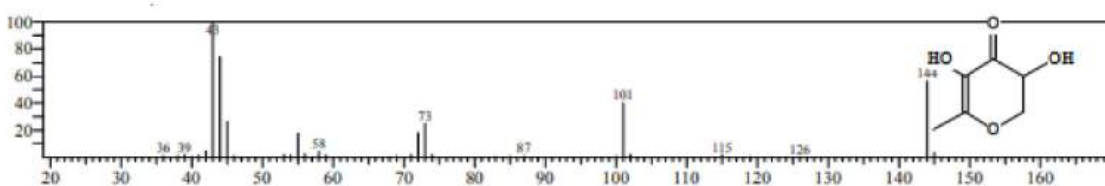
Phenylacetaldehyde



Maltol



3,5-Dihydroxy-6-methyl-2,3-dihydropyran-4-one



Glutamine

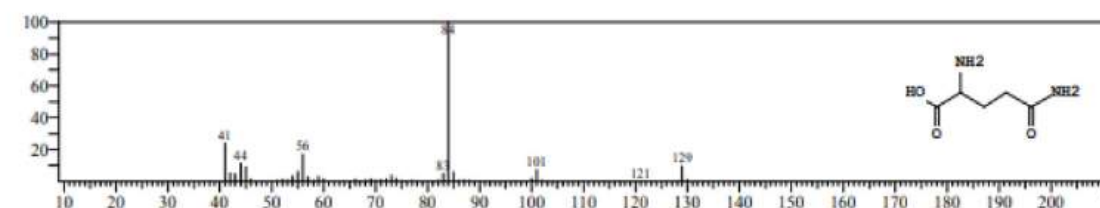
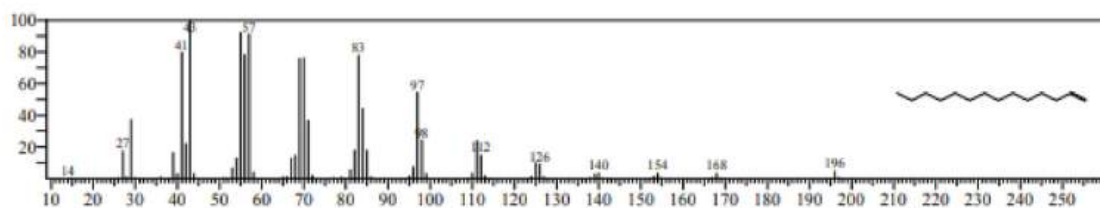
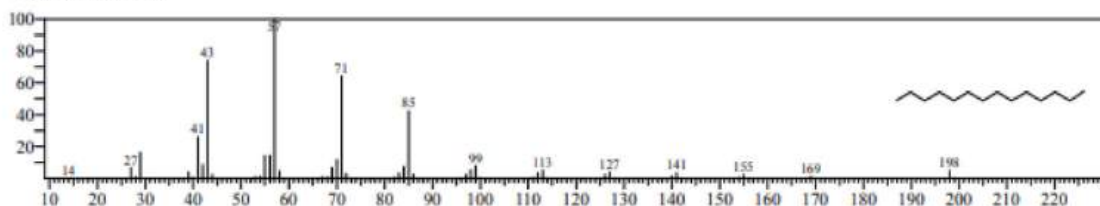


Fig. 17 (i): Mass spectra of compounds detected by GC/MS analysis of methanolic extract of *M. xanthopus*

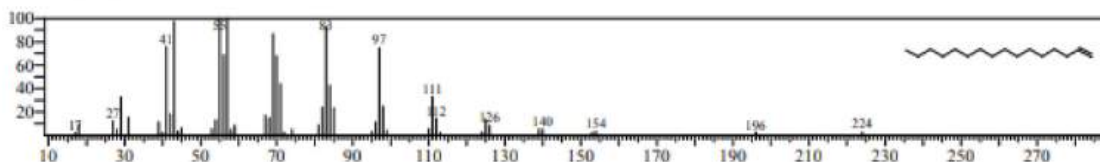
Tetradecene



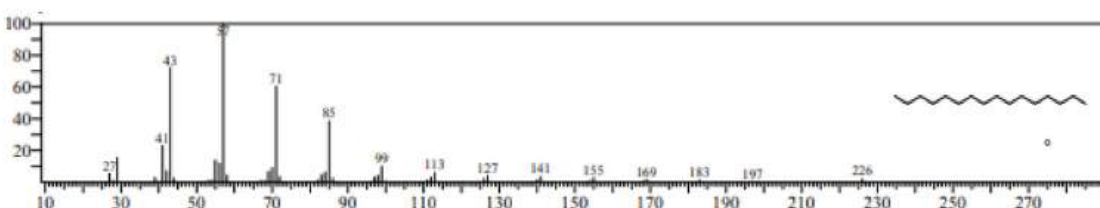
Tetradecane



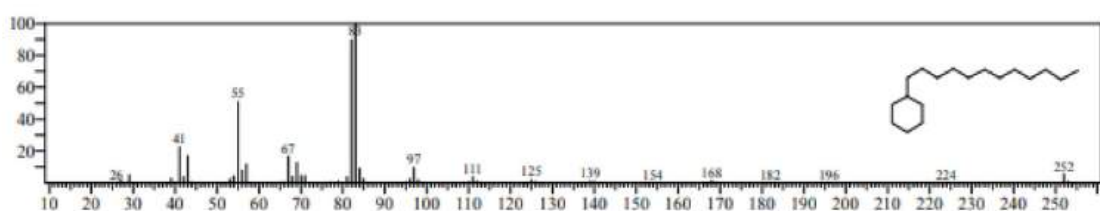
Hexadecene



Hexadecane



Dodecyl cyclohexane



8-Pentadecanone

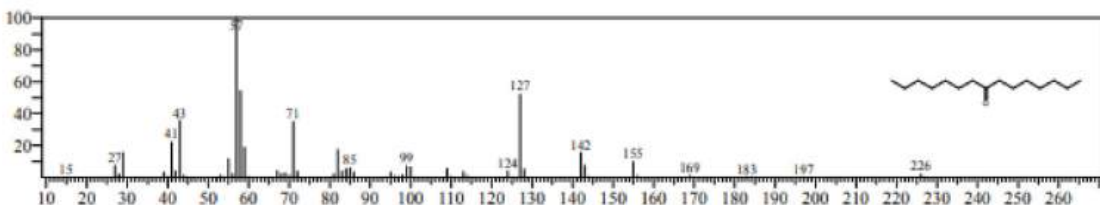
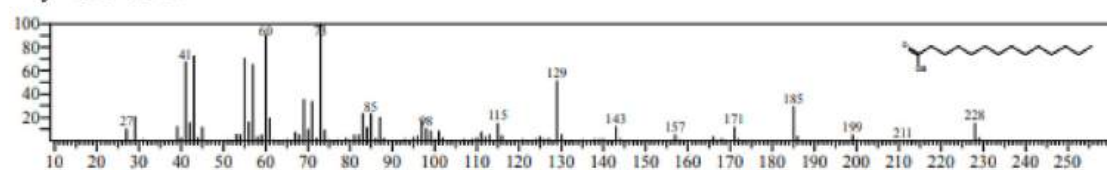
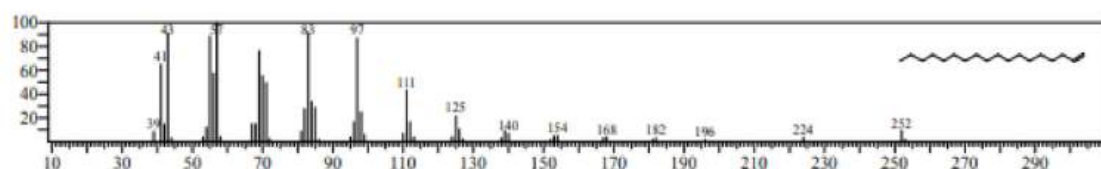


Fig. 17 (ii): Mass spectra of compounds detected by GC/MS analysis of methanolic extract of *M. xanthopus*

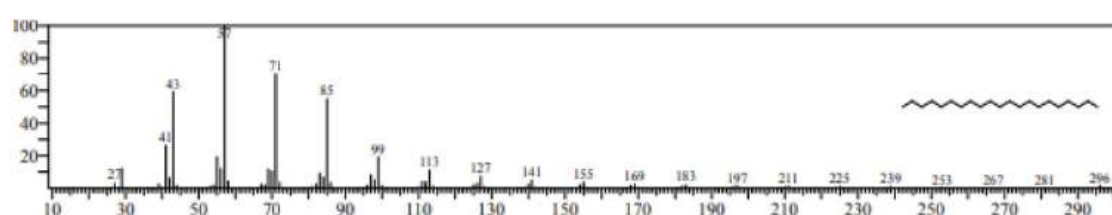
Myristic acid



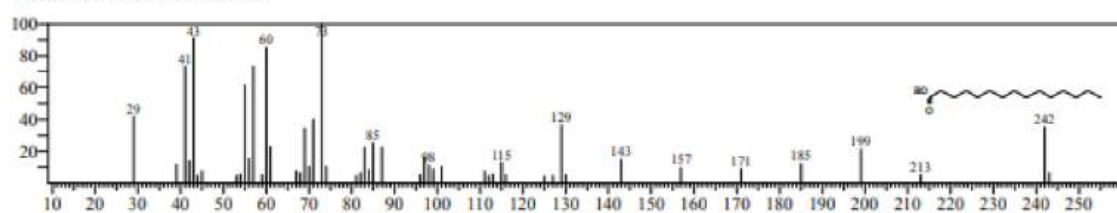
Octadecene



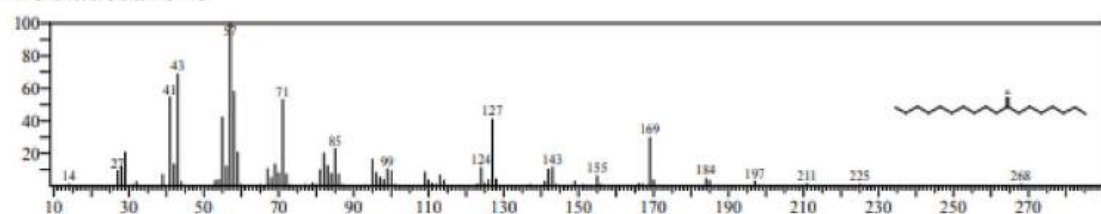
Heneicosane



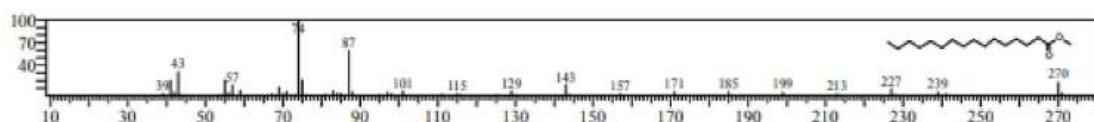
Pentadecanoic acid



8-Octadecanone



Methyl palmitate



Benzenepropanoic acid 3,5-bis (1,1-dimethylethyl)-4-hydroxy methyl ester

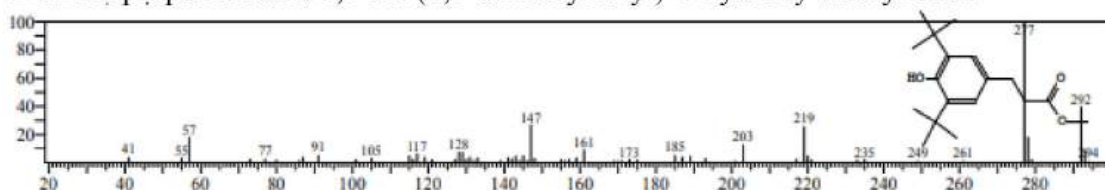
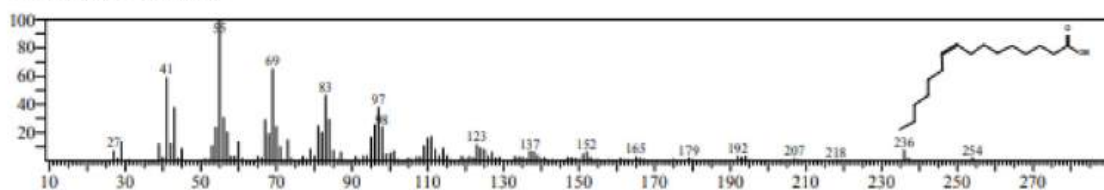
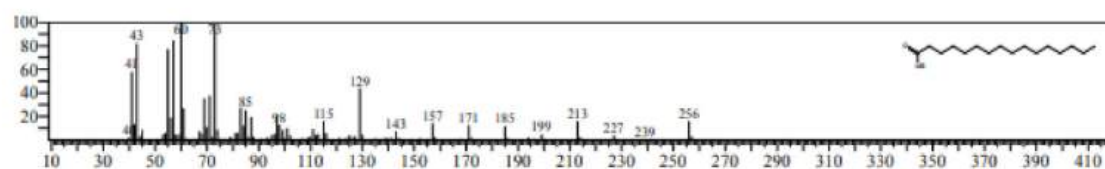


Fig. 17 (iii): Mass spectra of compounds detected by GC/MS analysis of methanolic extract of *M. xanthopus*

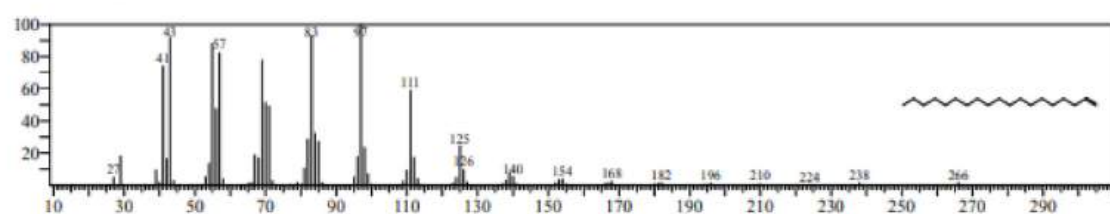
Palmitoleic acid



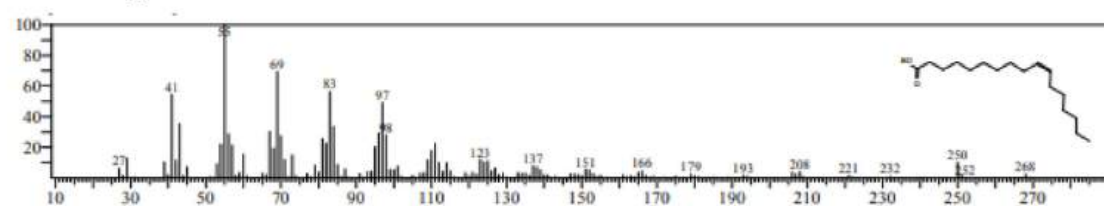
Palmitic acid



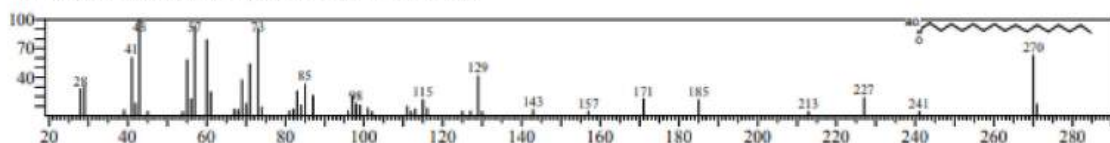
Nonadecene



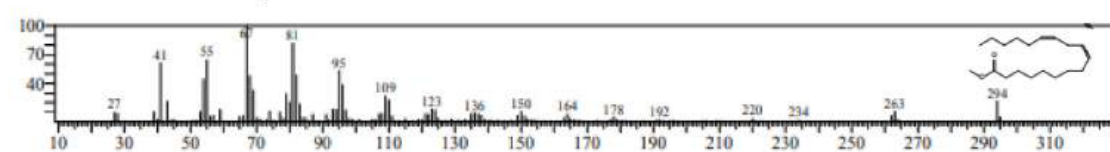
Cis-10-heptadecenoic acid



Margaric acid (Heptadecenoic acid)



Linoleic acid methyl ester



Oleic acid methyl ester

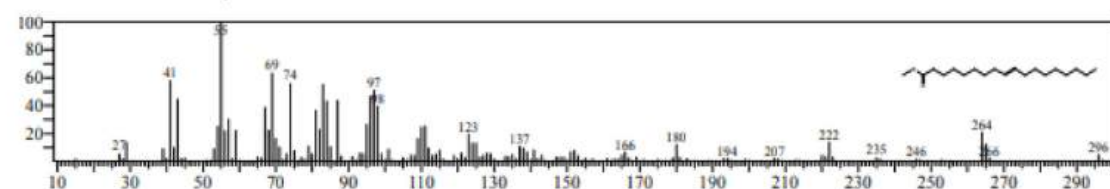
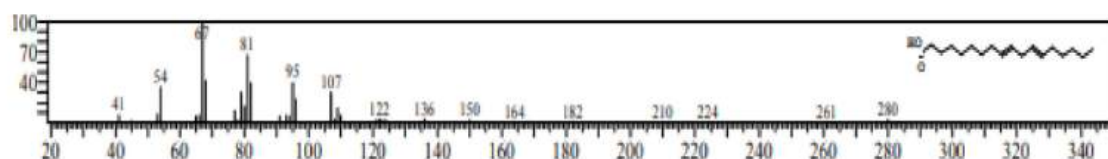
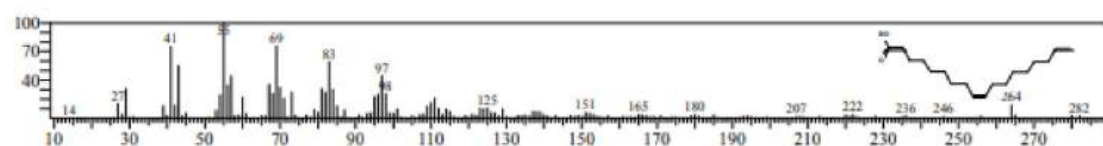


Fig. 17 (iv): Mass spectra of compounds detected by GC/MS analysis of methanolic extract of *M. xanthopus*

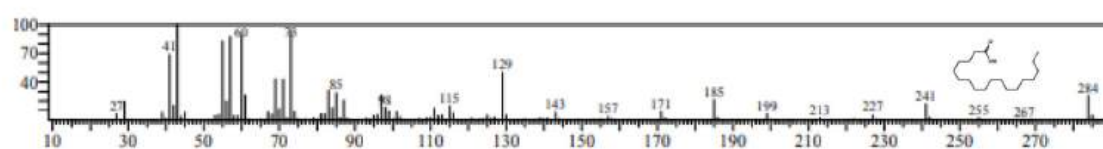
Linoleic acid



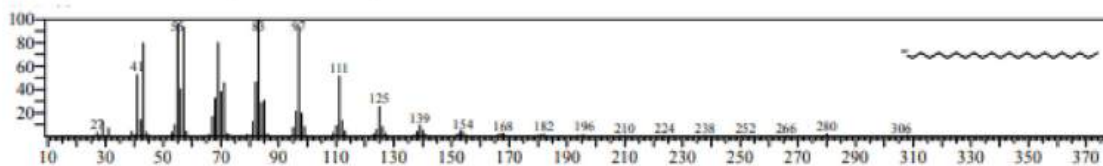
Oleic acid



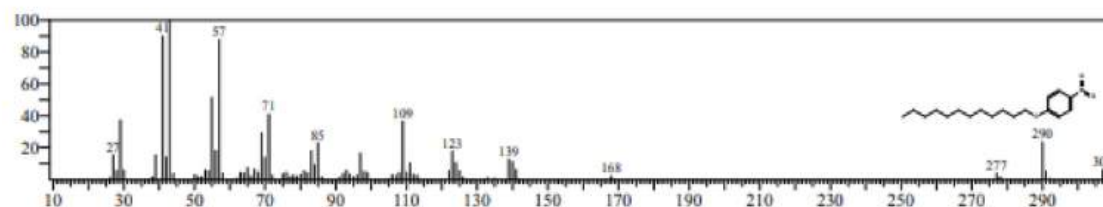
Stearic acid (Octadecanoic acid)



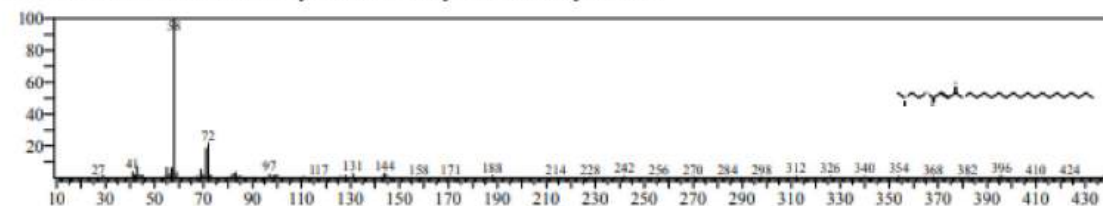
Behenic alcohol (Docosanol)



Dodecyl 4-nitrophenyl ether



Fumaric acid 2-dimethylaminoethyl octadecyl ester



9,12-Octadecadienal dimethyl acetal

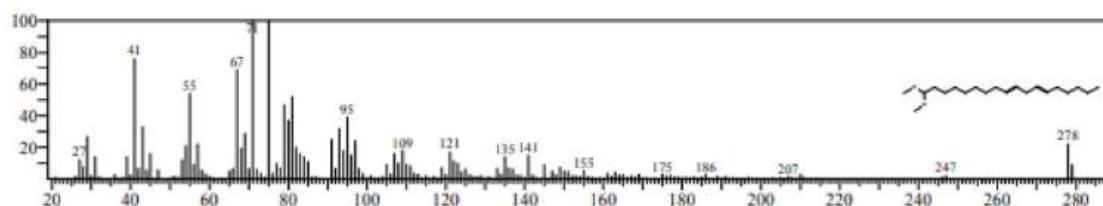
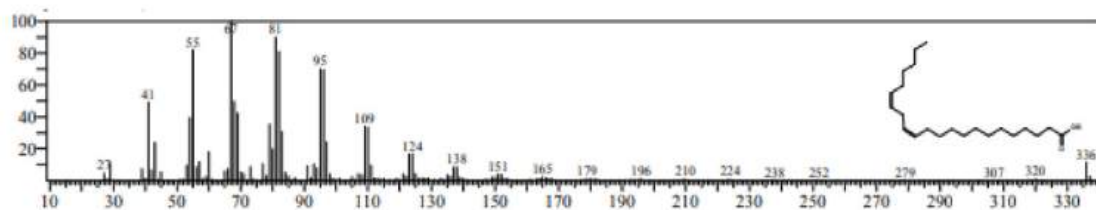
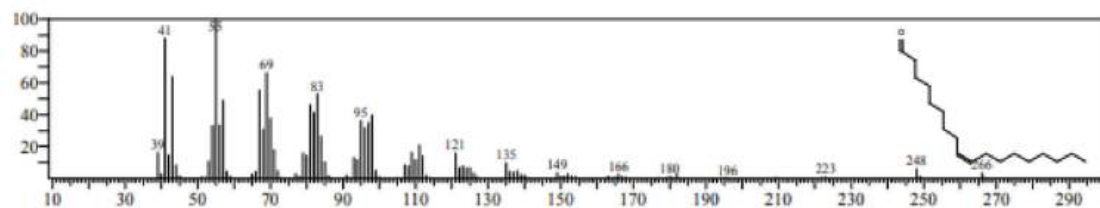


Fig. 17 (v): Mass spectra of compounds detected by GC/MS analysis of methanolic extract of *M. xanthopus*

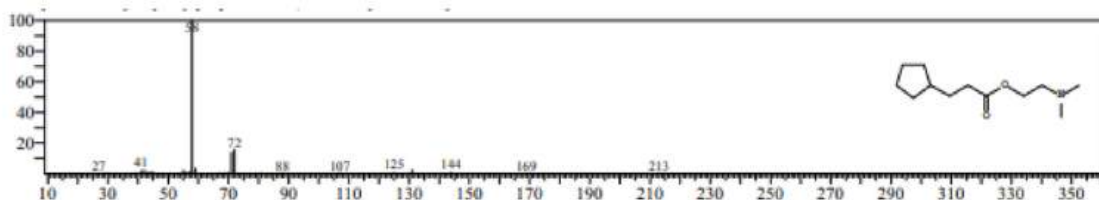
13,16- Docosadienoic acid



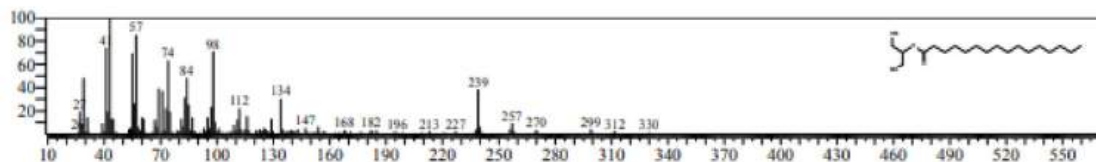
9-Octadecenal



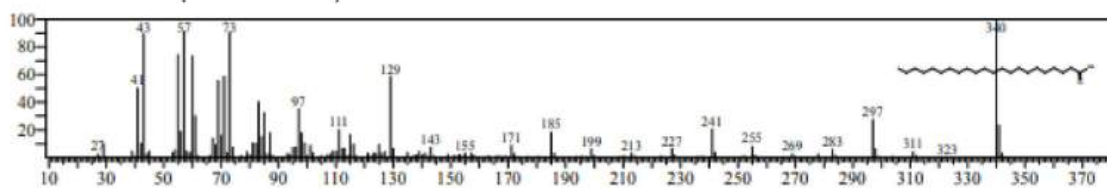
3-Cyclopentylpropionic acid 2-dimethylamino ethyl ester



2- Monopalmitin



Behenic acid (Docosanoic acid)



Tetracontane

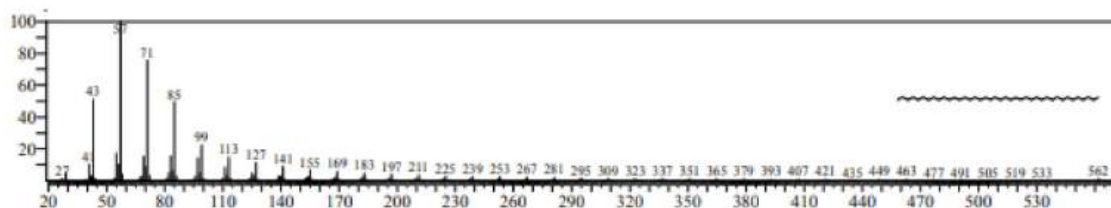
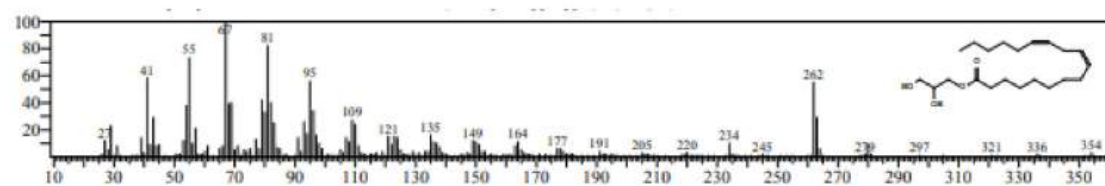
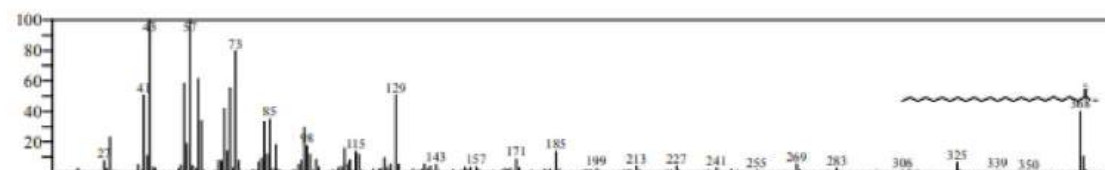


Fig. 17 (vi): Mass spectra of compounds detected by GC/MS analysis of methanolic extract of *M. xanthopus*

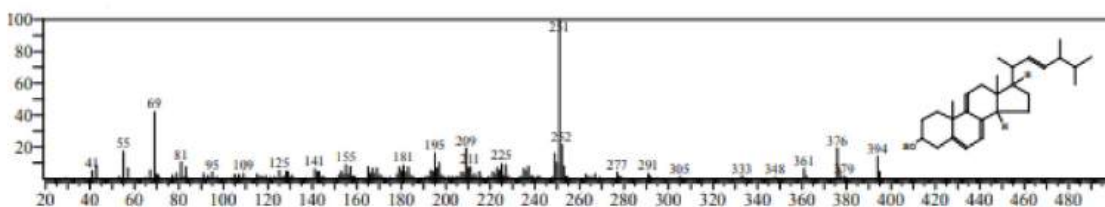
Monoolein



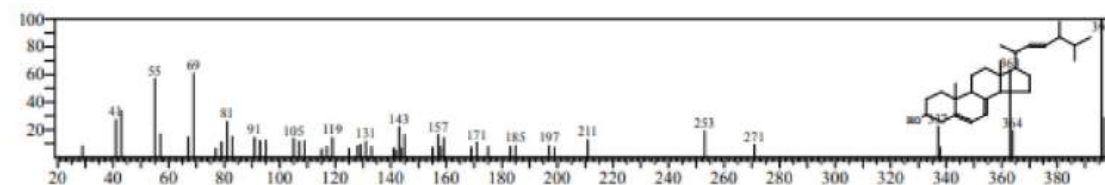
Lignoceric acid (Tetracosanoic acid)



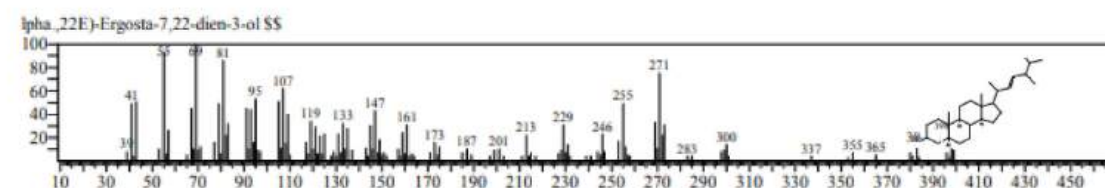
Dehydroergosterol



Ergosterol



Stellasterol



Fungisterol

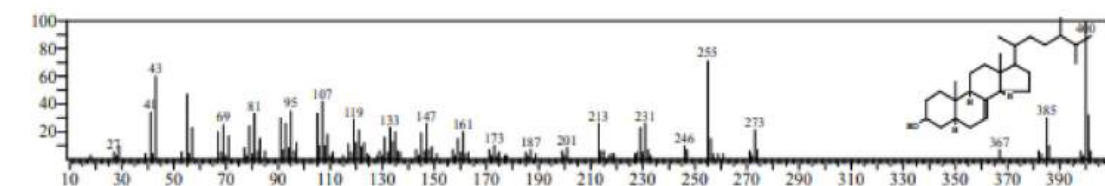


Fig. 17 (vii): Mass spectra of compounds detected by GC/MS analysis of methanolic extract of *M. xanthopus*

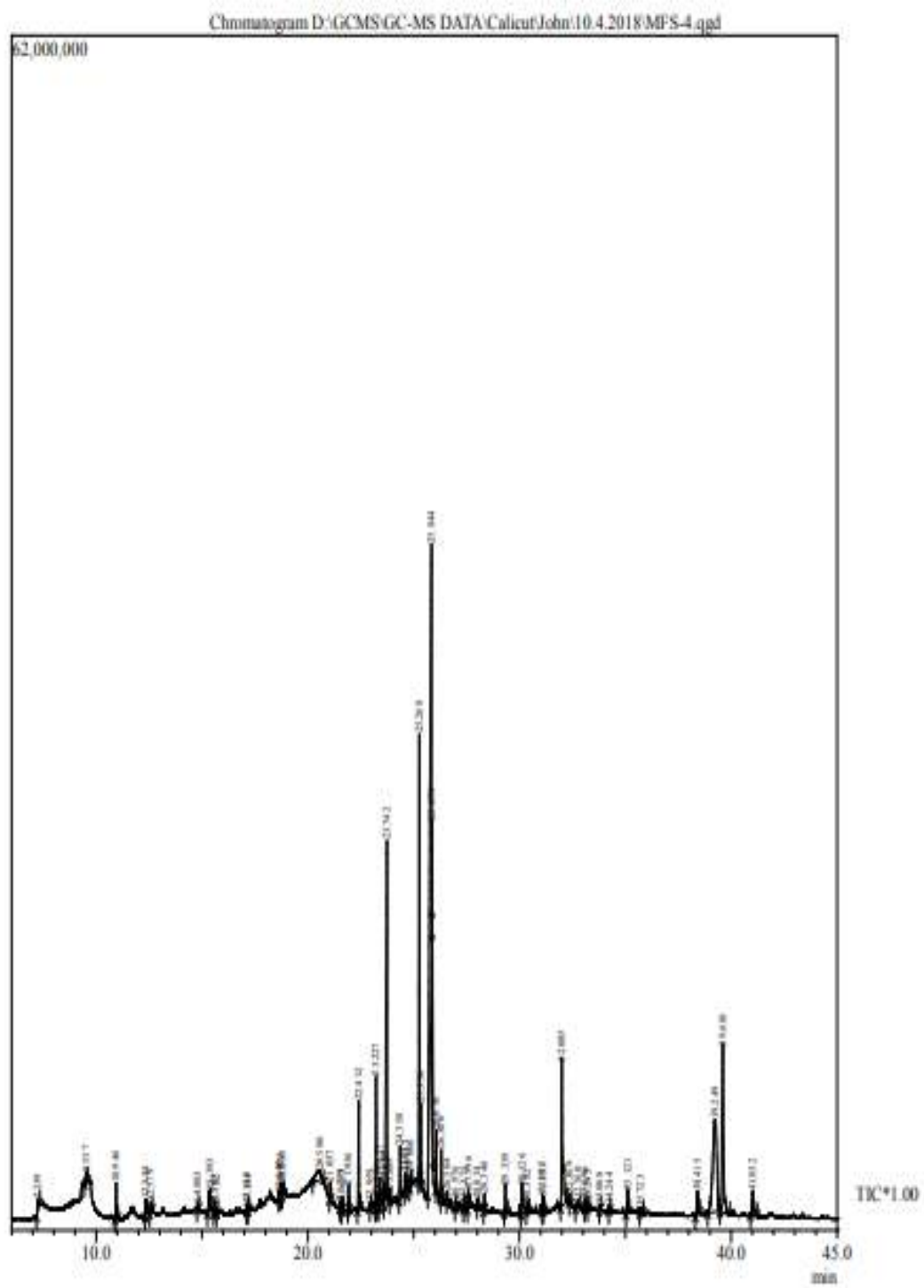
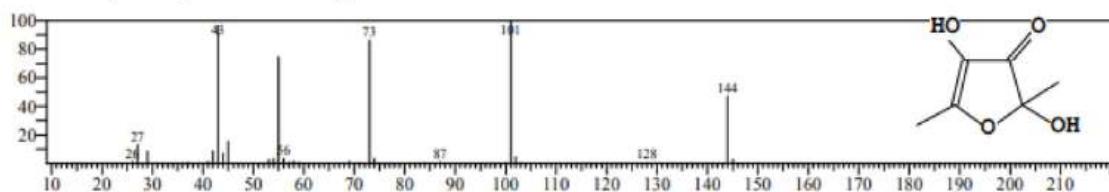
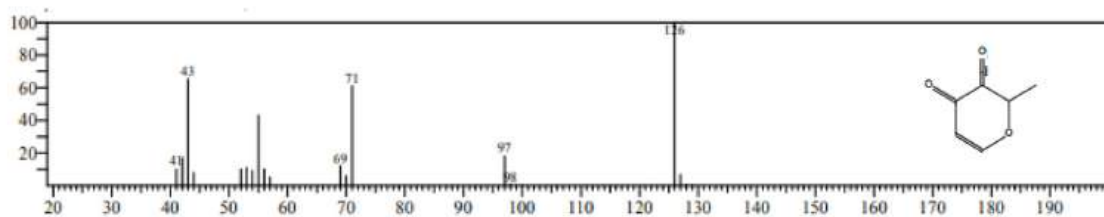


Fig. 16b: Gas chromatogram of methanolic extract of *M. affinis*

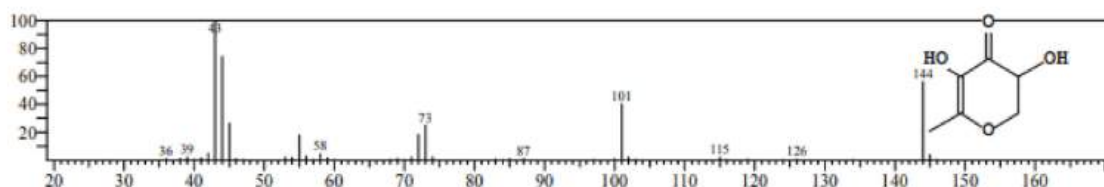
2,4-dihydroxy-2,5-dimethylfuran-3-one



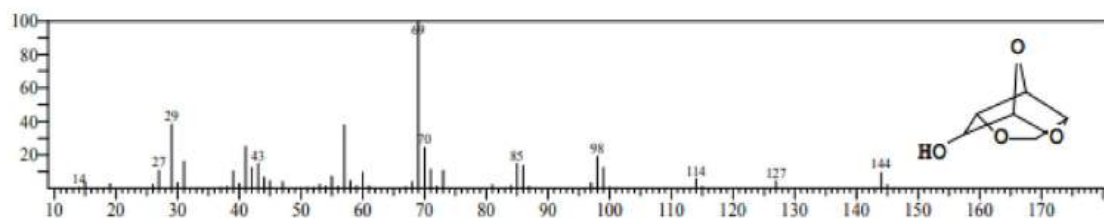
Maltol



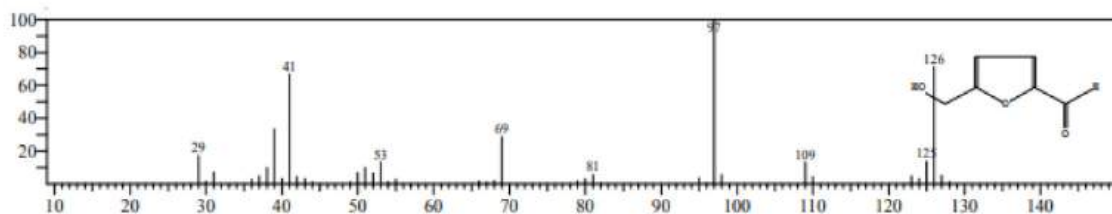
3,5-Dihydroxy-6-methyl-2,3-dihydropyran-4-one.



1,4:3,6- Dianhydro- α -d-glucopyranose



5-Hydroxymethylfurfural



Neric acid

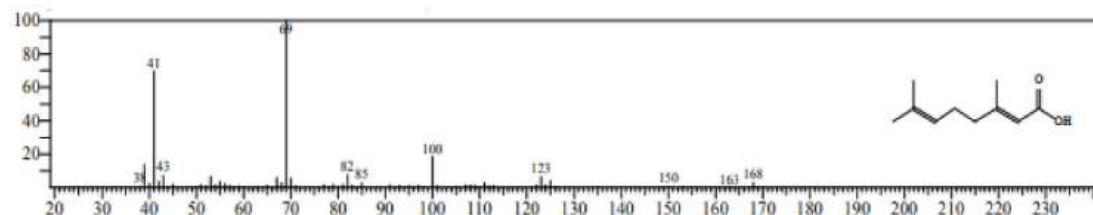
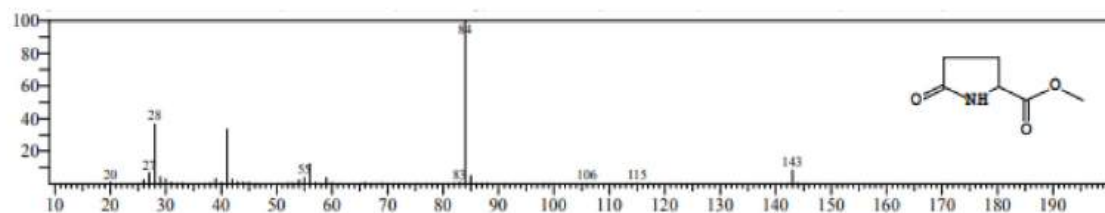
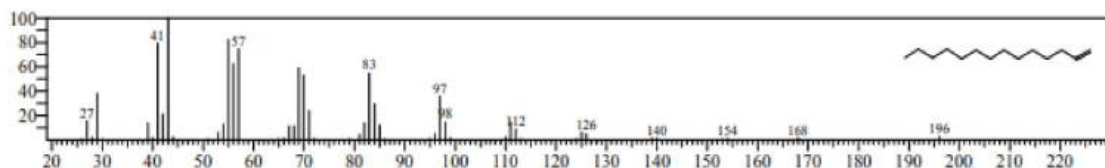


Fig. 18 (i): Mass spectra of compounds detected by GC/MS analysis of methanolic extract of *M. affinis*

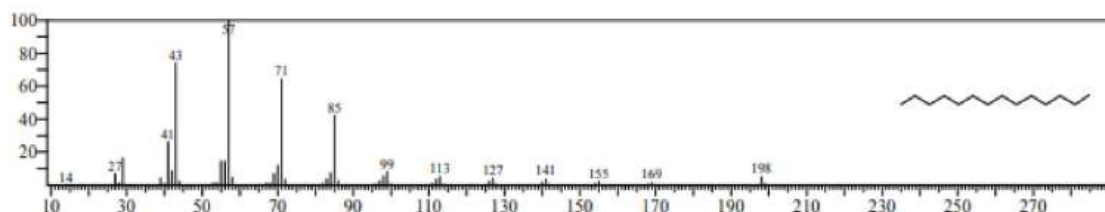
5-Oxo-DL- proline methyl ester



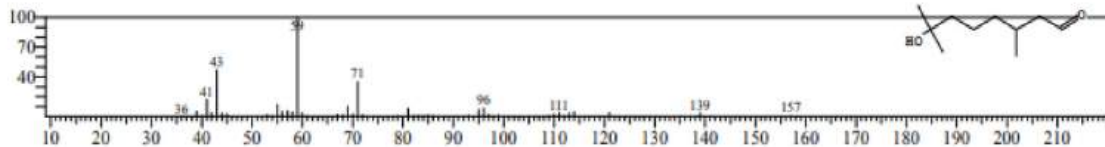
1-Tetradecene



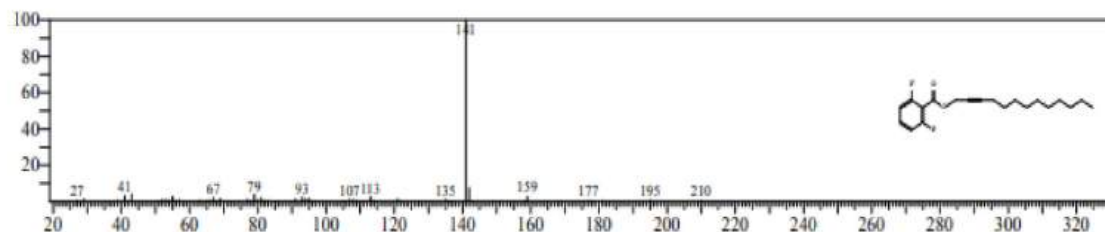
Tetradecane



Hydroxycitronellal



2,6- Difluorobenzoic acid tridec-2-ynyl ester



1-Heptadecene

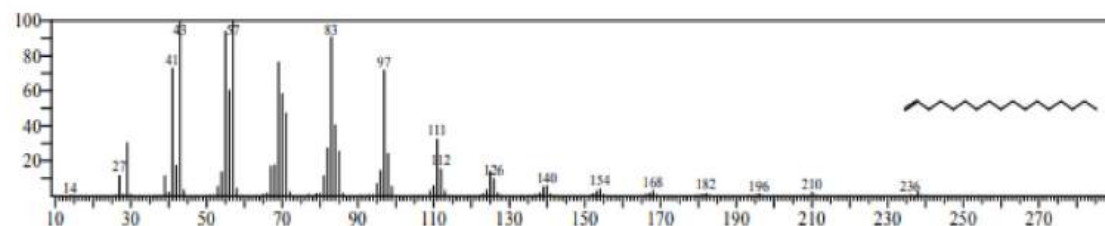
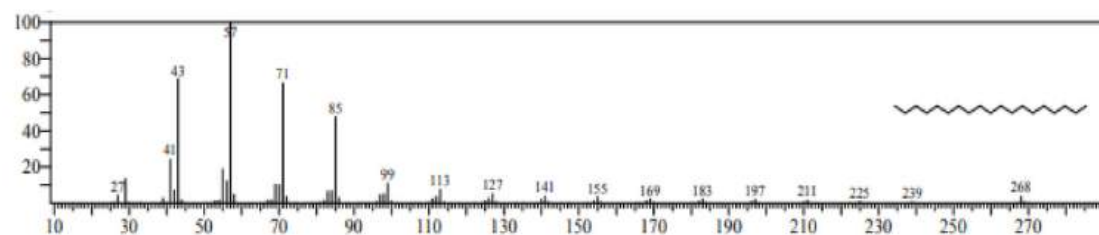
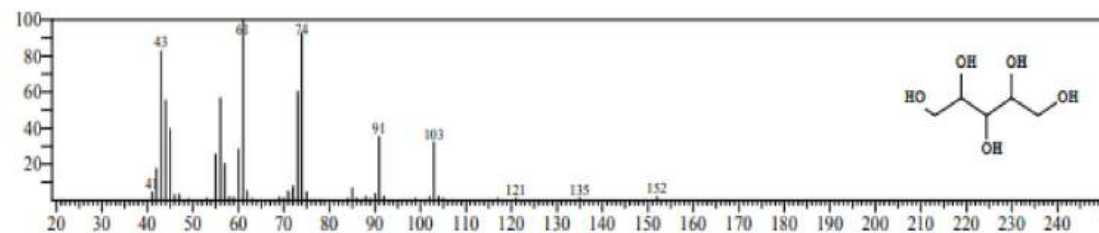


Fig. 18 (ii): Mass spectra of compounds detected by GC/MS analysis of methanolic extract of *M. affinis*

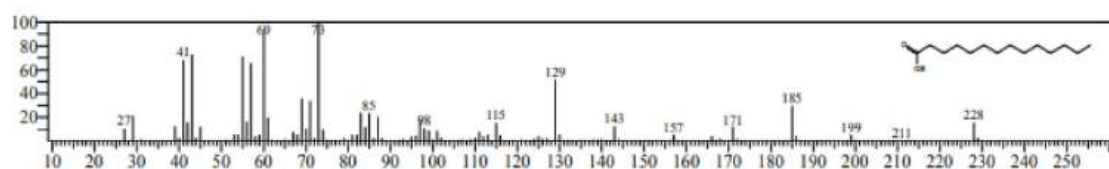
Nonadecane



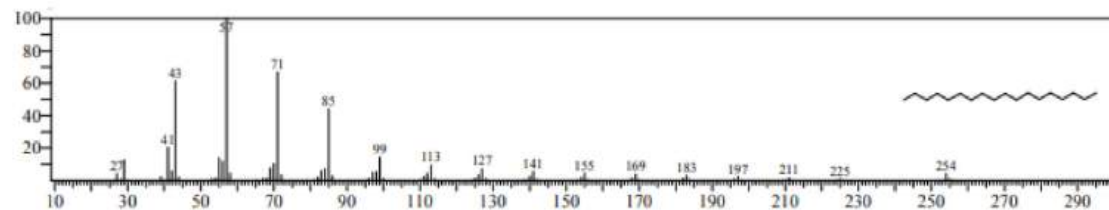
Ribitol



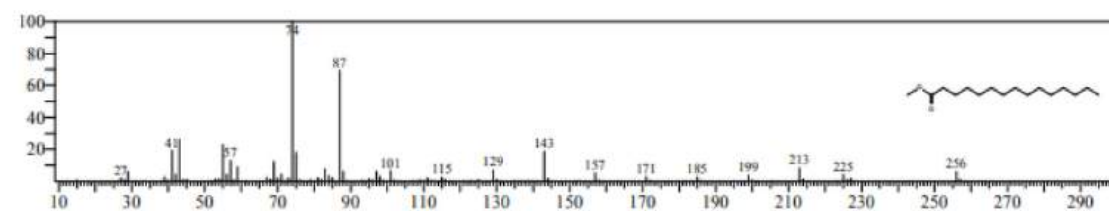
Myristic acid (Tetradecanoic acid)



Octadecane



Pentadecanoic acid methyl ester



Pentadecanoic acid

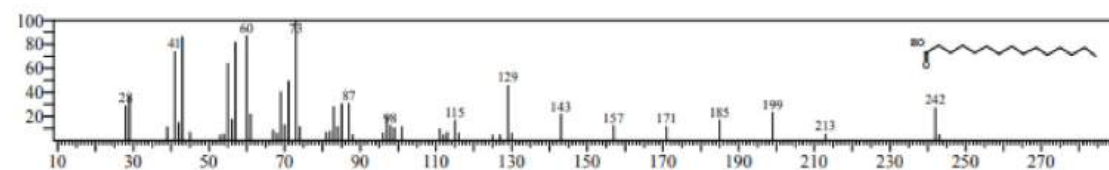
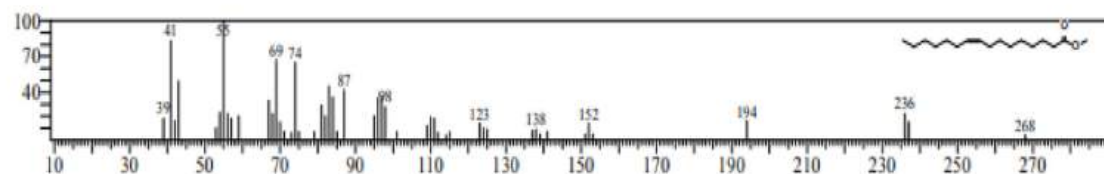
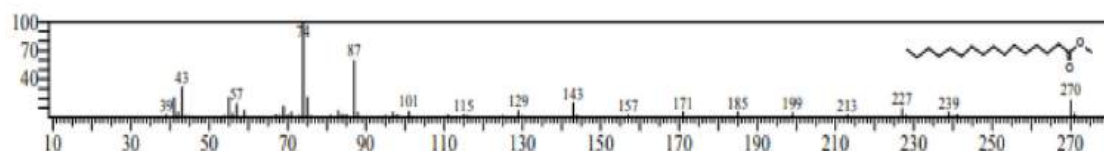


Fig. 18 (iii): Mass spectra of compounds detected by GC/MS analysis of methanolic extract of *M. affinis*

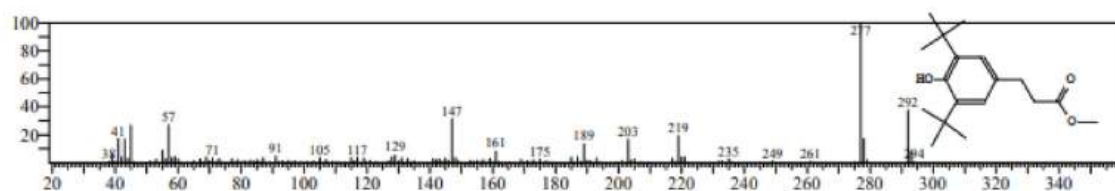
Methyl palmitoleate



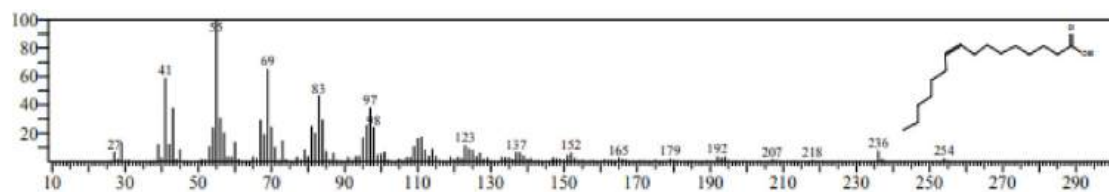
Methyl palmitate



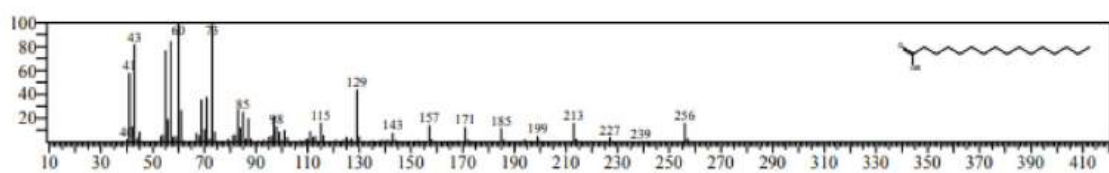
Benzene propanoic acid 3,5-bis (1,1 dimethyl) -4-hydroxy ester



Palmitoleic acid



Palmitic acid



2-Hydroxycyclopentadecanone

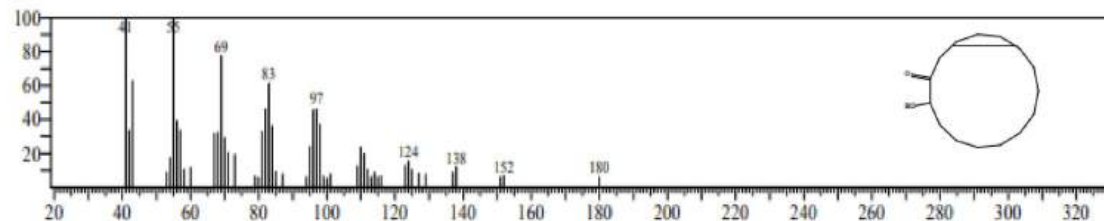
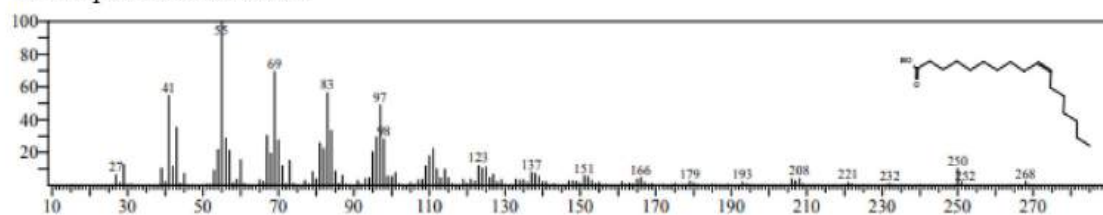
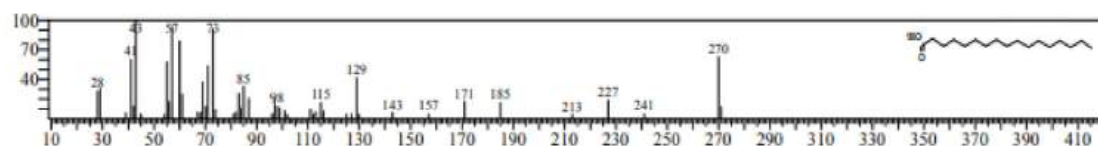


Fig. 18 (iv): Mass spectra of compounds detected by GC/MS analysis of methanolic extract of *M. affinis*

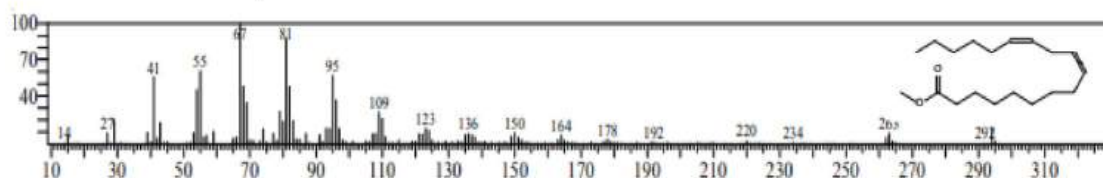
10-Heptadecenoic acid



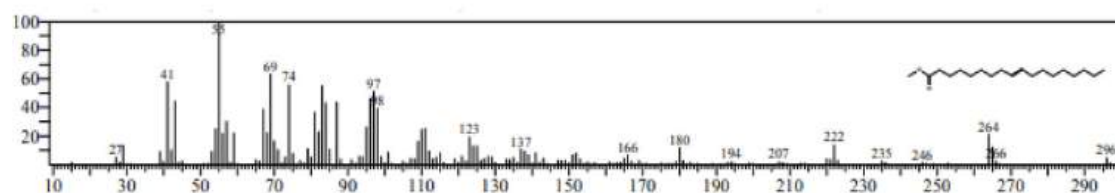
Margaric acid (Heptadecanoic acid)



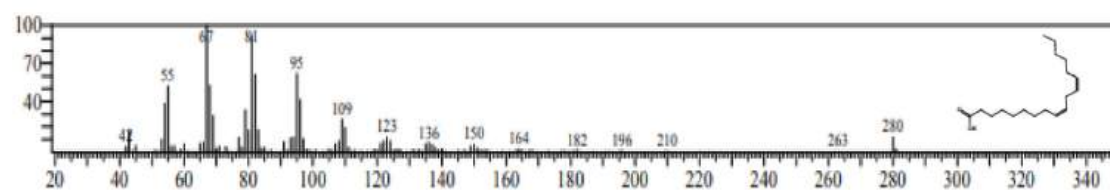
Linoleic acid methyl ester



Oleic acid methyl ester



Linoleic acid



Oleic acid

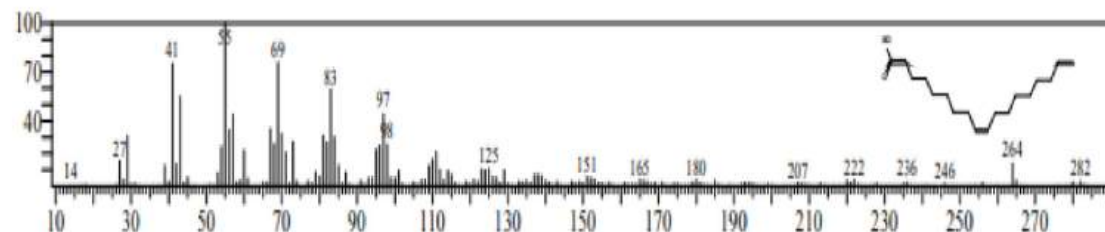
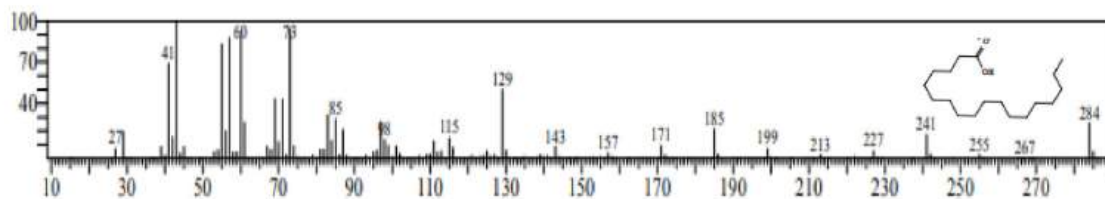
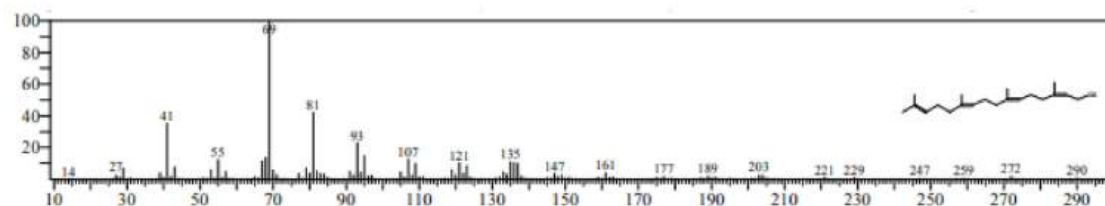


Fig. 18 (v): Mass spectra of compounds detected by GC/MS analysis of methanolic extract of *M. affinis*

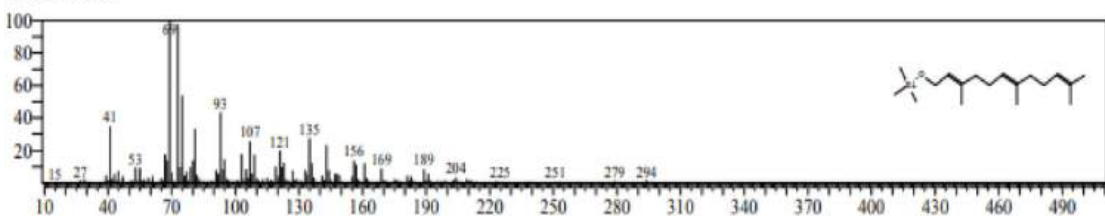
Stearic acid



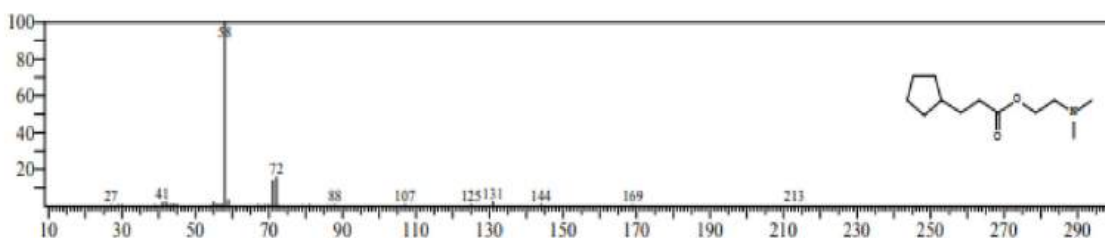
Geranylgeraniol



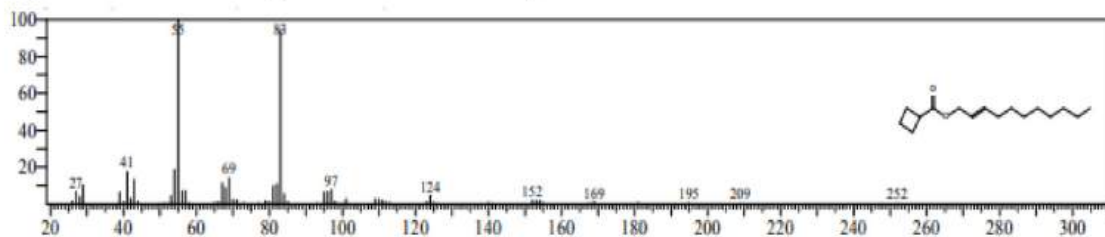
Farnesol



3-Cyclopentylpropionic acid 2-dimethylaminoethyl ester



Cyclobutane carboxylic acid undec-2-enyl ester



2-Monopalmitin

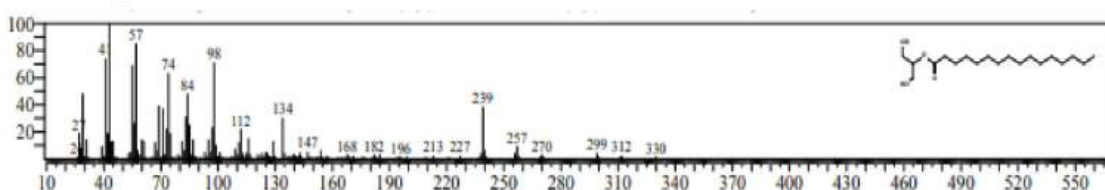
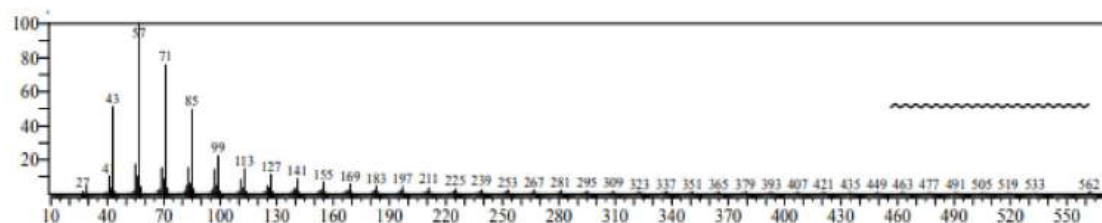
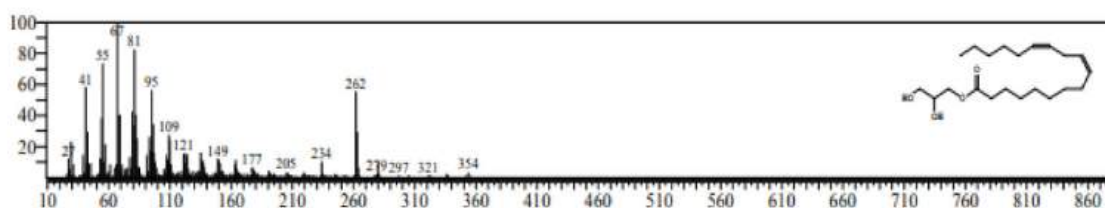


Fig. 18 (vi): Mass spectra of compounds detected by GC/MS analysis of methanolic extract of *M. affinis*

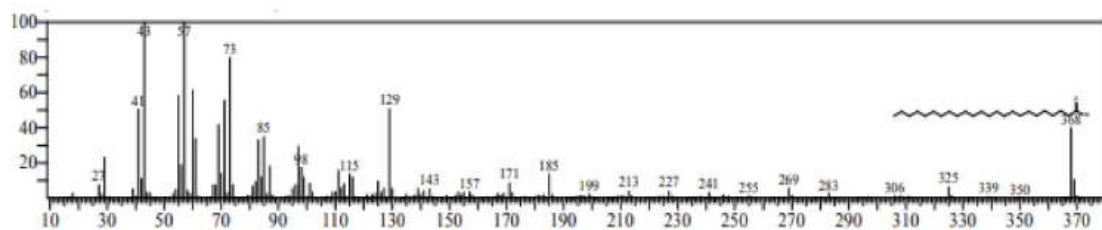
Tetracontane



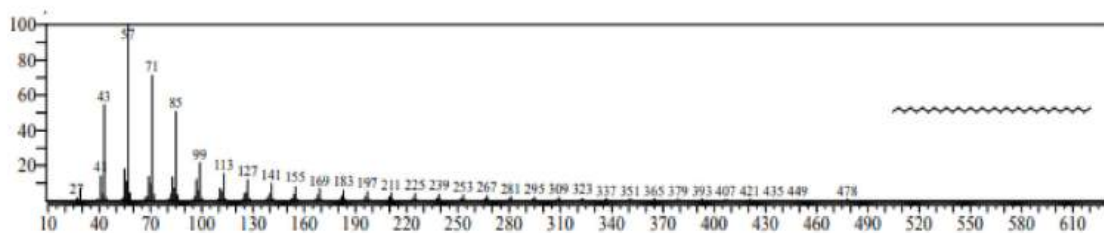
Monoolein



Tetracosanoic acid (Lignoceric acid)



Tetratriacontane



1- Heptacosanol

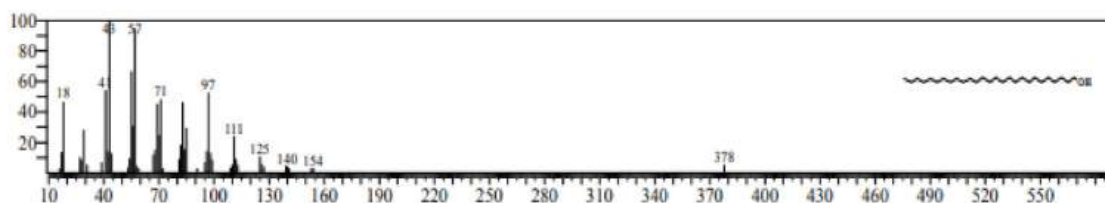
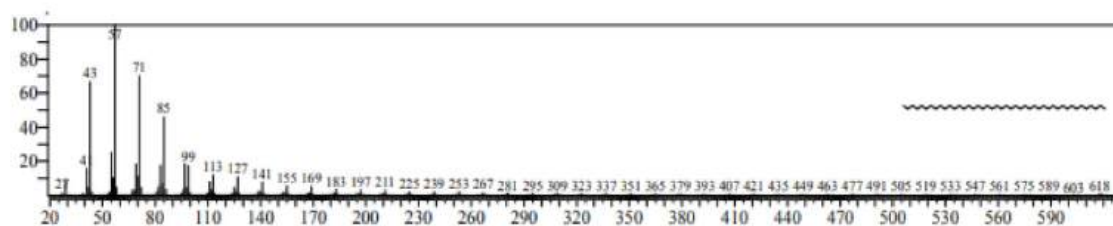
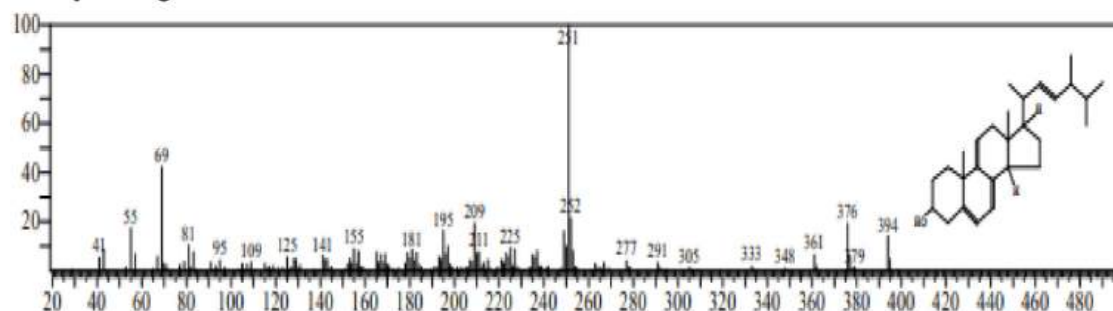


Fig. 18 (vii): Mass spectra of compounds detected by GC/MS analysis of methanolic extract of *M. affinis*

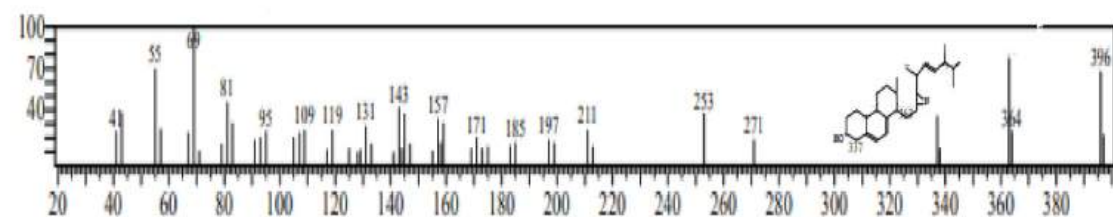
Tetratetracontane



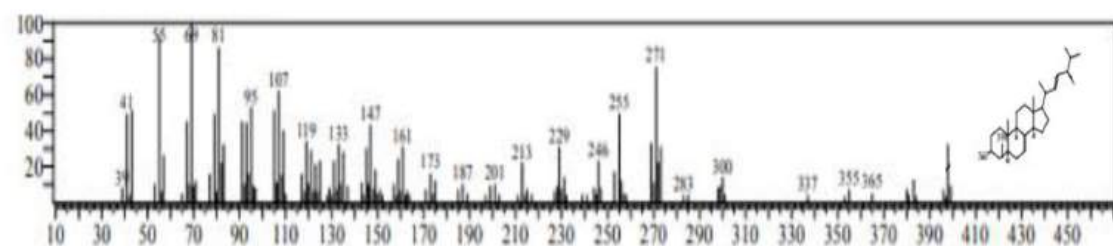
Dehydroergosterol



Ergosterol



Stellasterol



Fungisterol

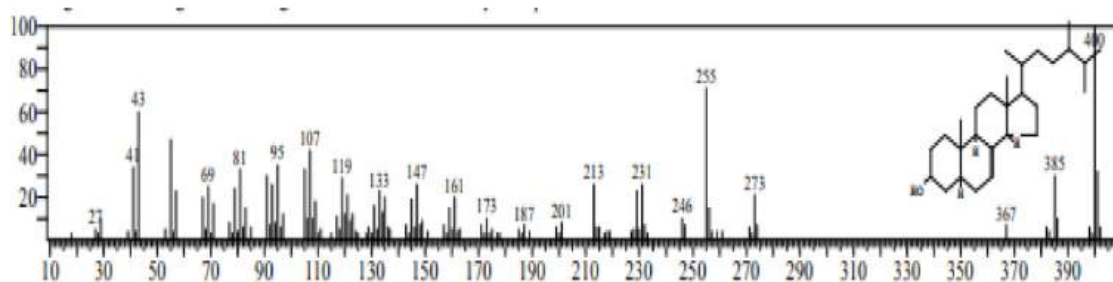


Fig. 18 (viii): Mass spectra of compounds detected by GC/MS analysis of methanolic extract of *M. affinis*

34	27.375	3-Cyclopentylpropionic acid 2-dimethylaminoethyl ester	Fatty acid ester	0.127
35	28.014	Cyclobutane carboxylic acid undec-2-enyl ester	Ester	0.124
36	30.126	2-Monopalmitin	Fatty acid ester	11.733
37	31.151	Tetracontane	Alkane	0.238
38	32.005	Monoolein	Fatty acid ester	5.938
39	32.760	Tetracosanoic acid (Lignoceric acid)	Fatty acid	0.330
40	33.096	Tetratriacontane	Alkane	0.181
41	33.808	1- Heptacosanol	Alcohol	0.127
42	34.244	Tetratetracontane	Alkane	0.146
43	35.121	Dehydroergosterol	Sterol	0.829
44	39.249	Ergosterol	Sterol	1.180
45	39.610	Stellasterol	Sterol	9.610
46	41.032	Fungisterol	Sterol	1.460

PHASE II: BIOACTIVITY SCREENING

a) Free radical scavenging assays (Antioxidant activities)

Antioxidants are the substances that neutralize free radicals, which are unstable molecules that can damage cells. Free radicals are the byproducts of body's metabolism and other processes, and they become harmful if their levels become too high. Antioxidants donate electrons to free radicals, thereby stabilizing and preventing them from causing damage. Thus, antioxidants are often called free radical scavengers. Excess free radicals, leading to oxidative stress, have been linked to various health issues such as heart diseases, cancer, aging etc. Hence the importance of antioxidants is increasing day by day. The efficacy of methanolic extract of two species of *Microporus*, *M. xanthopus* and *M. affinis* has been assessed by using four different assays. The important antioxidant assays tested are reducing power activity, DPPH radical, hydroxyl radical and nitric oxide radical scavenging activities.

i. DPPH radical scavenging assay

The DPPH (2, 2-Diphenyl-2-picryl hydrazyl) radical was scavenged by the methanolic extract of both *M. xanthopus* and *M. affinis*. Activity was dose dependent against various concentrations of the samples tested (12.5, 25, 50, 100 and 200 µg/mL). Ascorbic acid was used as the standard. Scavenging potential was measured based on the discolouration of purple colour of DPPH to yellow. IC₅₀ values were calculated for both samples and standard. Inhibition percentage measured at a maximum concentration of 200 µg/mL for *M. xanthopus* and *M. affinis* was 41.32 ± 0.32 and 63.95 ± 0.02 respectively. IC₅₀ value of standard was calculated as 38.42 ± 0.04 µg/mL. IC₅₀ values for *M. xanthopus* was 232.73 ± 0.24 µg/mL and for *M. affinis* was 140.26 ± 0.16 µg/mL. Though in a moderate range, *M. affinis* has better DPPH scavenging potential in comparison with the standard. The results are graphically plotted (Fig. 19).

ii. Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was assessed on both the species of *Microporus* by using various concentrations of the methanolic extract (125, 250, 500,

1000 and 2000 $\mu\text{g/mL}$). Percentage of inhibition at maximum concentration (2000 $\mu\text{g/mL}$) for *M. xanthopus* was 72.55 ± 0.15 and for *M. affinis* was 79.08 ± 0.16 %. Percentage inhibition of the samples and the standard was plotted graphically (**Fig. 20**). IC_{50} values of the standard gallic acid and the extracts of *M. xanthopus* and *M. affinis* were 477.49 ± 0.19 $\mu\text{g/mL}$, 802.31 ± 0.24 $\mu\text{g/mL}$ and 371.37 ± 0.13 $\mu\text{g/mL}$ respectively. In this study, the extract of *M. affinis* was found to be more potent in scavenging hydroxyl radicals when compared to the standard gallic acid used, which seems to be significant.

iii. Nitric oxide radical scavenging assay

Different concentrations (125, 250, 500, 1000 and 2000 $\mu\text{g/mL}$) of the fungal extracts were tested for nitric oxide scavenging assay. Gallic acid was used as the standard. At a maximum concentration of 2000 $\mu\text{g/mL}$, percentage of inhibition was found to be 79 ± 0.23 and 80.46 ± 0.37 for *M. affinis* and *M. xanthopus* respectively.

Maximum percentage of inhibition observed for gallic acid was 92.9 ± 0.26 % (**Fig. 21**). The IC_{50} values of the standard gallic acid, extracts of *M. xanthopus* and *M. affinis* were 108.81 ± 0.13 , 742.00 ± 0.22 and 477.04 ± 0.24 respectively. The extract of *M. affinis* was found to possess more scavenging activity for nitric oxide radicals than that of *M. xanthopus*.

iv. Reducing power activity

The reducing power assay is often used to evaluate the ability of an antioxidant to donate an electron. The reduction of Fe^{3+} (Ferric ion) to Fe^{2+} (Ferrous ion) resulted in the change of test solution from green to blue. Dose dependent effect was noticed for different concentrations of the extract. The absorbance was found to increase from 0.0597 ± 0.002 to 0.1474 ± 0.002 for *M. affinis*. While, *M. xanthopus* showed an increase in reducing power from 0.0521 ± 0.002 to 0.347 ± 0.01 with respect to increase in concentration (**Fig. 22**).

Among the antioxidant activities tested in various concentrations of the fungal extract against respective standards, *M. affinis* was found to be more potent than *M. xanthopus*.

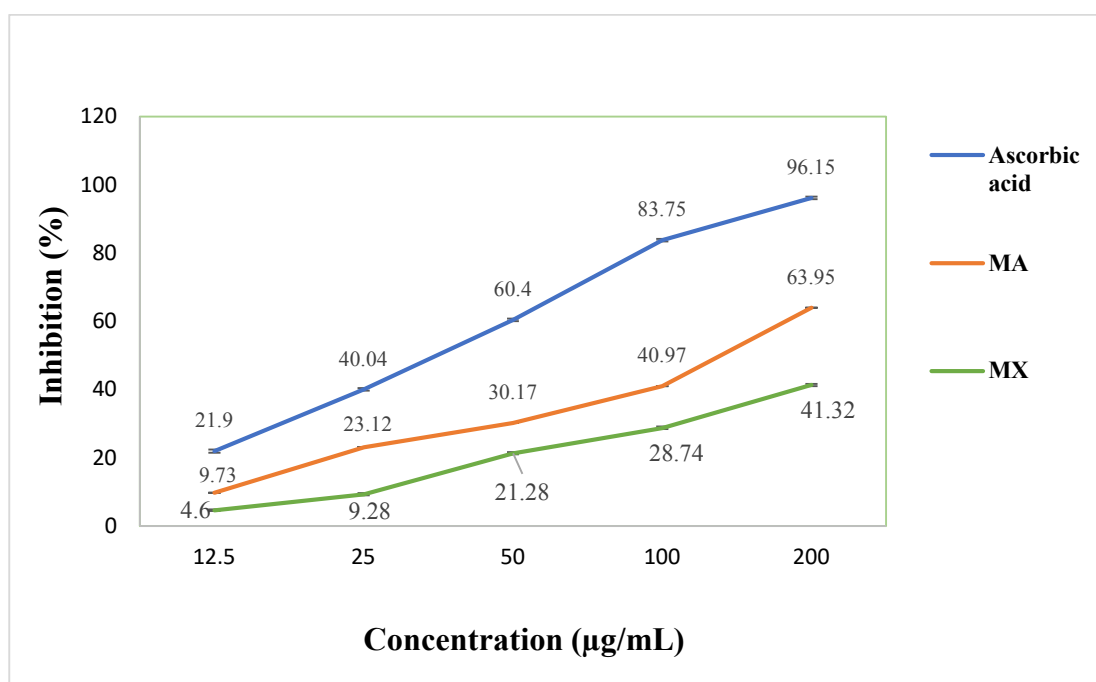


Fig. 19: *In vitro* DPPH free radical scavenging activity of *M. affinis* (MA) and *M. xanthopus* (MX) against standard ascorbic acid.

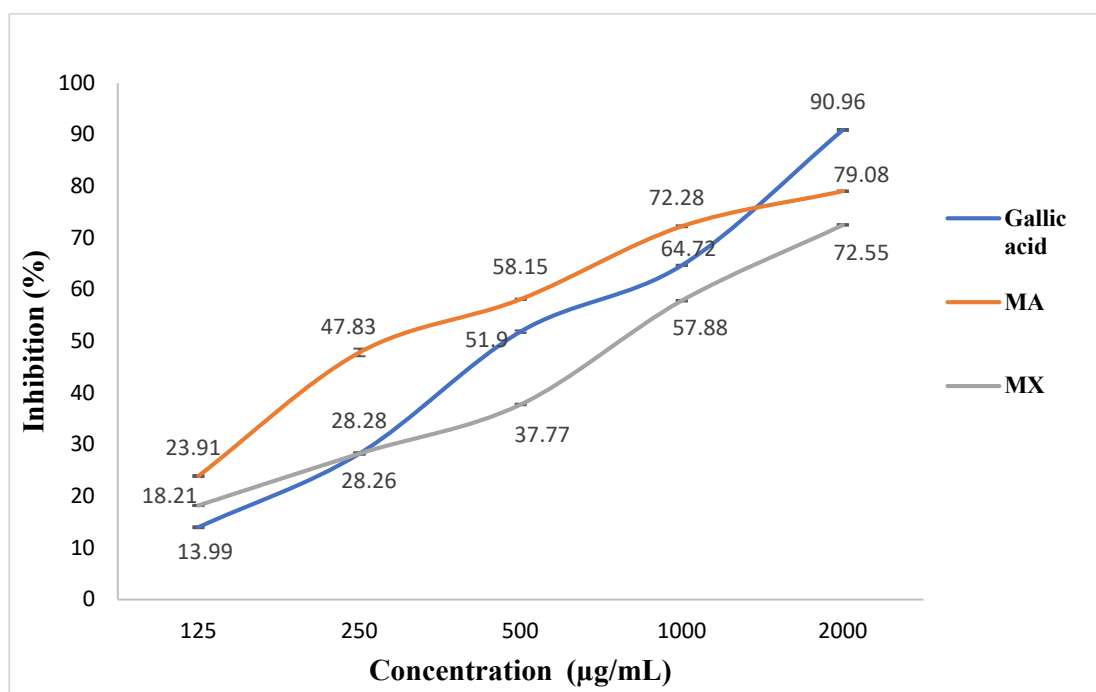


Fig. 20: *In vitro* hydroxyl free radical scavenging activity of *M. affinis* (MA) and *M. xanthopus* (MX) against standard gallic acid.

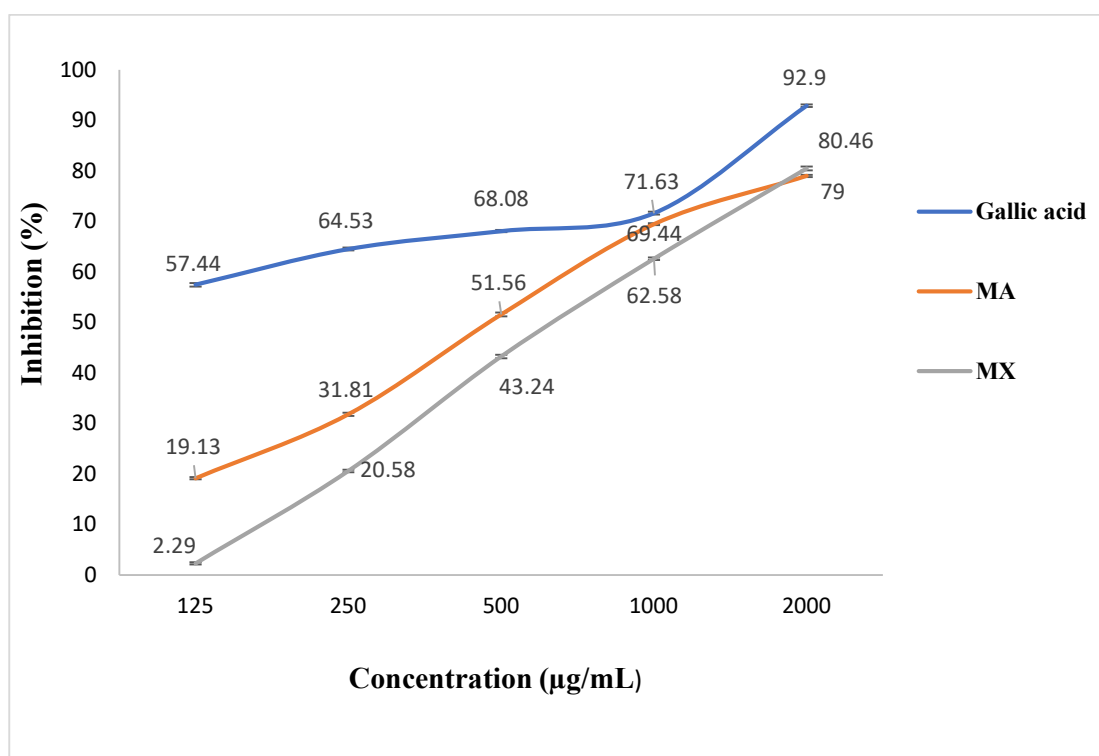


Fig. 21: *In vitro* nitric oxide free radical scavenging activity of *M. affinis* (MA) and *M. xanthopus* (MX) against standard gallic acid.

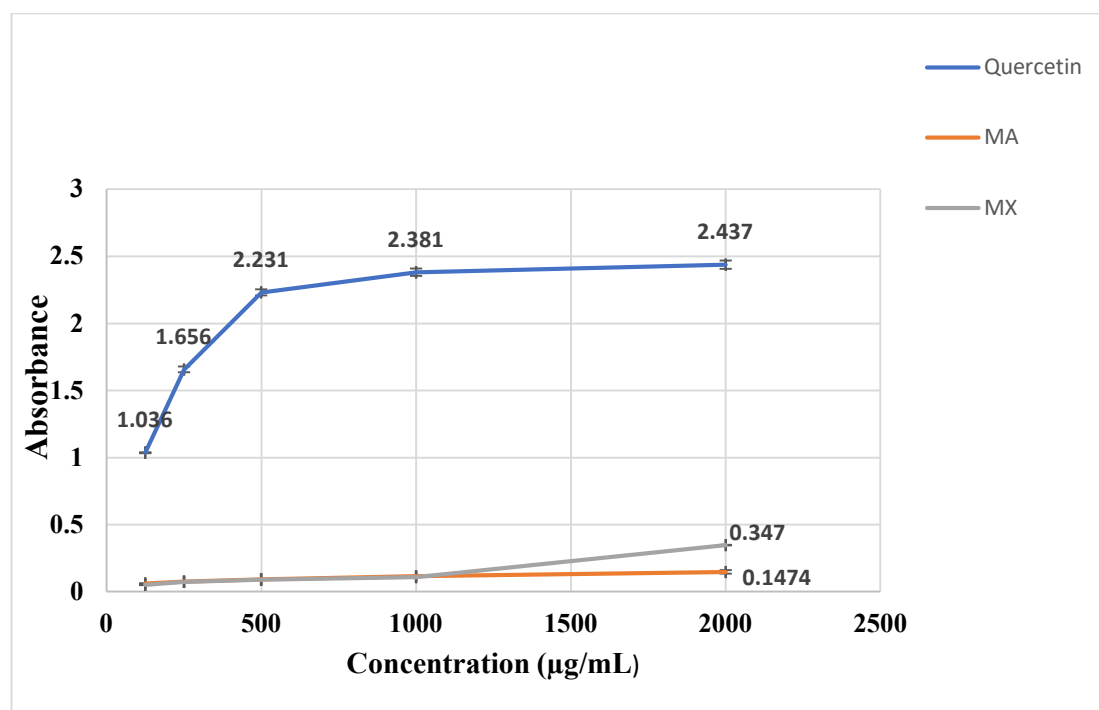


Fig. 22: *In vitro* reducing power activity of *M. affinis* (MA) and *M. xanthopus* (MX) against standard quercetin.

b) Cytotoxicity studies using *Allium cepa* bioassay:

For the study of induced cytotoxicity and chromosomal aberrations, the root meristems of *Allium cepa* L. are most frequently used as experimental materials. In the present study, root tip meristems of *A. cepa* were treated with the different concentrations (05, 10, 50 and 100 µg/mL) of methanolic extracts of two species of *Microporus* for different time intervals (1 h, 12 h and 24 h). Distilled water was taken as the negative control and hydrogen peroxide (100 µL/mL) as the positive control. Mitotic index and chromosome aberrations were the two important parameters taken to study the cytotoxicity potential of fungal extracts on *A. cepa* root tip meristems. Reduction in mitotic index and increase in the abnormality percentage are regarded as the indicators of cytotoxicity potential. The aberrations induced were apparently different from that of normal stages of mitosis. In both the species of *Microporus*, mitotic index was found to exhibit a dose dependent reduction as the concentration of extract increases. While abnormality percentage was found to be increasing as the concentration increases. An increase in the period of time was also found to have a significant effect on these parameters. The results are depicted in **Fig. 23-25** and **Plates 4-12**.

Mitotic index (MI) and chromosomal aberration percentage are the widely accepted parameters to measure the cytotoxic potential of fungal extracts. Mitotic index and percentage of chromosomal aberrations were found to be concentration dependent. After 1 h incubation for lowest concentration (5 µg/mL), mitotic index was found to be 85 ± 0.33 % and 75 ± 0.88 % for *M. xanthopus* and *M. affinis* respectively. At the highest concentration (100 µg/mL), mitotic index declined and was found to be 38 ± 0.33 % and 41 ± 0.88 % for *M. xanthopus* and *M. affinis* respectively (**Fig. 23a**). While chromosomal aberration percentage at 1 h treatment for *M. xanthopus* ranges from 29 ± 0.33 % to 79 ± 0.33 % and for *M. affinis* it ranges from 25 ± 0.33 % to 67 ± 0.33 % (**Fig. 23b**). It was compared with the high percentage of aberration (90 ± 1.52 %) of positive control.

At 12 h incubation, mitotic index showed further reduction with respect to concentration. At lowest concentration, it was found to be 82 ± 0.88 % and 72 ± 1.15

% for *M. xanthopus* and *M. affinis* respectively. It was found to be 33 ± 0.88 % and 36 ± 0.24 % for *M. xanthopus* and *M. affinis* respectively at highest concentration of 100 µg/mL (**Fig. 24a**). At the same time period, percentage of aberration was found in the range of 32 ± 1.2 % to 81 ± 0.88 % for *M. xanthopus* and for *M. affinis*, it was in the range of 28 ± 1.45 % to 69 ± 1.2 % (**Fig. 24b**).

At the time period of 24 h incubation, there was a notable reduction in the mitotic index as concentration increased. At the lowest concentration, mitotic index was found to be 82 ± 1.52 % and 70 ± 0.88 % for *M. xanthopus* and *M. affinis* respectively. While at highest concentrations, it was declined to 31 ± 0.88 % for *M. xanthopus* and 36 ± 1.76 % for *M. affinis* (**Fig. 25a**). While in case of chromosomal aberrations at the same time period, highest concentration showed 82 ± 1.15 % for *M. xanthopus* and 71 ± 0.88 % for *M. affinis* (**Fig. 25b**). It was highly comparable to that of positive control (90 ± 0.88 %) used for the highest concentration. From these, it is clear that mitotic index and abnormality percentage are time and dose dependent. Mitotic index was found to be inversely proportional and percentage of aberration was found to be directly proportional to the concentration of both fungal extracts used. Nevertheless, positive control showed greater percentage of aberration irrespective of concentration and time period in all the treatments.

Cytotoxic study on two species of *Microporus* revealed wide range of chromosomal aberrations, which are broadly classified into clastogenic and non-clastogenic aberrations. Chromosome stickiness, pulverization, nuclear lesions, nuclear erosion, nuclear extrusion, nuclear disintegration, nuclear budding, nuclear peak, giant cell formation, coagulated chromosomes, chromosome gap, chromosome bridges, chromosome fragmentation, ring chromosomes, contorted chromosomes, hyperchromasia, cytomixis etc., are the important clastogenic aberrations observed. Non-clastogenic aberrations include hypoploid, polyploid, vagrants, laggards, shift in microtubule organizing center (MTOC), ball metaphase, misorientation, binucleate cell, stellate chromosomes, C-metaphase, diagonal arrangement of chromosomes, disturbed chromosomes, stathmo anaphase, scattering of chromosomes, micronuclei,

early movement of chromosomes, somatic pairing, tropokinesis, macro and micro cell formation, chained chromosomes etc. (plates 4-12).

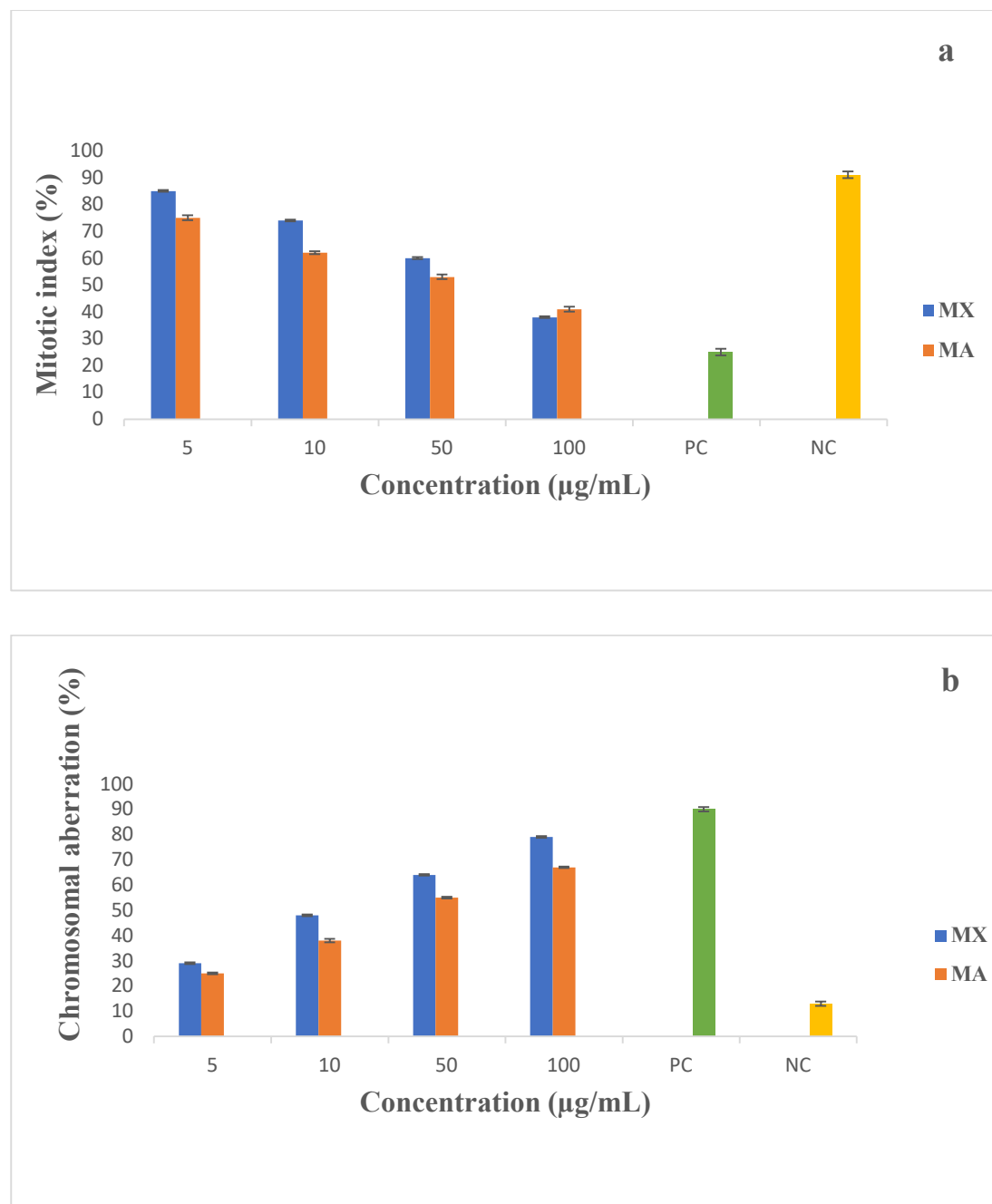


Fig. 23: The effect of varying concentrations of methanolic extracts of selected species of *Microporus* on *Allium cepa* root tip meristems after a period of 1 h treatment. a. Mitotic index b. chromosome aberrations

(MX- *Microporus xanthopus*, MA- *Microporus affinis*, PC-positive control, NC-negative control)

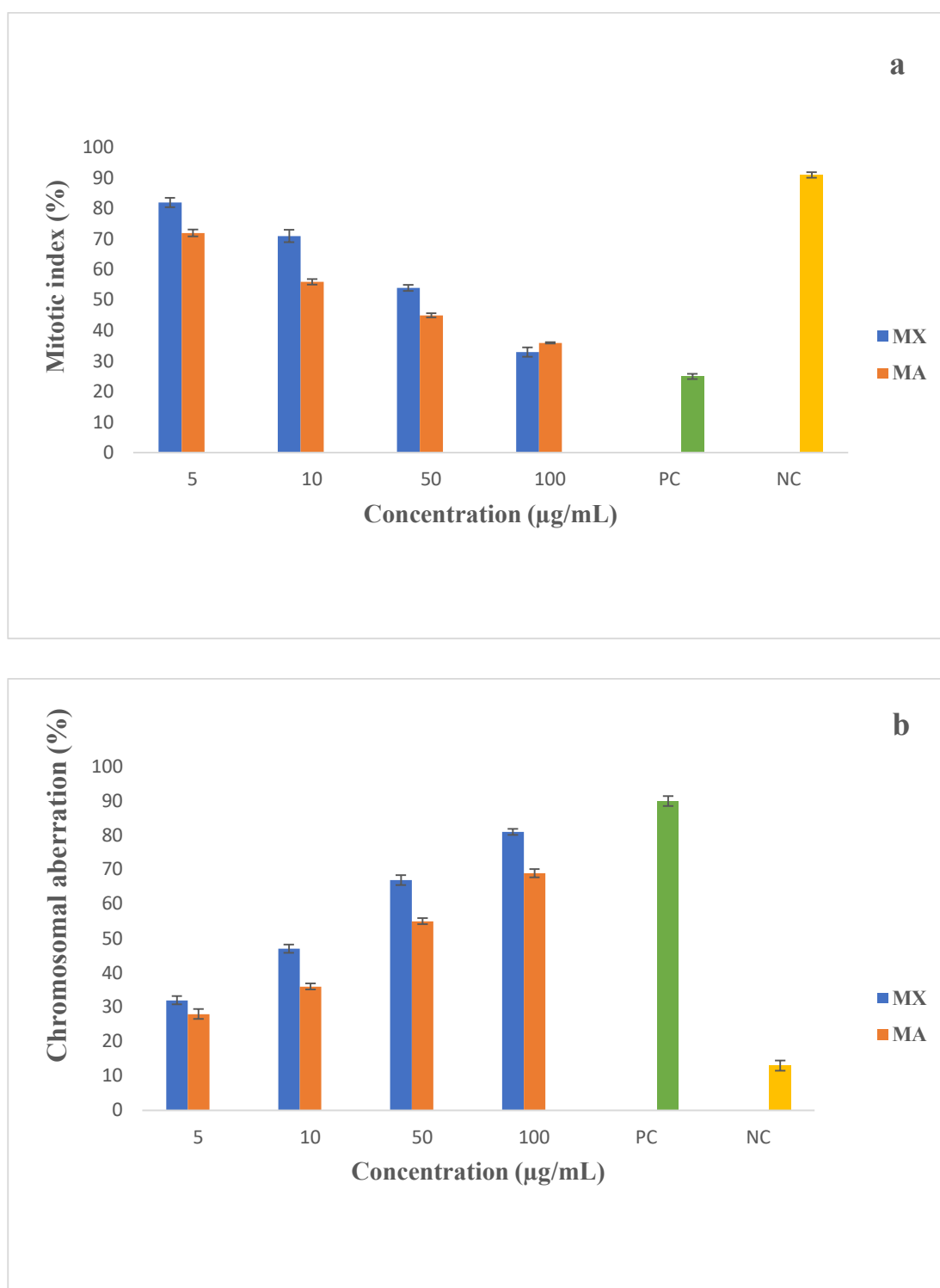


Fig. 24: The effect of varying concentrations of methanolic extracts of selected species of *Microporus* on *Allium cepa* root tip meristems after a period of 12 h treatment. a. Mitotic index b. chromosome aberrations

(MX- *Microporus xanthopus*, MA- *Microporus affinis*, PC-positive control, NC-negative control)

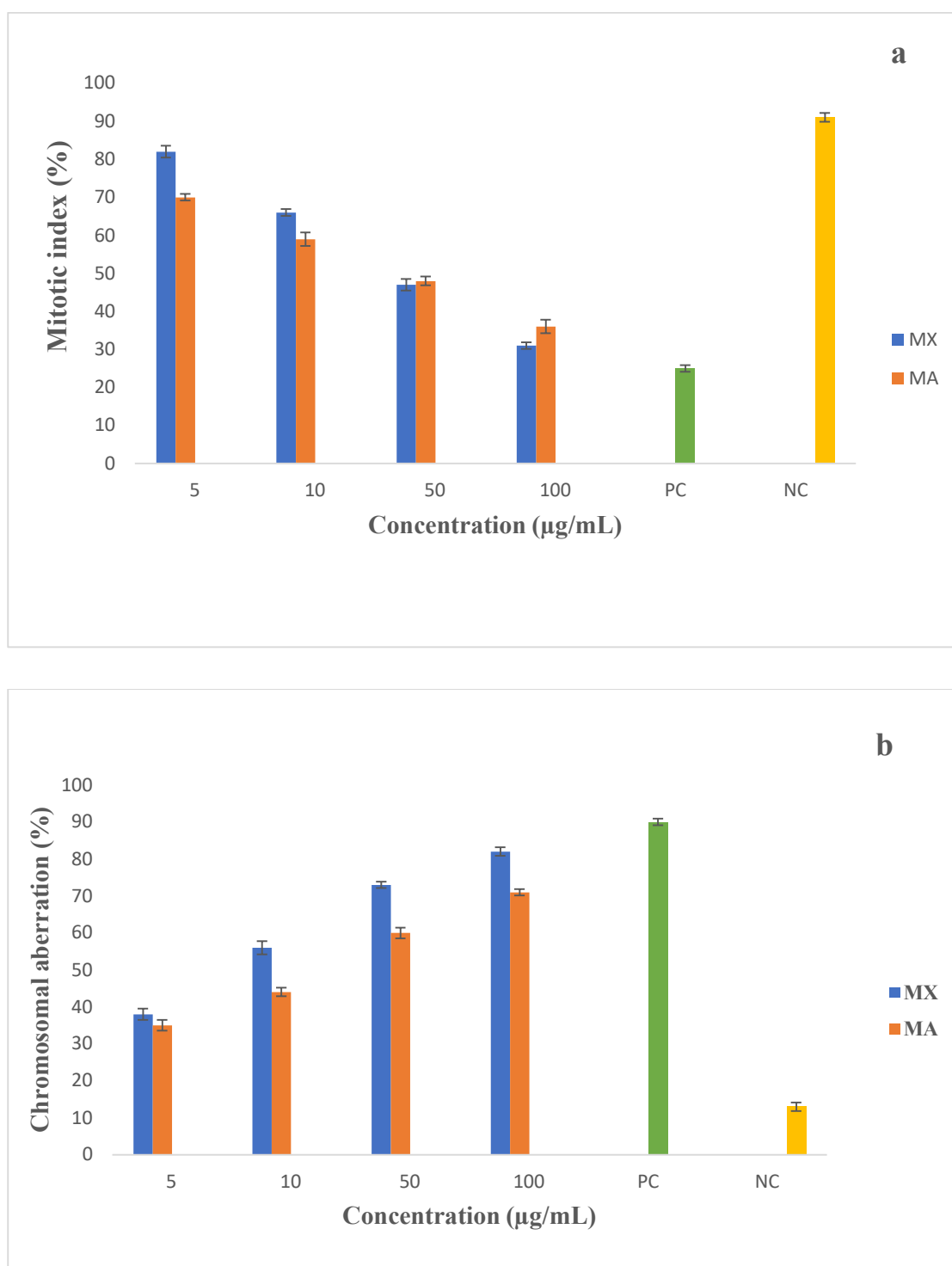


Fig. 25: The effect of varying concentrations of methanolic extracts of selected species of *Microporus* on *Allium cepa* root tip meristems after a period of 24 h treatment. a. Mitotic index b. chromosome aberrations

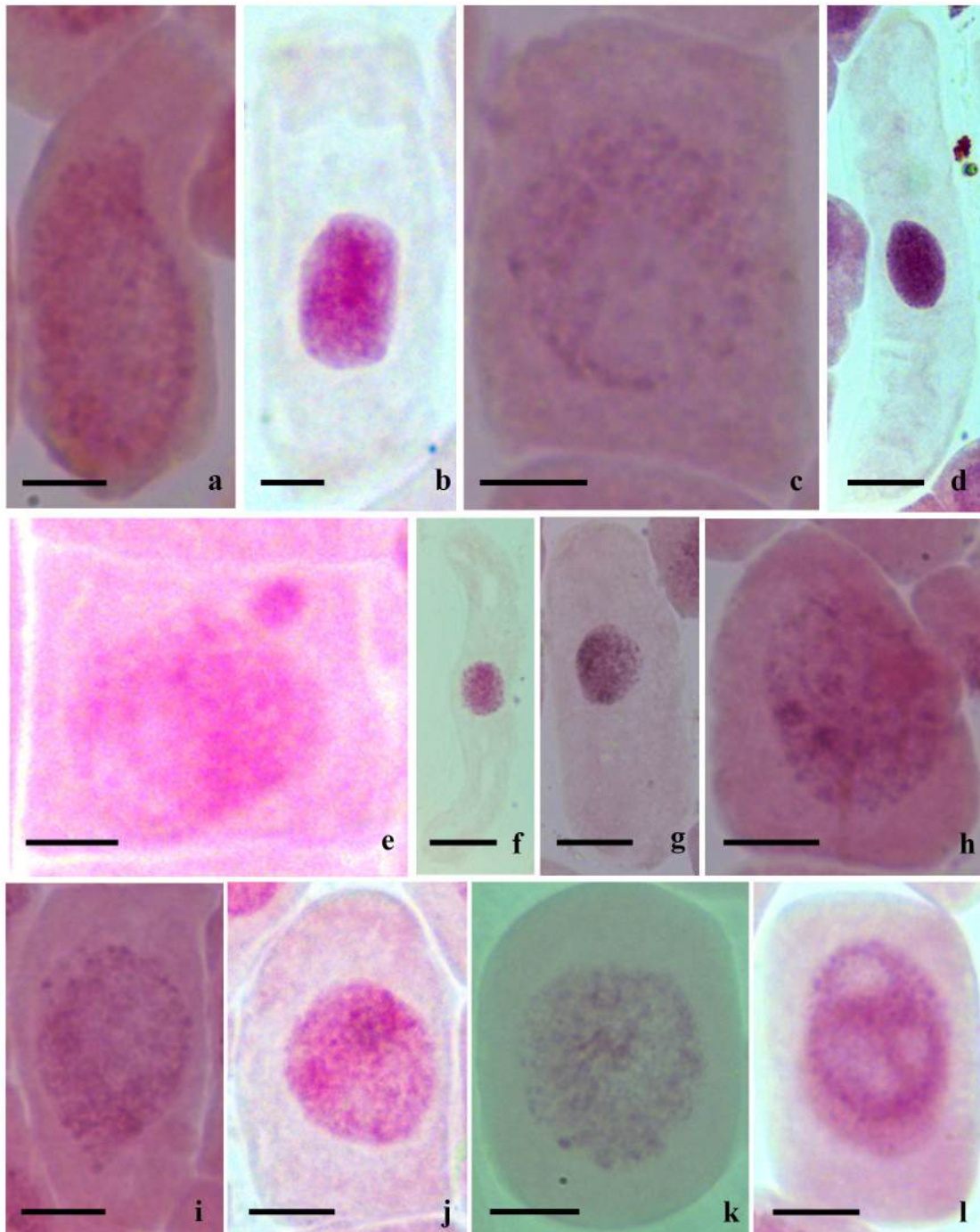
(MX- *Microporus xanthopus*, MA- *Microporus affinis*, PC-positive control, NC-negative control)

PLATE 3



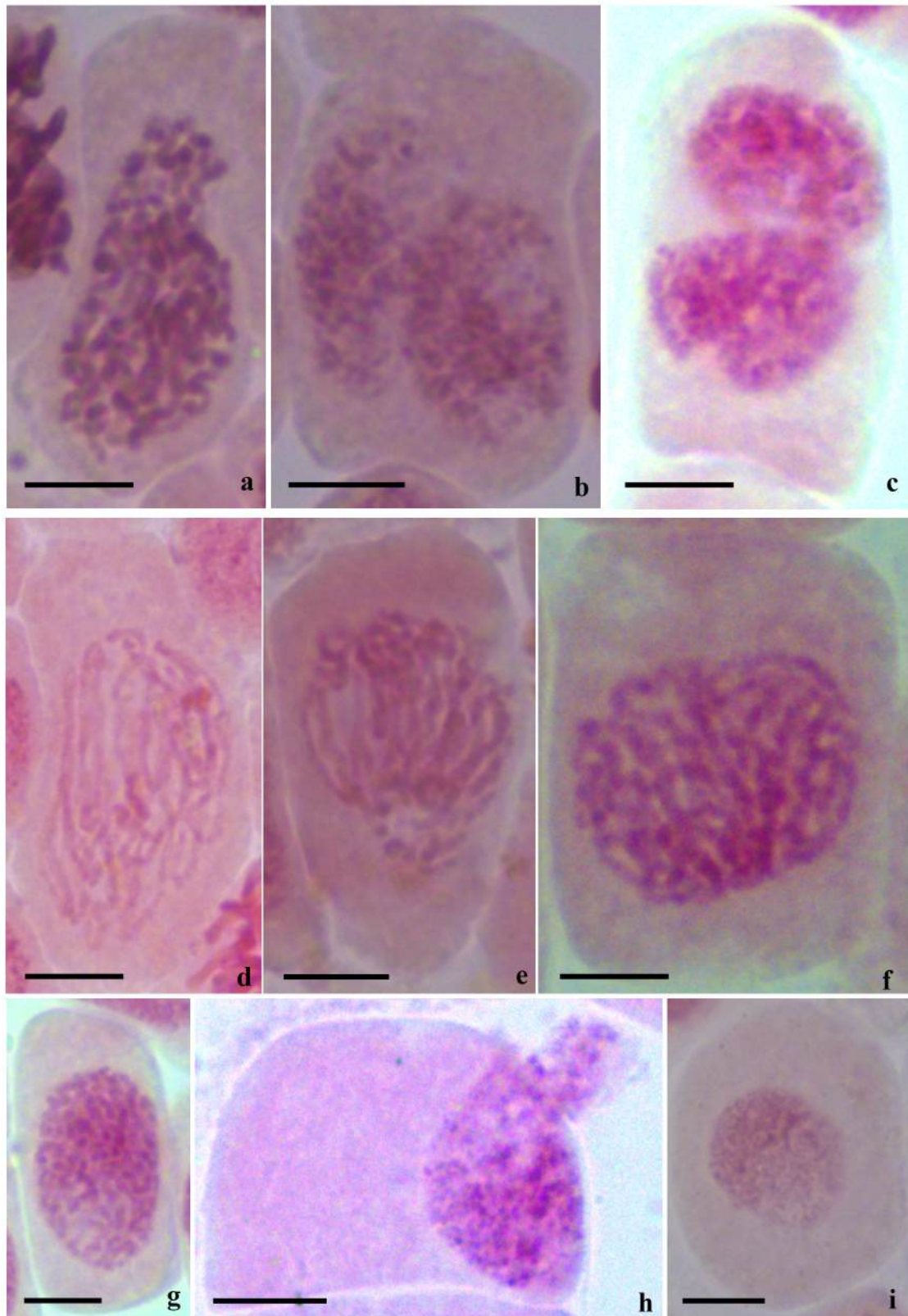
Normal stages of mitosis in *Allium cepa* root tip meristem: a. Interphase; b. Prophase; c. Metaphase; d. Anaphase; e. Telophase; f. Cytokinesis. Scale bar-10 μ m

PLATE 4



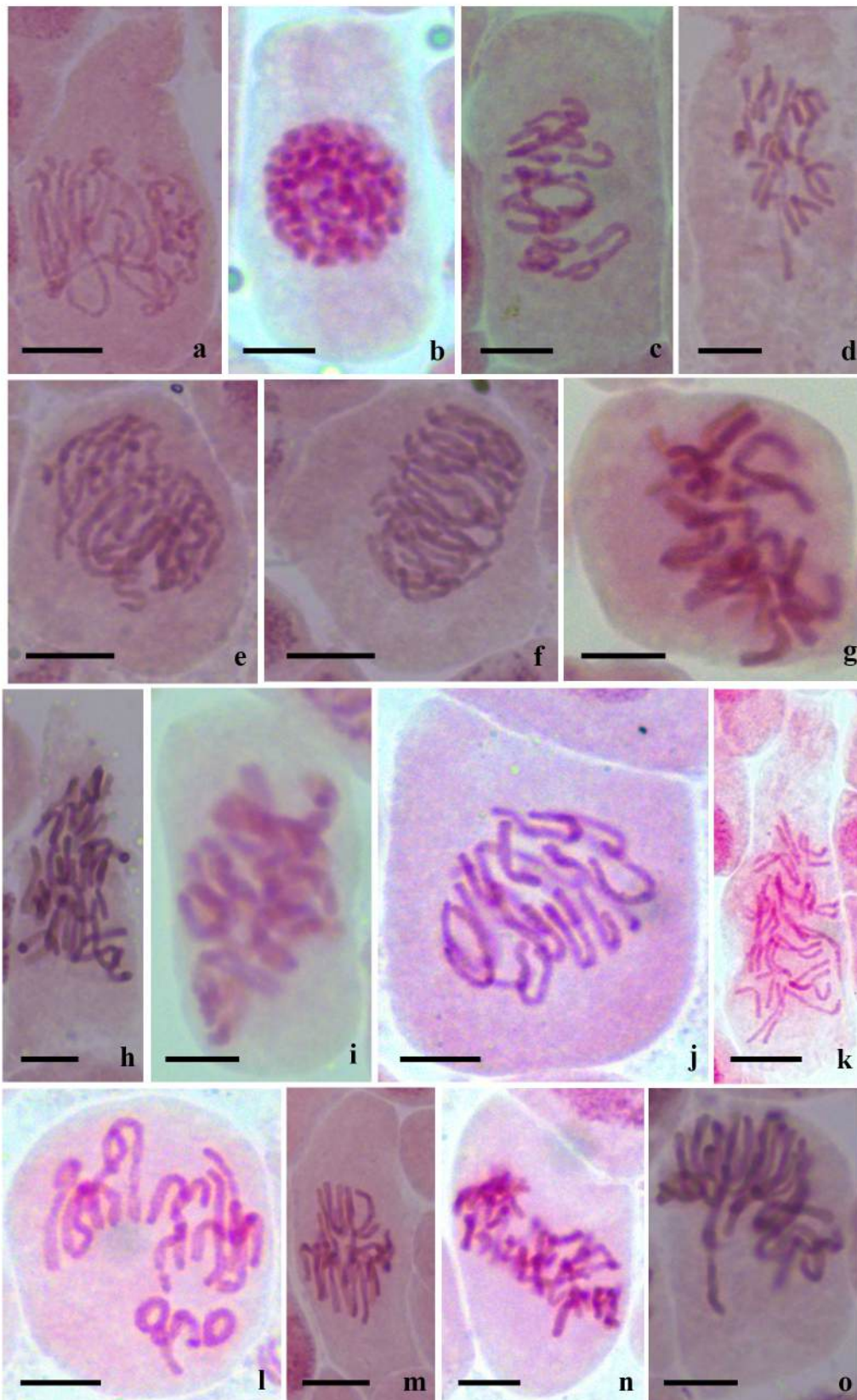
Chromosomal aberrations induced by the extracts of two species of *Microporus* in *A. cepa*. Interphase: **a.** Apoptotic nuclear enlargement and chromatin disintegration at interphase; **b.** Cytoplasmic shrinkage at interphase; **c.** Ghost cell formation at interphase; **d.** Hyperchromasia at interphase; **e.** Micronucleus at interphase; **f.** Nuclear and cytoplasmic disintegration at interphase; **g.** Nuclear bulging at interphase; **h.** Nuclear expulsion at interphase; **i.** Nuclear peak and formation of lesion at interphase; **j.** Pulverized chromatin at interphase; **k.** Pulverization at interphase; **l.** Triple nuclear lesions at interphase. Scale bar-10 μm

PLATE 5



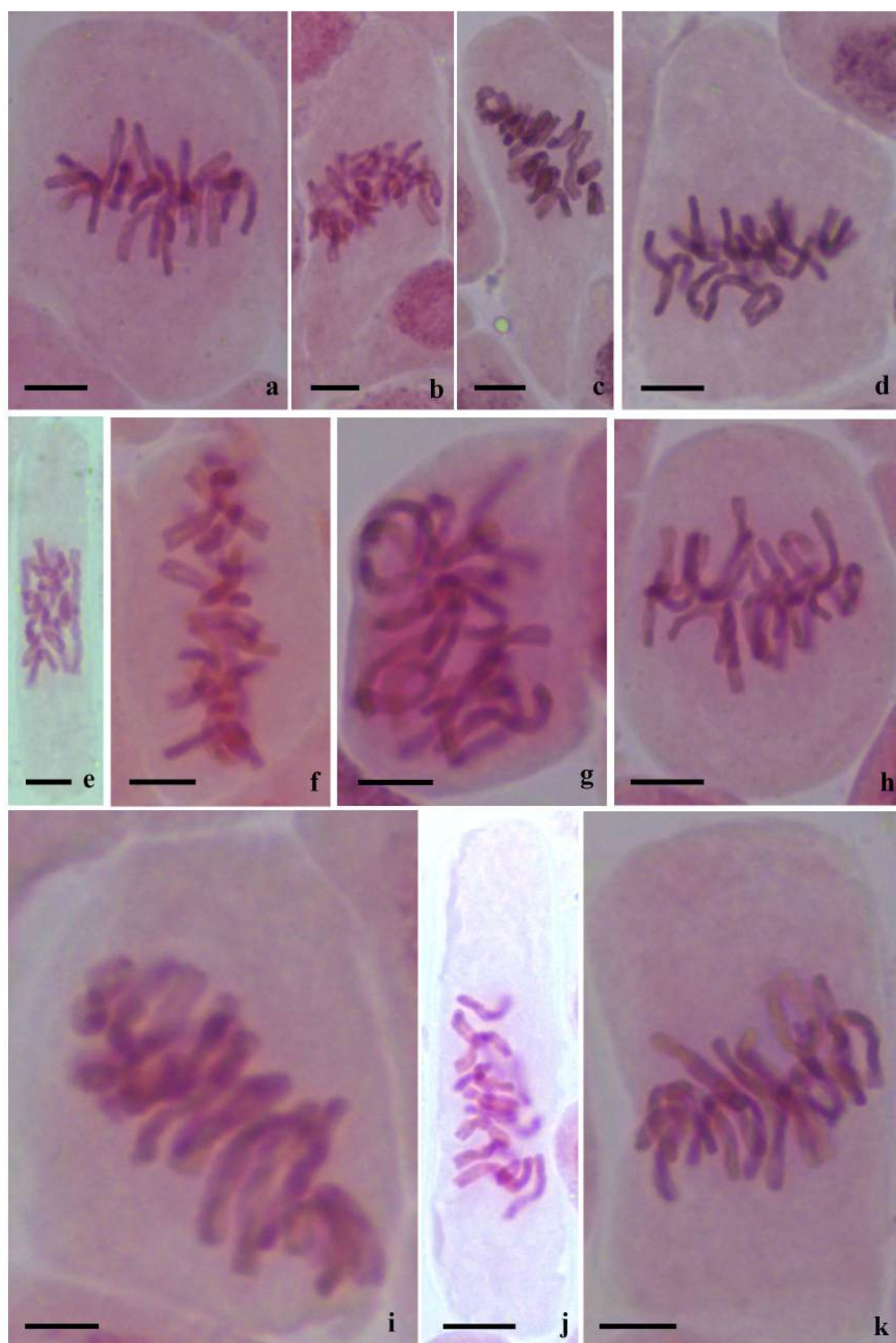
Chromosomal aberrations induced by the extracts of two species of *Microporus* in *A. cepa*. Prophase: **a.** Abnormal condensation of chromatin at late prophase; **b.** Apoptotic disintegration of the chromatin at prophase; **c.** Bridged binucleate cell at late prophase; **d.** Chromatin erosion at late prophase; **e.** Chromatin fragmentation at late prophase; **f.** Chromatin gaps at late prophase; **g.** Formation of nuclear lesion at prophase; **h.** Nuclear extrusion at prophase; **i.** Pulverized chromatin at prophase. Scale bar-10 μ m

PLATE 6



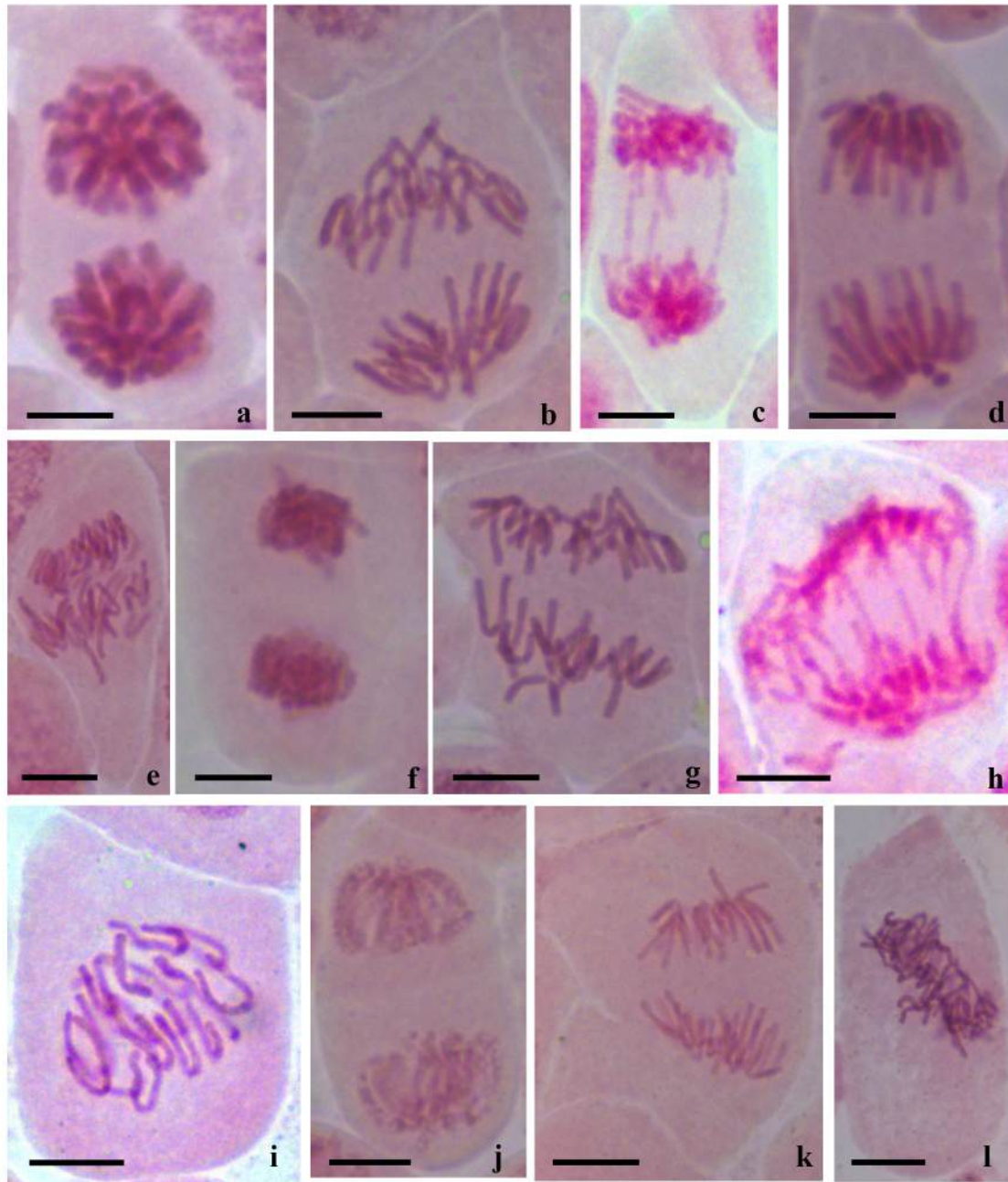
Chromosomal aberrations induced by the extracts of two species of *Microporus* in *A. cepa*. **Metaphase:** **a.** Apoptotic fragmentation of chromosomes and cell at early metaphase; **b.** Ball metaphase showing chromosome erosion; **c.** Chromosome fragments at early metaphase; **d.** Chromosome gaps at early metaphase; **e.** Chromosomes showing decondensation at metaphase; **f.** Diagonal ball metaphase in a hyperploid cell; **g.** Diagonal metaphase; **h.** Disturbed metaphase in a polyploid cell; **i.** Disturbed and sticky somatic pairing at metaphase; **j.** Misorientation at metaphase; **k.** Partial somatic pairing at metaphase in a polyploid cell; **l.** Ring chromosomes at metaphase; **m.** Stellate metaphase; **n.** Sticky chained metaphase; **o.** Tropokinesis at dislocated metaphase showing vagrants. Scale bar-10 μ m

PLATE 7



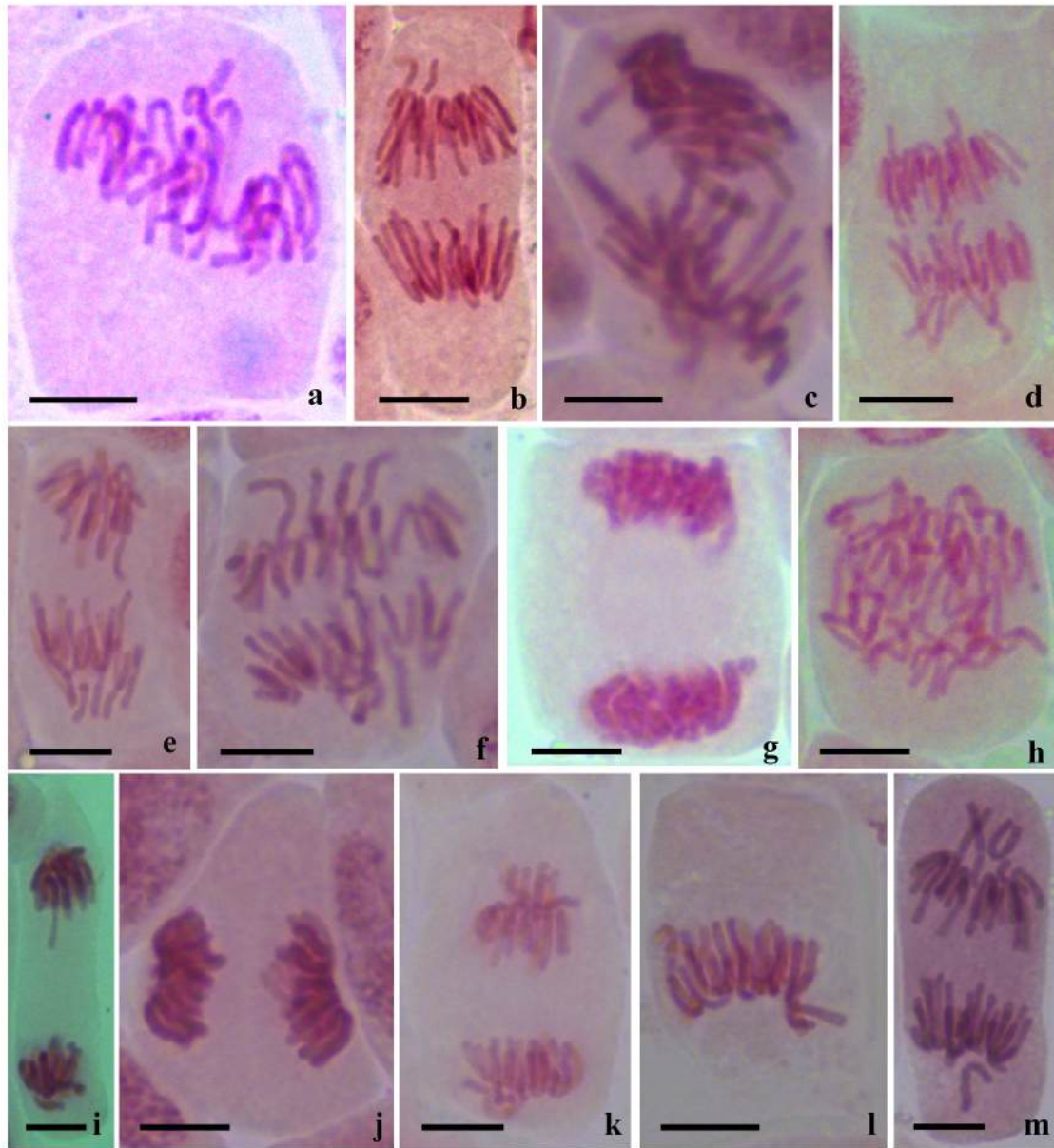
Chromosomal aberrations induced by the extracts of two species of *Microporus* in *A. cepa*. C-Metaphase: a. C-metaphase; **b.** C-metaphase showing tropokinesis; **c.** Disturbed C-metaphase in a hypoploid cell; **d.** Partial C-metaphase; **e.** Partial sticky C- metaphase; **f.** Pole to pole C-metaphase; **g.** Scattered partial C-metaphase; **h.** Stellate C-metaphase; **i.** Sticky diagonal C-metaphase; **j.** Strap cell showing partial C- metaphase; **k.** Tilted equatorial plate showing chromosome erosion at C-metaphase. Scale bar-10 μm

PLATE 8



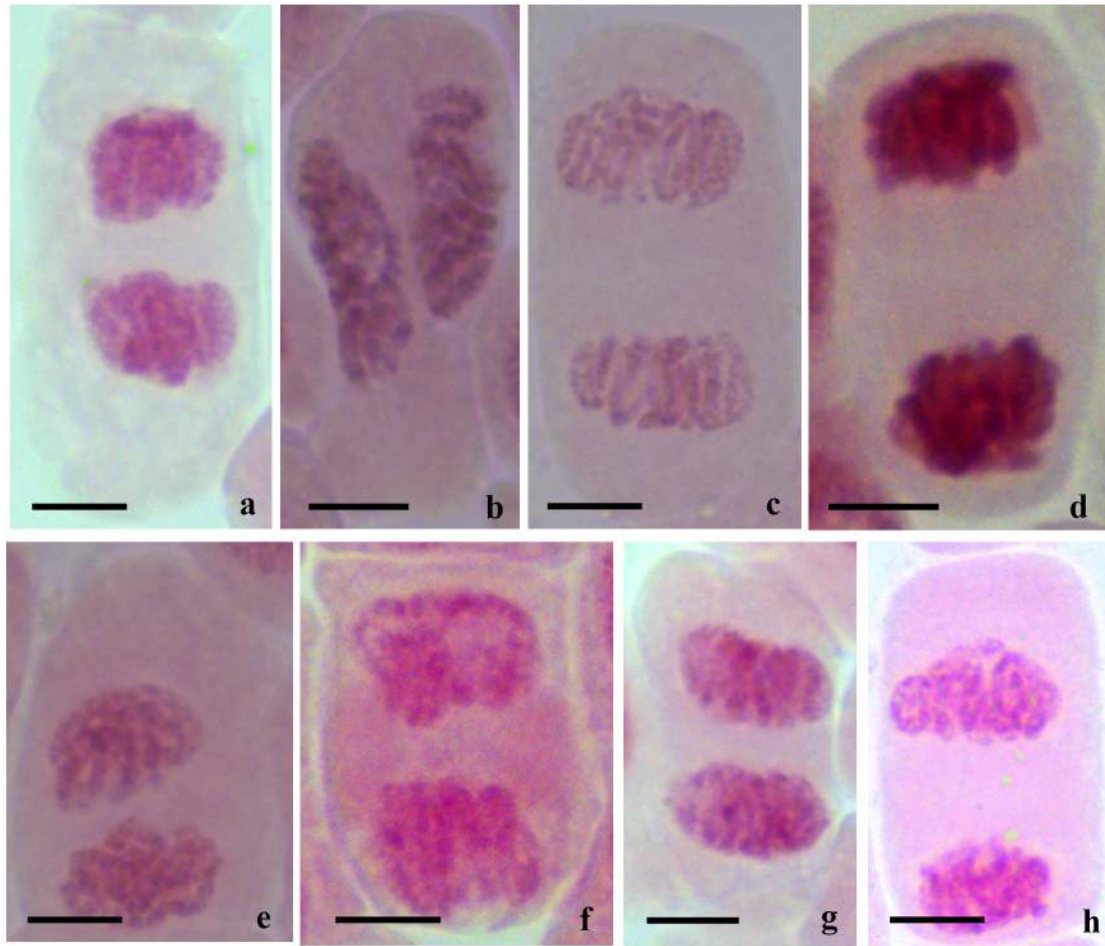
Chromosomal aberrations induced by the extracts of two species of *Microporus* in *A. cepa*. Anaphase: **a.** Ball anaphase; **b.** Chained anaphase; **c.** Chromosome bridge and broken bridges at anaphase; **d.** Chromosome fragments at anaphase; **e.** Chromosome laggards and vagrants at anaphase; **f.** Coagulated anaphase showing chromosome vagrants; **g.** Diagonal anaphase; **h.** Diagonal sticky anaphase showing multiple bridges; **i.** Diagonal unipolar anaphase in a hypoploid cell; **j.** Disintegrated chromosomes at anaphase showing early cell plate formation; **k.** Disturbed anaphase showing chromosome gaps; **l.** Disturbed diagonal stathmo-anaphase. Scale bar-10 μm

PLATE 9



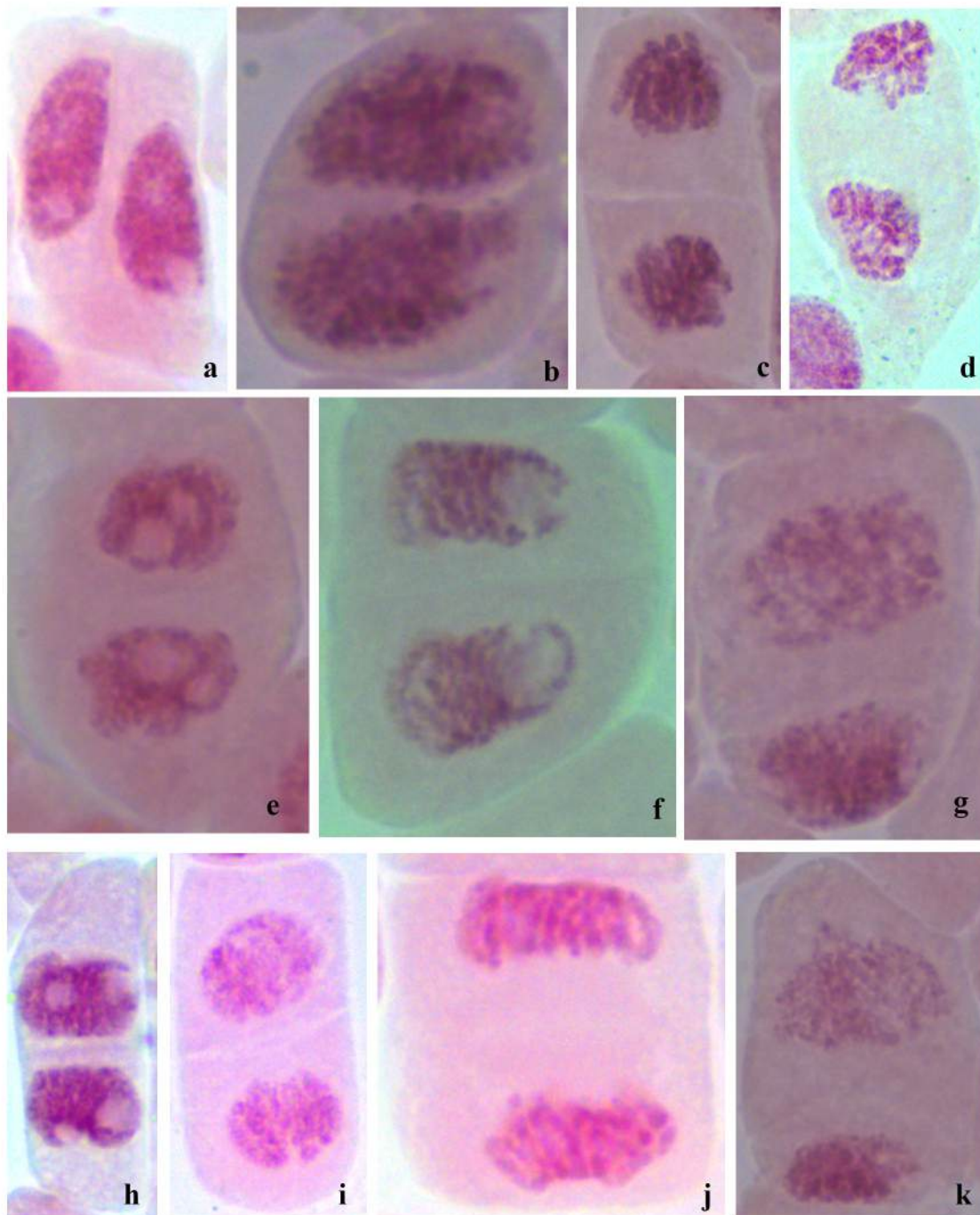
Chromosomal aberrations induced by the extracts of two species of *Microporus* in *A. cepa*. Anaphase: **a.** Disturbed unipolar anaphase; **b.** Early movement of chromosome at anaphase; **c.** Hemi-stellate anaphase showing misorientation; **d.** Misorientation at anaphase showing vagrants; **e.** Partial C-anaphase; **f.** Scattered diagonal anaphase; **g.** Shift in MTOC at anaphase; **h.** Stathmo-anaphase; **i.** Sticky late anaphase; **j.** Sticky unequal equatorial separation at anaphase; **k.** Unequal separation of chromosomes at sticky anaphase; **l.** Unipolar partial C- anaphase in a hypoploid cell; **m.** Vagrants at anaphase. Scale bar-10 μm

PLATE 10



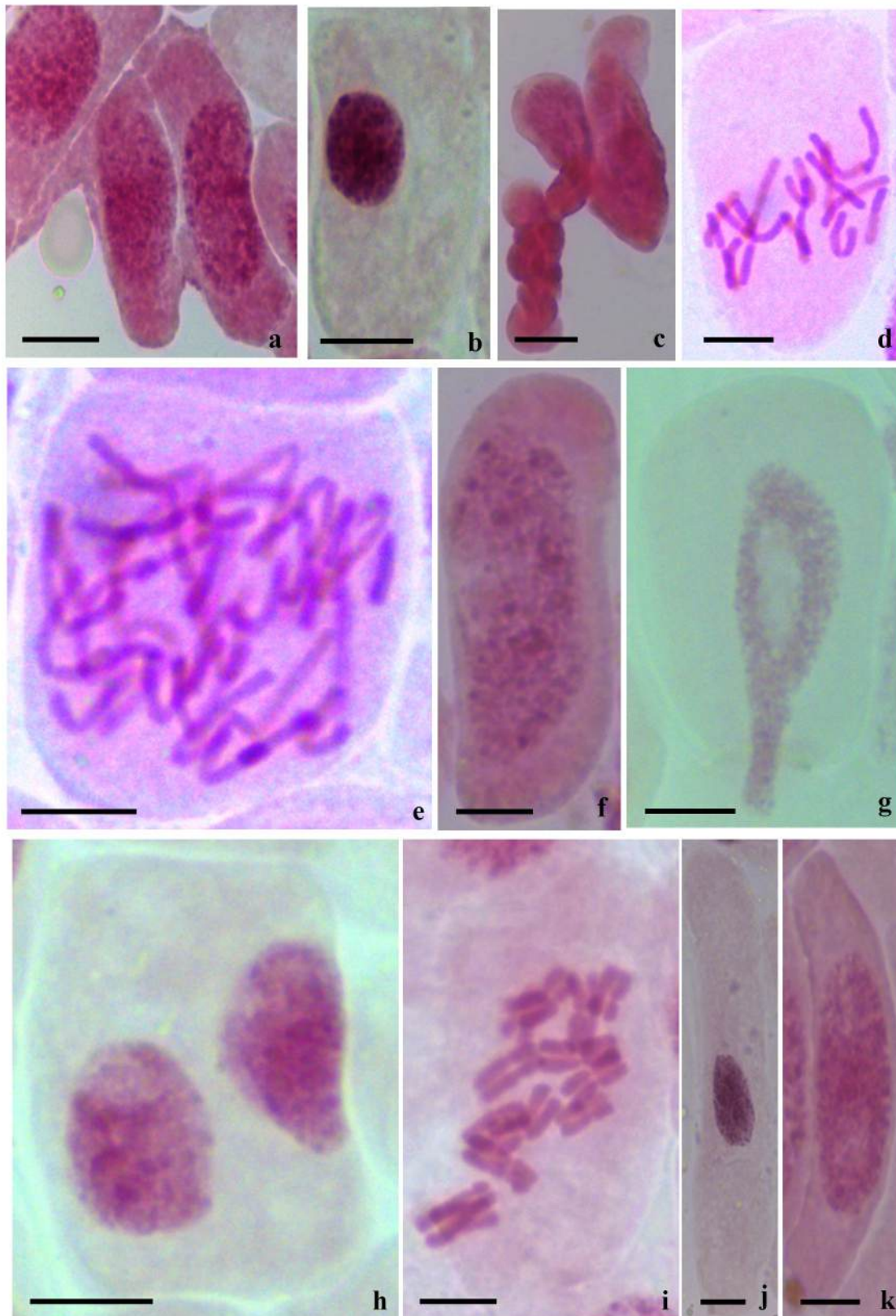
Chromosomal aberrations induced by the extracts of two species of *Microporus* in *A. cepa*. Telophase: **a.** Abnormal chromosome decondensation at telophase; **b.** Chromosome disintegration at telophase after equatorial separation; **c.** Chromosome erosion at telophase; **d.** Coagulated telophase; **e.** Misorientation at telophase showing pulverized chromosomes; **f.** Pulverized and coagulated early telophase; **g.** Pulverized chromatin at telophase; **h.** Sticky telophase. Scale bar-10 μm

PLATE 11



Chromosomal aberrations induced by the extracts of two species of *Microporus* in *A. cepa*. Cytokinesis: **a.** Abnormal cytokinesis showing nuclear lesions and pole to pole cell plate formation; **b.** Chromatin disintegration at cytokinesis showing oblique cell plate; **c.** Chromatin fragment at cytokinesis; **d.** Displaced chromatin groups at cytokinesis after shift in MTOC; **e.** Double nuclear lesions at cytokinesis; **f.** Erosion at cytokinesis; **g.** Macro and micro cell with chromatin disintegration at cytokinesis; **h.** Nuclear lesion and erosion at late cytokinesis; **i.** Pulverized chromatin at cytokinesis; **j.** Sticky cytokinesis; **k.** Unequal chromatin content showing disintegration at cytokinesis. Scale bar-10 μ m

PLATE 12



Chromosomal aberrations induced by the extracts of two species of *Microporus* in *A. cepa*. Rare Cytotoxicity Aberrations: **a.** Cytomixis at interphase; **b.** Hyperchromasia at interphase; **c.** Contorted strap cell showing disintegrated nucleus; **d.** Cytostasis in a hypoploid cell; **e.** Cytostasis in a polyploid cell; **f.** Giant nucleus with abnormal chromatin condensation; **g.** Nuclear lesion and extrusion; **h.** Sticky chromatin and diagonal cell plate formation at cytokinesis; **i.** Somatic pairing in a hyperploid cell; **j.** Strap cell showing nuclear fragmentation; **k.** Strap cell with giant nucleus showing lesions. Scale bar-10 μm

c). Antiproliferative efficacy of *Microporus* species

i) *In vitro* activity on DLD1 cell line using MTT assay

As a safety measure, *in vitro* cytotoxic tests on mammalian cell lines are primarily adopted before proceeding into animal models. In the present study, antiproliferative activity of *M. xanthopus* and *M. affinis* against human adenocarcinoma cell line, DLD1 was tested by using MTT assay. MTT assay was used for the colorimetric determination of the toxic potential of the extracts. DLD1 cell lines were incubated in various concentrations (6.25, 12.5, 25, 50 and 100 $\mu\text{g/mL}$) of fungal extracts for 24 h. LC_{50} values for each fungal extract was determined. A dose dependent toxicity was obtained and reflected in the percentage of viable cells in the cultures (**Fig. 26**). The morphology of the treated cells was also affected similarly and microphotographs were taken (**Plates: 13- 14**). The highest percentage of toxicity was shown at 100 $\mu\text{g/mL}$ concentration of *M. affinis* with 66.81 ± 0.11 cell viability percentage and *M. xanthopus* with a 67.15 ± 0.12 percentage of viability. LC_{50} values were calculated as 171.76 ± 0.43 $\mu\text{g/mL}$ in *M. affinis* and 193.24 ± 0.32 $\mu\text{g/mL}$ in *M. xanthopus* (**Table: 9**). This clearly depicted that the lower LC_{50} value will be more efficient against cancer cell lines. Morphological investigations of the treated cells clearly displayed many apoptotic characters like cell rounding, blebbing, shrinkage and formation of apoptotic bodies.

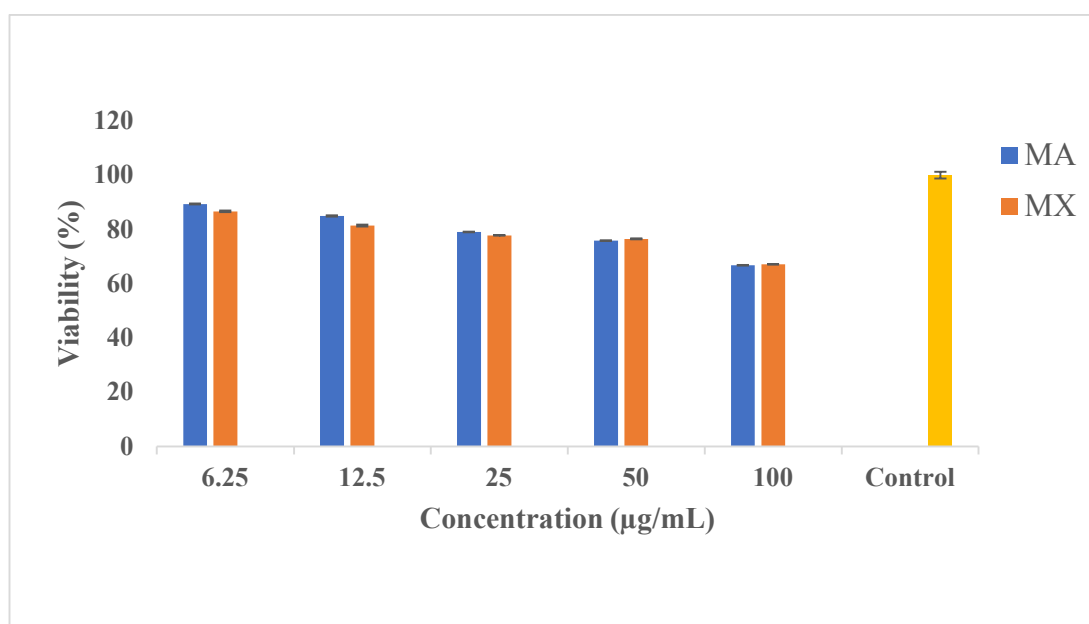


Fig. 26: Effect on the cell viability of DLD1 cell line treated with methanolic extracts of *M. xanthopus* and *M. affinis* using MTT assay

ii). *In vitro* activity on L929 cells using MTT assay

Fungal extracts of *M. xanthopus* and *M. affinis* were analysed for cytotoxicity on normal L929 cells (normal mouse fibroblast cell line) by MTT assay. Different concentrations in the range of 6.25 µg/mL to 100 µg/mL were tested and LC₅₀ was calculated. The cell viability was found to be decreasing in a dose dependent manner (Fig. 27). Images of cytotoxic effects were recorded for different concentrations (Plates: 15 - 16). Cell viability percentage at 100 µg/mL for *M. affinis* and *M. xanthopus* were observed as 68 ± 0.44 and 70.67 ± 0.26 respectively. LC₅₀ was found to be 166.98 ± 0.35 µg/mL and 164.61 ± 0.34 µg/mL for *M. affinis* and *M. xanthopus* respectively.

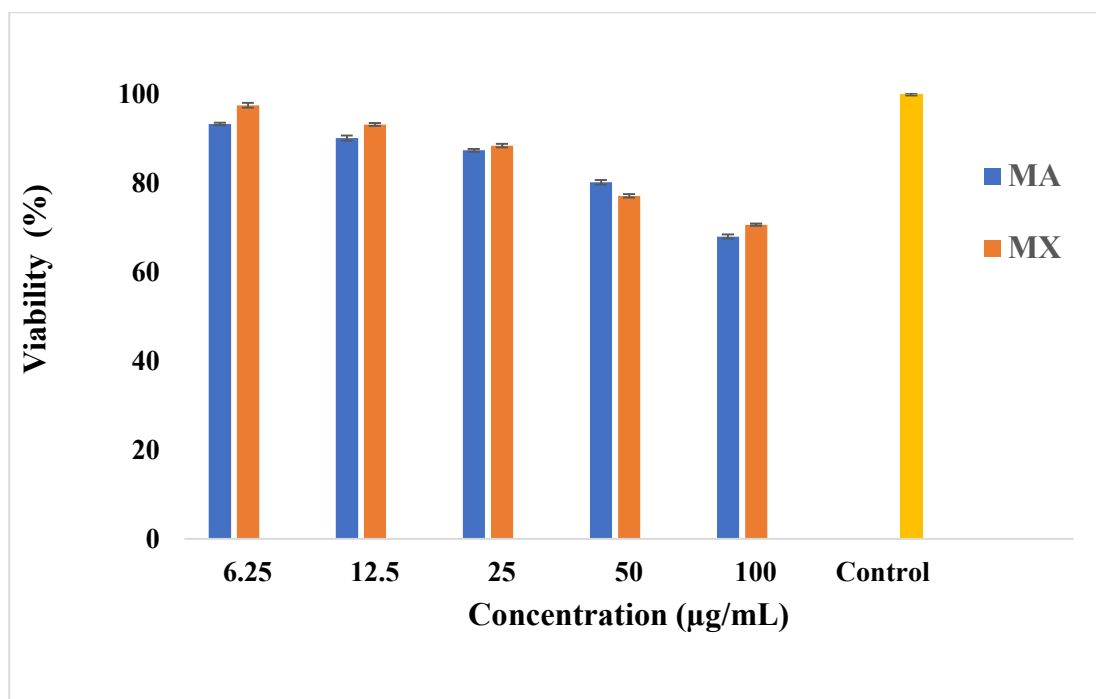


Fig. 27: Effect on the cell viability of L929 fibroblast cells treated with methanolic extracts of *M. xanthopus* and *M. affinis* using MTT assay

Table 9: LC₅₀ concentrations of methanolic extracts of *M. xanthopus* and *M. affinis* against DLD1 cell line and L929 fibroblast cells using MTT assay

Fungal species	LC ₅₀ - DLD1 cell line (µg/mL)	LC ₅₀ - L929 (µg/mL)
<i>M. xanthopus</i>	193.24 ± 0.32	164.61 ± 0.34
<i>M. affinis</i>	171.76 ± 0.43	166.98 ± 0.35

PLATE 13

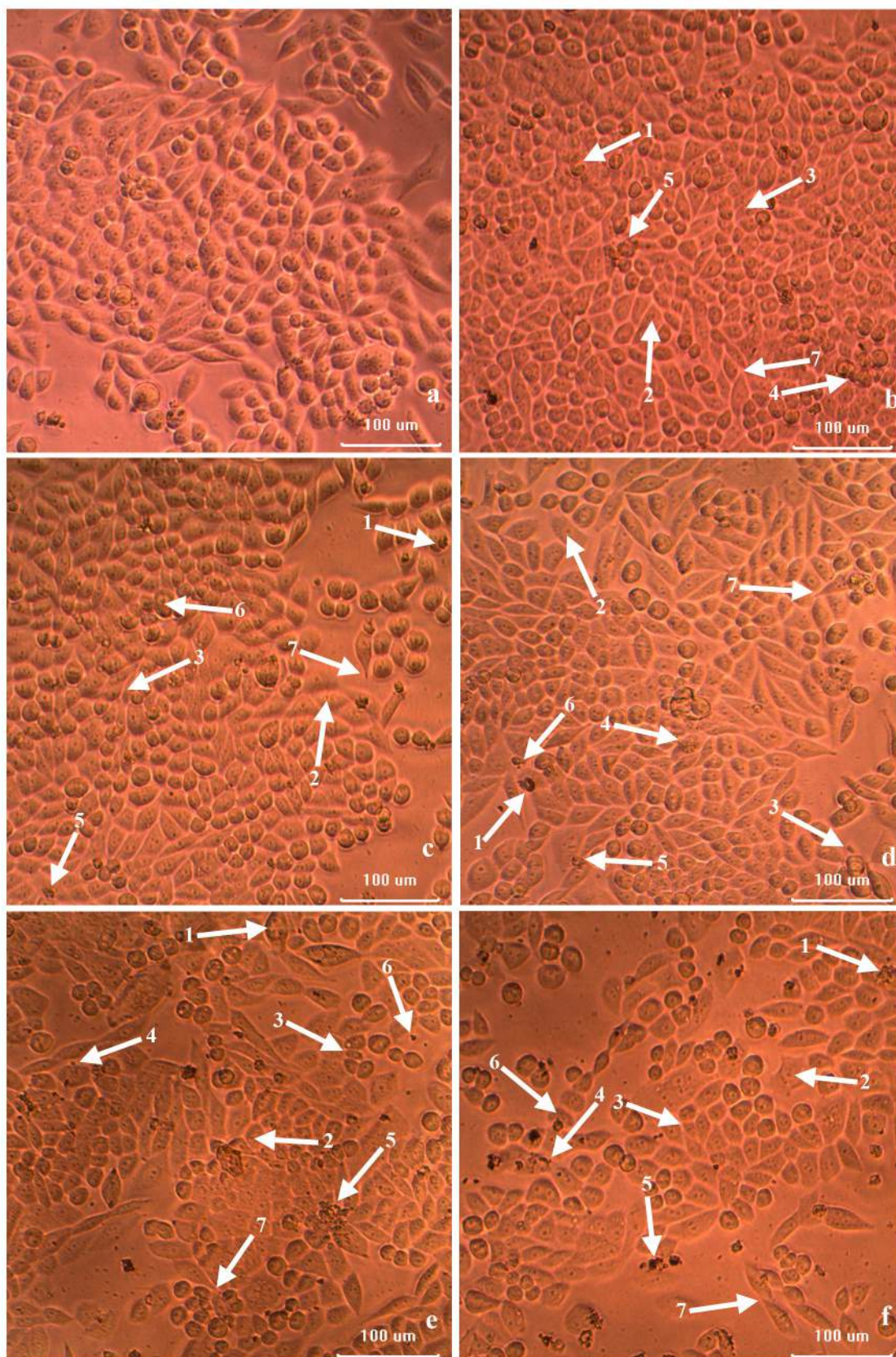


Plate 13: *In vitro* cytotoxic effects of different concentrations of methanolic extracts of *M. affinis* on DLD1 cell line using MTT assay **a.** Control; **b.** 6.25 µg/mL; **c.** 12.5 µg/mL; **d.** 25 µg/mL; **e.** 50 µg/mL; **f.** 100 µg/mL. **1.** Nuclear fragmentation; **2.** Condensed nuclei; **3.** Cell shrinkage; **4.** Membrane blebbing; **5.** Apoptotic bodies; **6.** Budding; **7.** Echinoid spikes. Scale bar 100 µm

PLATE 14

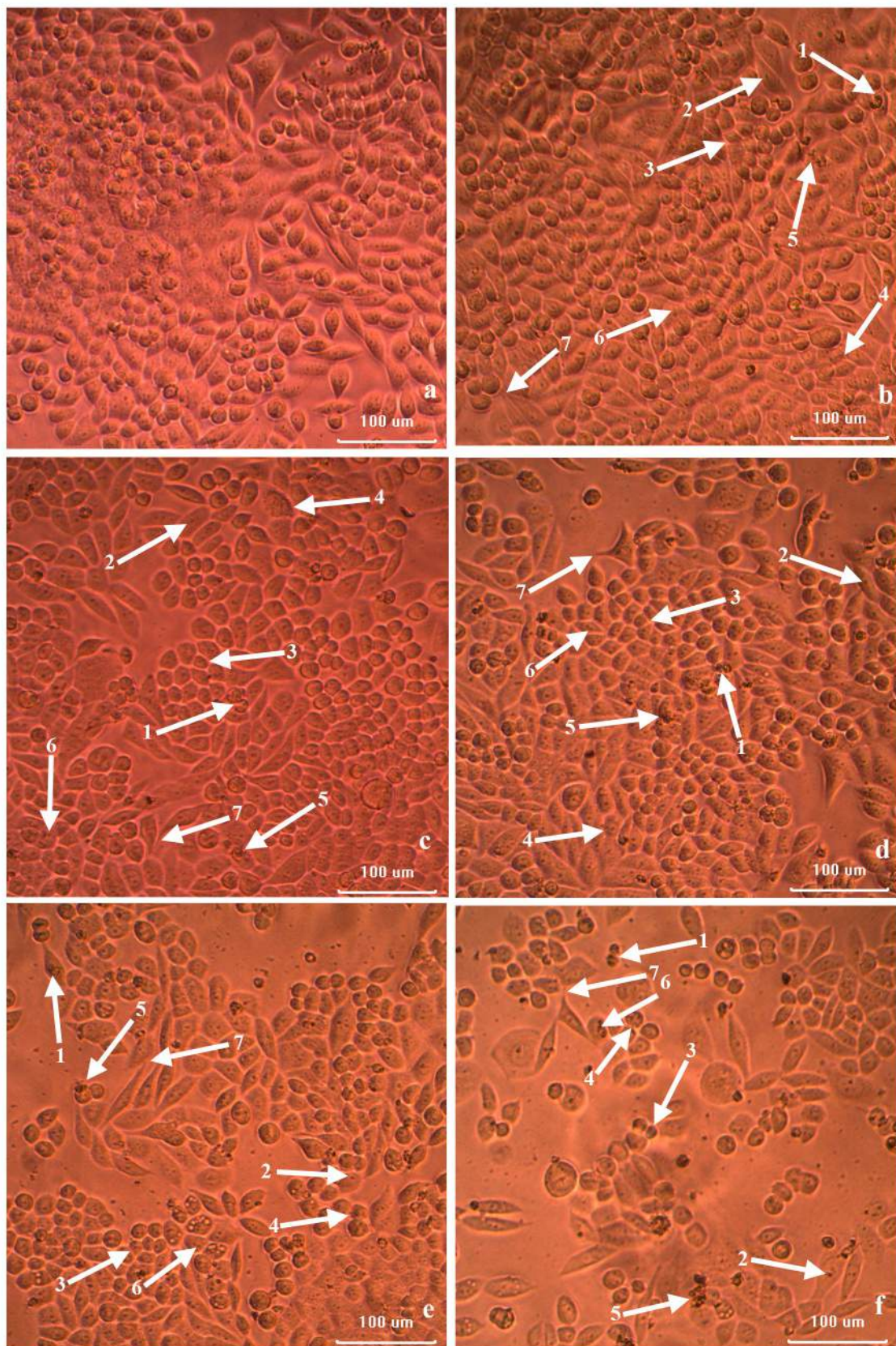


Plate 14: *In vitro* cytotoxic effects of different concentrations of methanolic extracts of *M. xanthopus* on DLD1 cell line using MTT assay **a.** Control; **b.** 6.25 µg/mL; **c.** 12.5 µg/mL; **d.** 25 µg/mL; **e.** 50 µg/mL; **f.** 100 µg/mL. **1.** Nuclear fragmentation; **2.** Condensed nuclei; **3.** Cell shrinkage; **4.** Membrane blebbing; **5.** Apoptotic bodies; **6.** Budding; **7.** Echinoid spikes. Scale bar 100 µm

PLATE 15

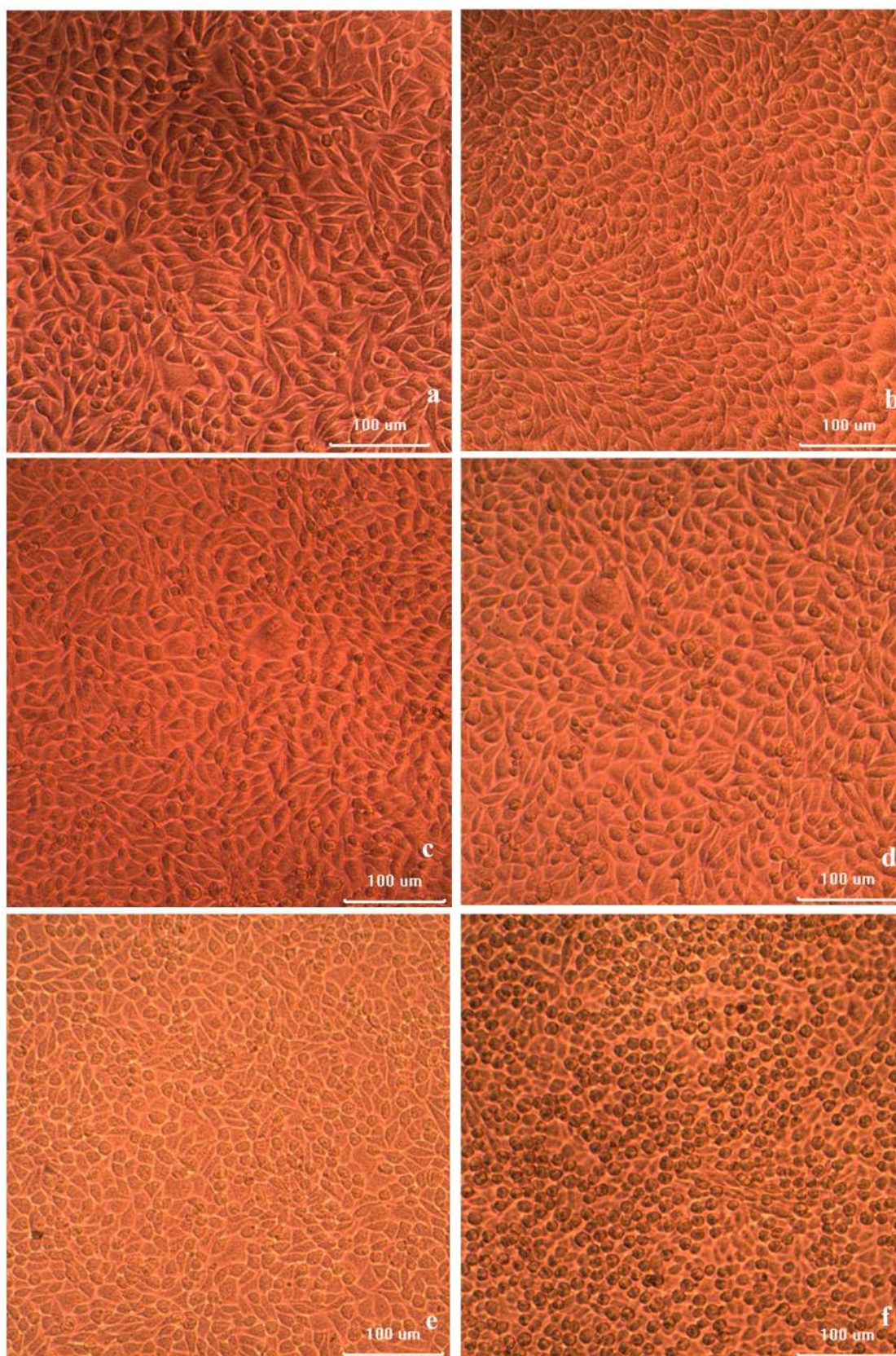


Plate 15: *In vitro* cytotoxic effects of different concentrations of methanolic extracts of *M. affinis* on L 929 cell line **a.** Control; **b.** 6.25 µg/mL; **c.** 12.5 µg/mL; **d.** 25 µg/mL; **e.** 50 µg/mL; **f.** 100 µg/mL. Scale bar 100 µm

PLATE 16

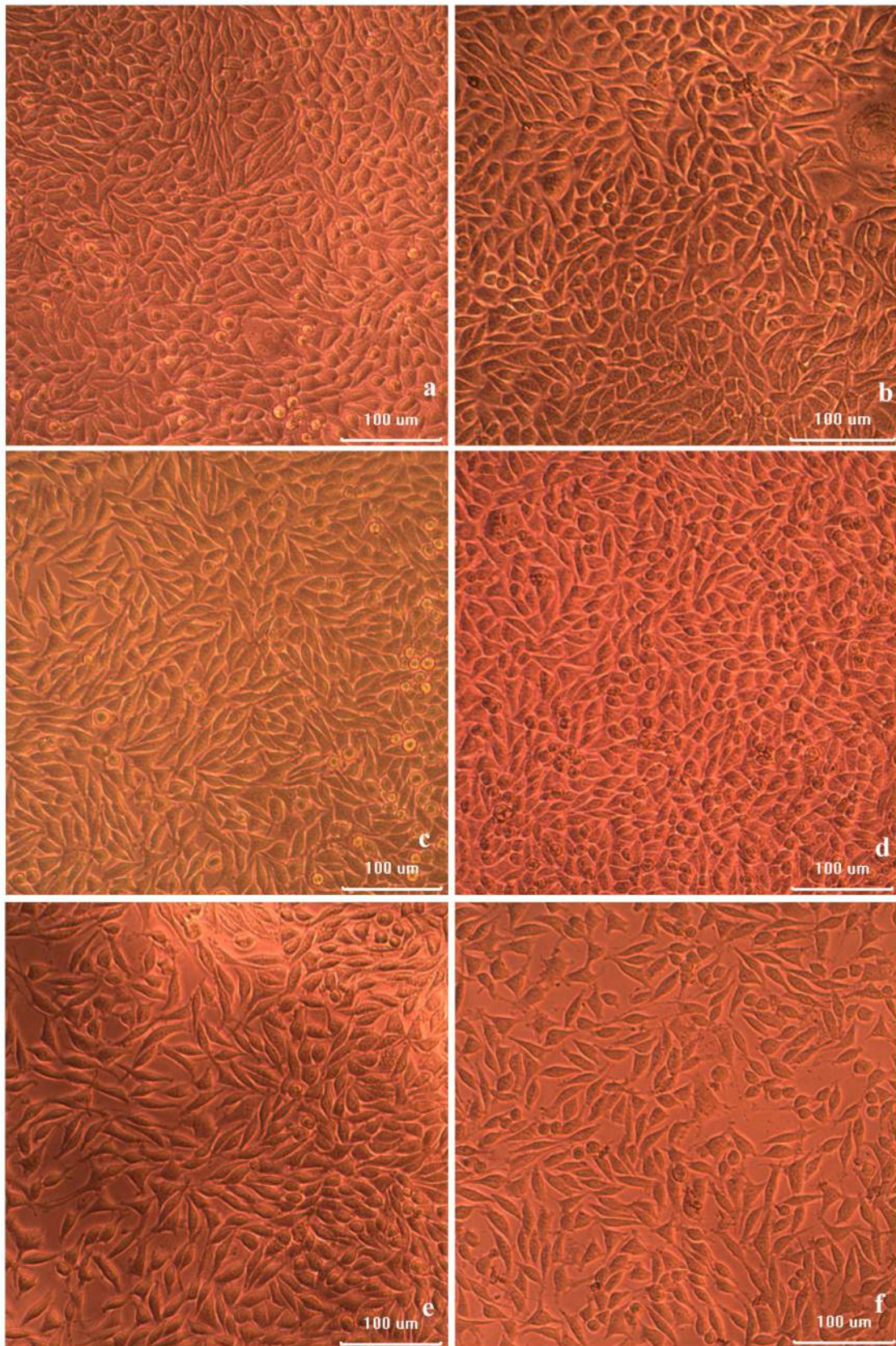


Plate 16: *In vitro* cytotoxic effects of different concentrations of methanolic extracts of *M. xanthopus* on L 929 cell line **a.** Control; **b.** 6.25 µg/mL; **c.** 12.5 µg/mL; **d.** 25 µg/mL; **e.** 50 µg/mL; **f.** 100 µg/mL. Scale bar 100 µm

PLATE 17

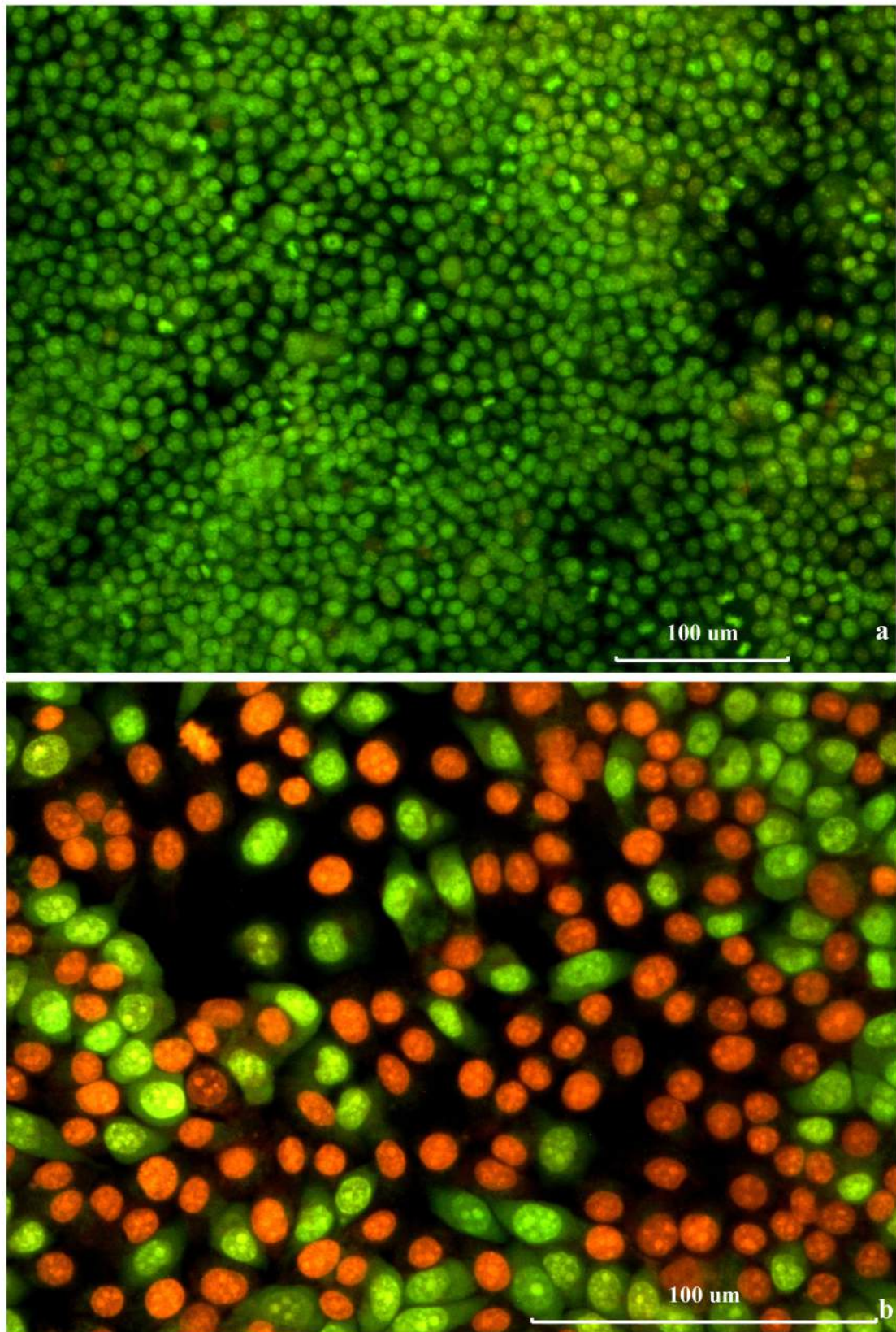


Plate 17: Detection of apoptosis by AO/EtBr staining on DLD1 cell line **a.** Control; **b.** Cells treated with methanolic extract of *M. xanthopus*. Scale bar 100 µm

PLATE 18

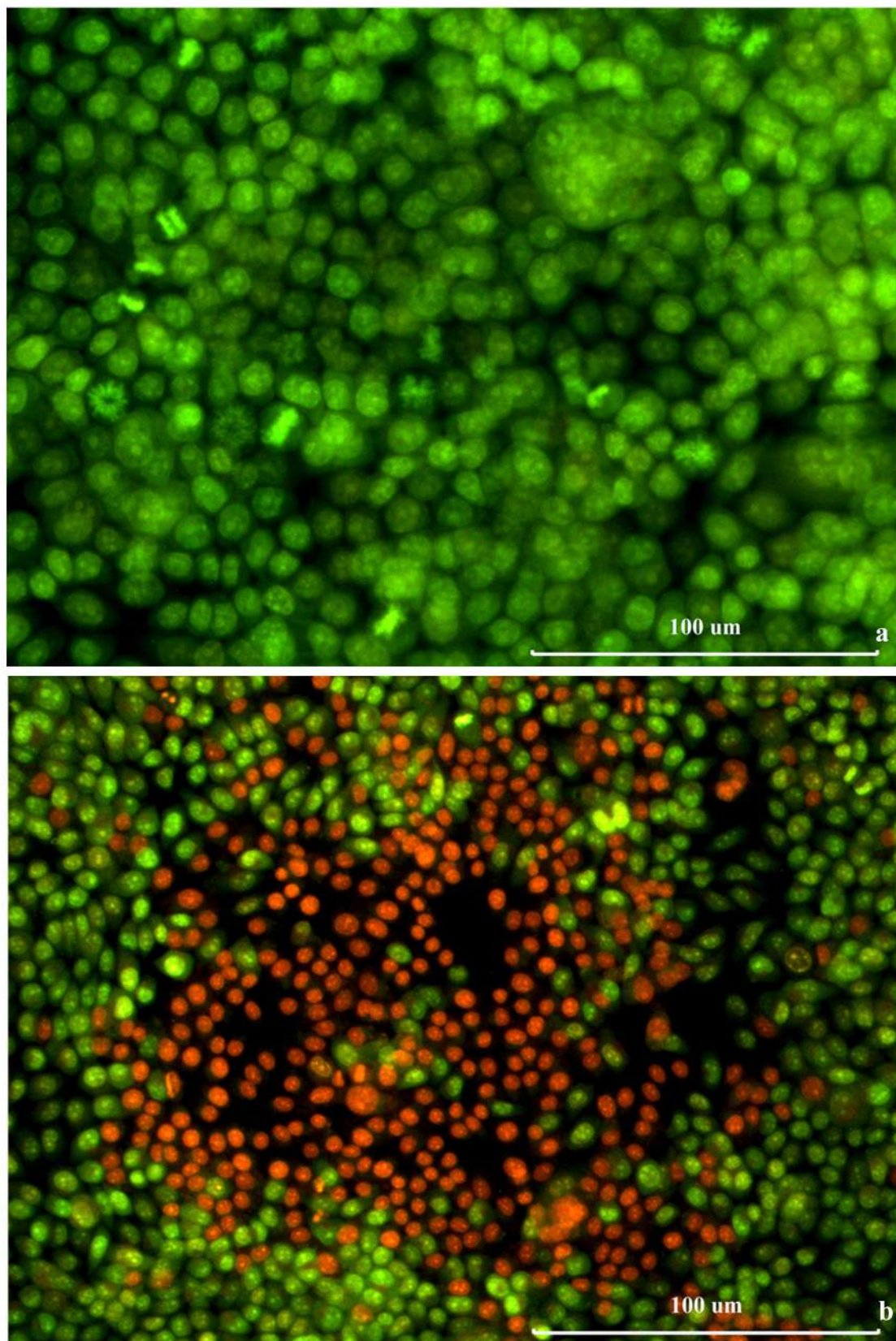


Plate 18: Detection of apoptosis by AO/EtBr staining on DLD1 cell line **a.** Control; **b.** Cells treated with methanolic extract of *M. affinis*. Scale bar 100 µm

Comparatively less LC_{50} value of *M. affinis* indicates its more cytotoxic potential towards DLD1 cell line than that of *M. xanthopus*.

iii). **Determination of apoptosis by using Acridine Orange (AO) and Ethidium Bromide (EtBr) double staining method**

DNA binding dyes Acridine Orange (AO) and Ethidium Bromide (EtBr) were used for the morphological detection of apoptotic and necrotic cells. Cell death can easily be distinguishable by using AO/EtBr staining. DLD1 cells were treated with LC_{50} concentration of each of the methanolic extracts. Clear-cut image of the live and dead cells will be obtained by this double staining method. LC_{50} concentrations of the methanolic extracts of two species of *Microporus* were treated with DLD1 cell lines and were stained using AO/EtBr double staining technique. This is the good method to differentiate apoptotic and necrotic cells from the normal cell lines. Morphologically 4 different cells can be observed after staining. The normal cells are having green nucleus which is clearly seen in the control. Bright green colour of the condensed or fragmented chromatin indicates the early apoptotic cells. While the late apoptotic cells have orange-red stained nucleus with chromatin condensation or fragmentation. Necrotic cells are uniformly stained with orange colour (**Plates: 17-18**).

d). **Preliminary neuroprotective study**

On a global scale, neurodegenerative diseases like Alzheimer's, Parkinson's, stroke etc., are increasing nowadays. Hence there is need for the studies on neuroprotective areas. Neuroprotective effects refer to the ability of substances or interventions to protect the nervous system from acute injuries or neurodegenerative diseases. Neuroprotective agents can work by various mechanisms, including inhibiting apoptosis, improving mitochondrial function, scavenging free radicals etc.

The preliminary study on neuroprotective effect of two species of *Microporus* was evaluated in IMR 32 neuroblastoma cell lines using different assays like MTT and apoptosis determination by AO/EtBr double staining.

i. *In vitro* neuroprotective effect determination by MTT assay

Neuroprotective effect of methanolic extract of *M. affinis* and *M. xanthopus* was determined using MTT assay in IMR 32 cell line. The cell viability of the fungal extract was compared with control and the cells were treated with amyloid. Cell viability was found to be declined in cells treated with amyloid (44.84 ± 0.33).

In the case of cells treated with the extract of *M. affinis*, percentage of cell viability increased from 51.70 ± 0.32 (for the concentration of $6.25 \mu\text{g/mL}$) to 57.87 ± 0.08 (for the concentration of $100 \mu\text{g/mL}$). Even though, there is an increase in percentage of viability, it is not concentration dependent as viability percentage was found to be greater in $25 \mu\text{g/mL}$ and $50 \mu\text{g/mL}$ than $100 \mu\text{g/mL}$ of the extract. Photographs of the morphology of treated cells are given in **Plate 19**.

There is a gradual increase in the percentage of cell viability for cells treated with the extract of *M. xanthopus* (**Fig. 28**). For the lowest concentration ($6.25 \mu\text{g/mL}$) it was found to be 46.20 ± 0.63 and for the highest concentration ($100 \mu\text{g/mL}$) viability percentage was increased to 84.51 ± 0.34 . This clearly indicates dose dependent efficiency of *M. xanthopus* in the recovery of degenerated neuronal cells. Microphotographs of the morphology of treated cells was also affected similarly and was recorded in **Plate 20**.

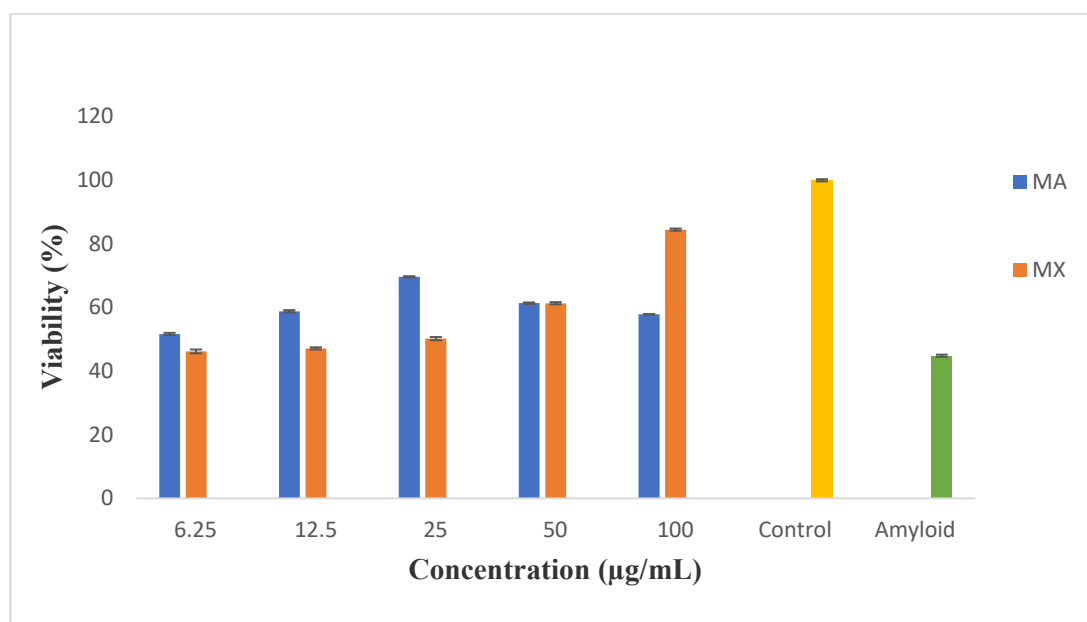


Fig. 28: Determination of cell viability percentage using MTT assay in the cells treated with amyloid, different concentrations of *Microporus* and the control.

PLATE 19

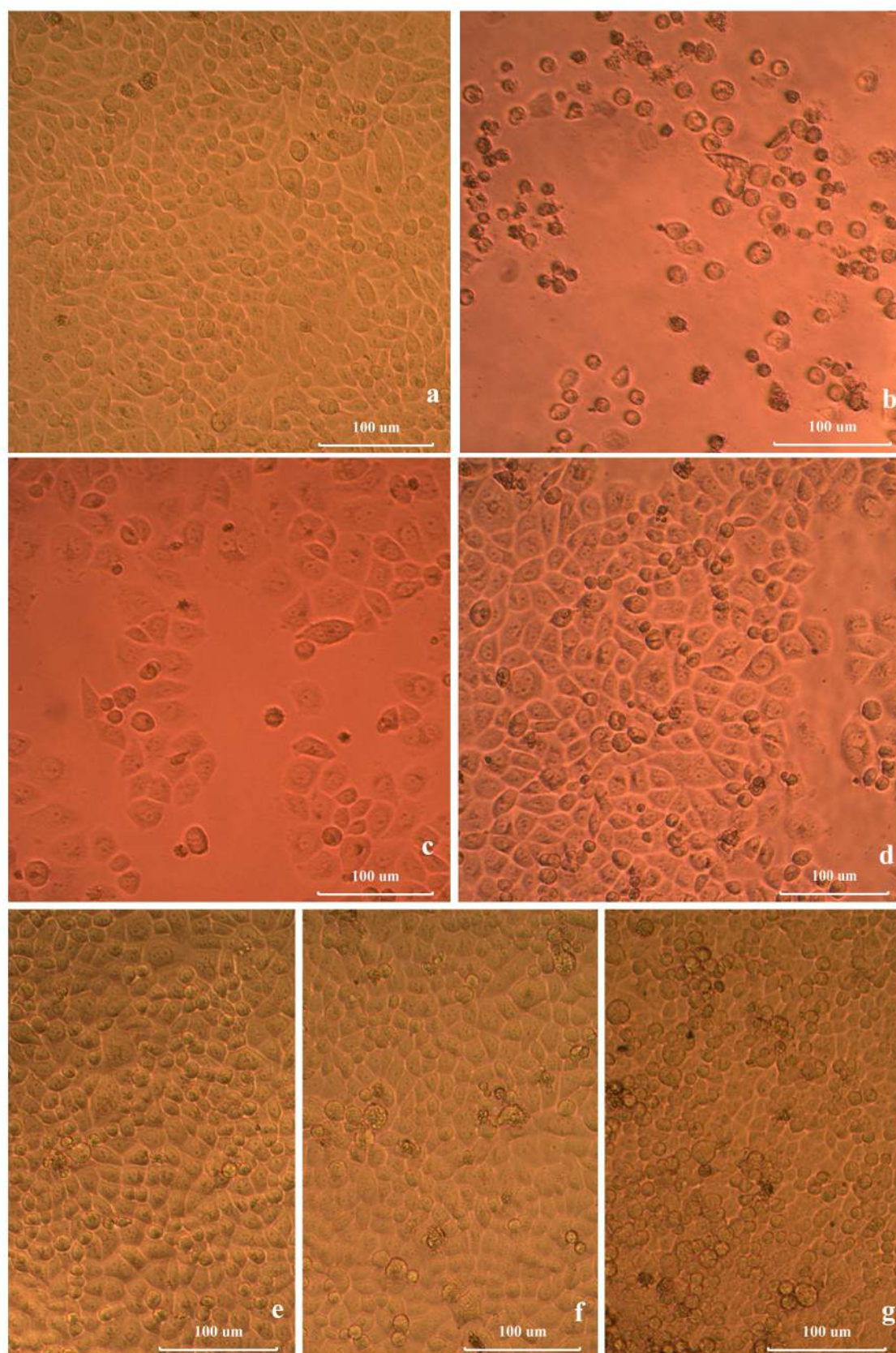


Plate 19: *In vitro* neuroprotective effect determination by MTT assay on methanolic extracts of *M. affinis* on IMR32 cell line **a.** Control; **b.** amyloid treated; **c-f:** cells treated with extracts of *M. affinis*; **c.** 6.25 µg/mL; **d.** 12.5 µg/mL; **e.** 25 µg/mL; **f.** 50 µg/mL; **g.** 100 µg/mL Scale bar 100 µm

PLATE 20

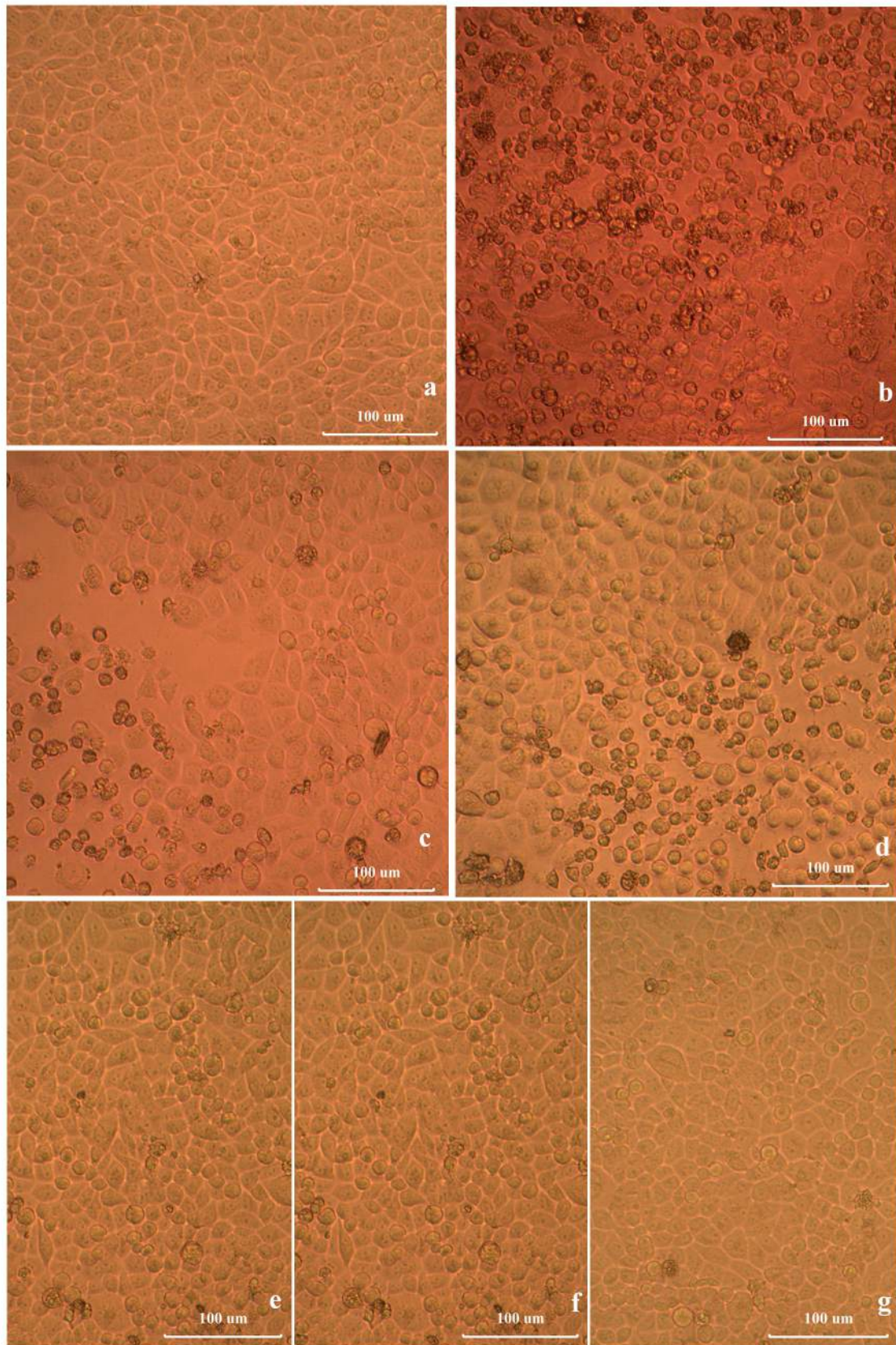


Plate 20: *In vitro* neuroprotective effect determination by MTT assay on methanolic extracts of *M. xanthopus* on IMR32 cell line **a.** Control; **b.** amyloid treated; **c-f:** cells treated with extracts of *M. affinis*; **c.** 6.25 µg/mL; **d.** 12.5 µg/mL; **e.** 25 µg/mL; **f.** 50 µg/mL; **g.** 100 µg/mL Scale bar 100 µm

PLATE 21

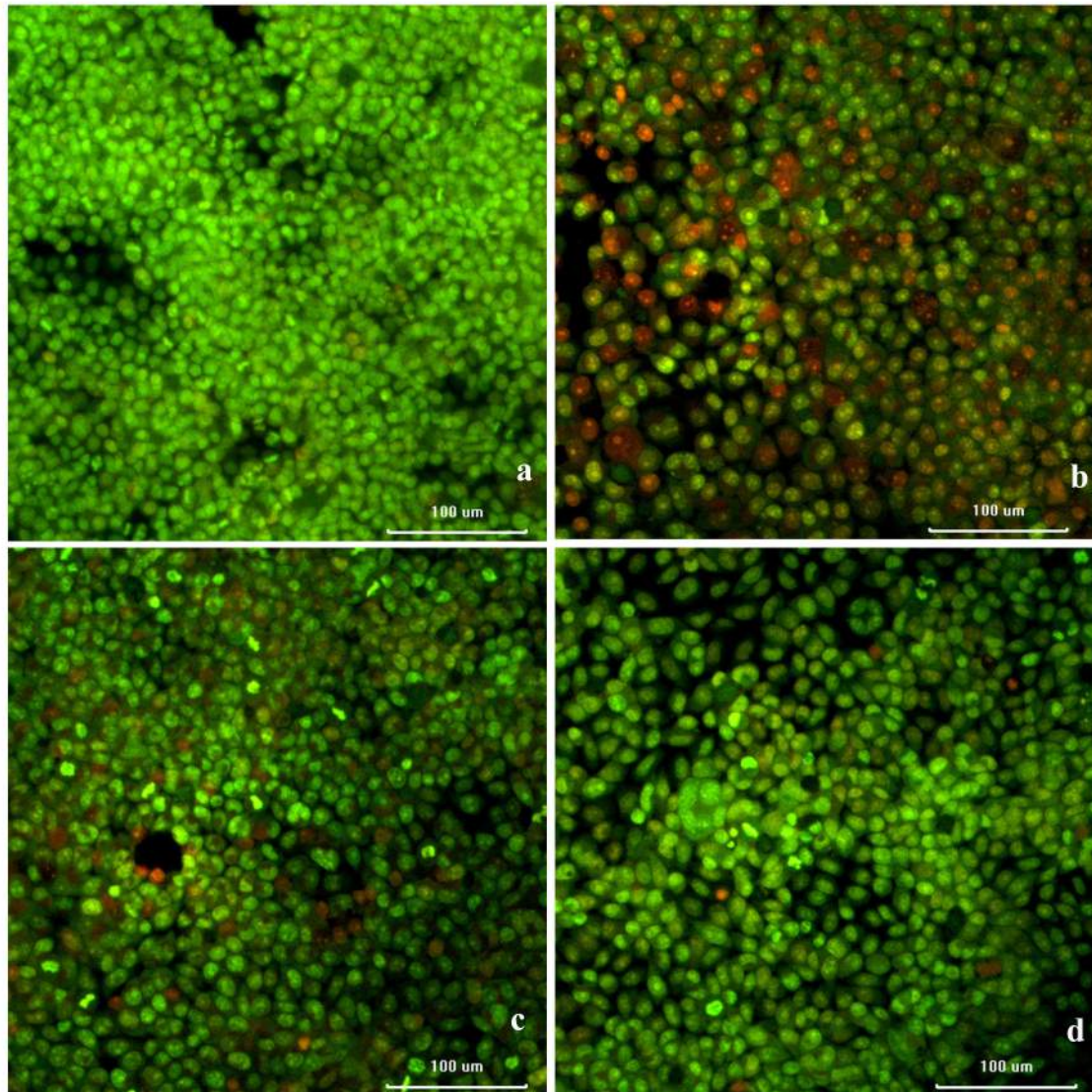


Plate 21: Determination of apoptosis by using AO/EtBr double staining on IMR32 cell line **a.** Control; **b.** Amyloid treated cells; **c.** Cells treated with the methanolic extracts of *M. affinis*; **d.** Cells treated with the methanolic extracts of *M. xanthopus*. Scale bar 100 µm

ii. Apoptosis determination by using acridine orange and ethidium bromide (AO-EtBr) double staining

For the morphological detection of apoptotic and necrotic cells, DNA-binding dyes Acridine Orange (AO) and Ethidium Bromide (EtBr) are used. AO is taken up by both viable and non-viable cells and emits green fluorescence. EtBr is taken up by only non-viable cells and emits red fluorescence. By observing the stained cells under fluorescence microscope, four categories of cells are obtained: living cells with normal green nuclei, early apoptotic cells with bright green nuclei, late apoptotic cells having orange stained nuclei with chromatin condensation or fragmentation and finally apoptotic cells with uniformly orange stained cell nuclei. In comparison with the extract of *M. affinis*, the extract of *M. xanthopus* was found to be more effective in neuroprotective aspects. The result was compared with the control and cells treated with amyloid. Thus, further studies are carried out in the extract of *M. xanthopus* only. Photographs taken are given in **Plate 21**.

PHASE III: DETAILED STUDY ON NEUROPROTECTION IN *MICROPORUS XANTHOPUS*

The preliminary study on neuroprotective effect of two species of *Microporus* was evaluated in IMR 32 neuroblastoma cell lines using different assays like MTT and apoptosis determination by AO/EtBr double staining. From these data it was clear that *M. xanthopus* is having a potential role in neuroprotection than *M. affinis*. Hence further studies are carried out on *M. xanthopus* only. Different assays were undertaken like ROS determination by using DCFDA, indirect ELISA, Autophagy, Annexin V-FITC apoptosis determination by flow cytometry and finally gene expression studies using qRT PCR.

i. *In vitro* ROS measurement using DCFDA

Dichlorodihydrofluoresceindiacetate (DCFDA) is initially a non-fluorescent, reduced form of dye that is permeable to cell membranes, that measures the ROS activity within the cell. After diffusion into the cell, DCFDA is deacetylated to another non - fluorescent compound, DCFH (2,7-Dichlorodihydrofluorescein), which is later oxidized to 2, 7 – Dichlorofluorescein (DCF). DCF exhibits strong green fluorescence. The intensity of the green fluorescence is directly proportional to the concentration of ROS within the cell (**Plate: 22**). Normally cells may contain ROS at low level. But as cells progresses with neurodegeneration, ROS increases.

The following represents the fluorescence measured by a fluorimeter at 470 nm excitation and emission at 635 nm (**Fig. 29**) and are expressed in arbitrary units (AU)

PLATE 22

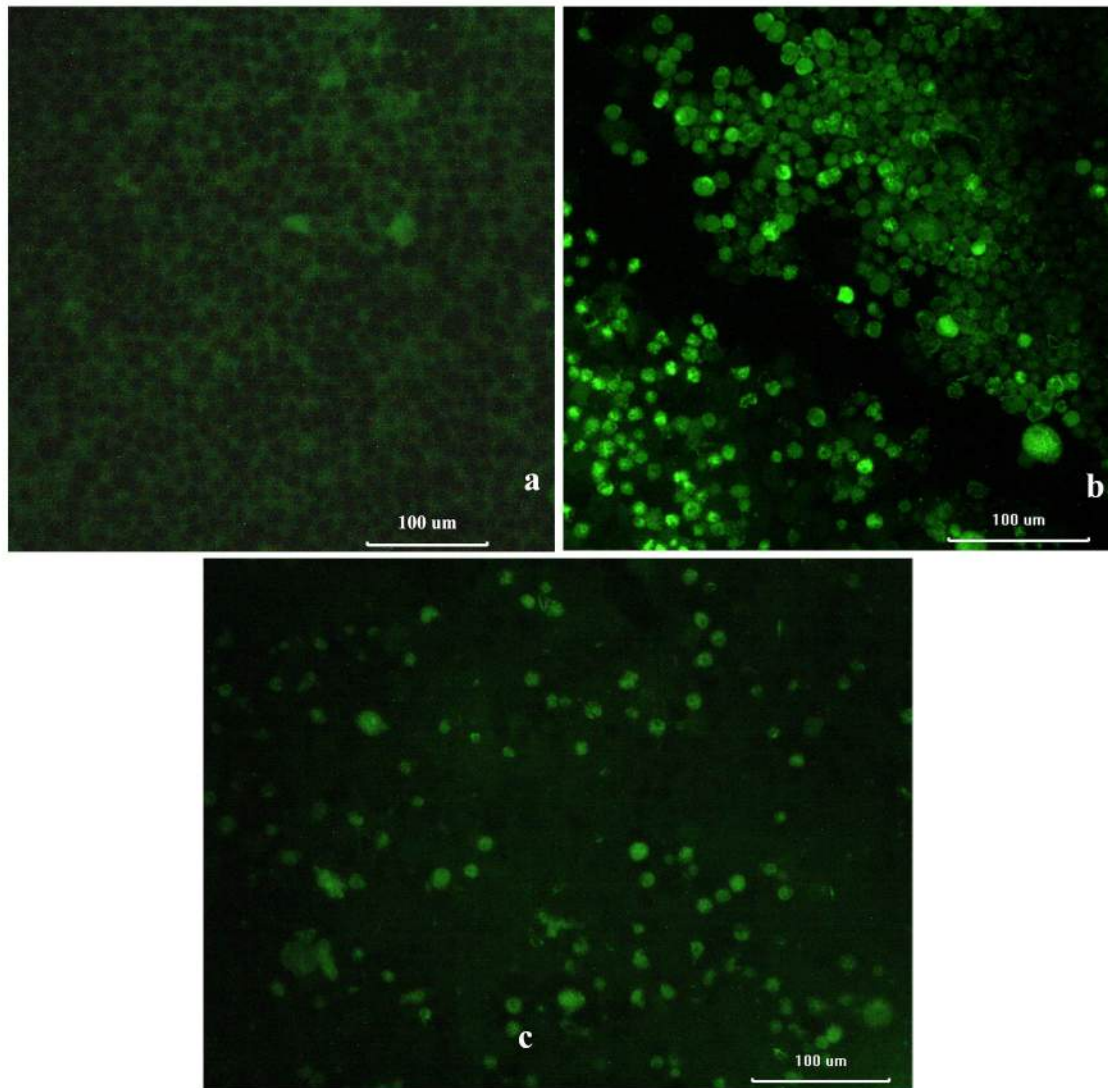


Plate 22: *In vitro* ROS measurement using DCFDA on IMR32 cell line **a.** Control; **b.** Amyloid treated cells; **c.** methanolic extract of *M. xanthopus* treated cells. Scale bar 100 µm

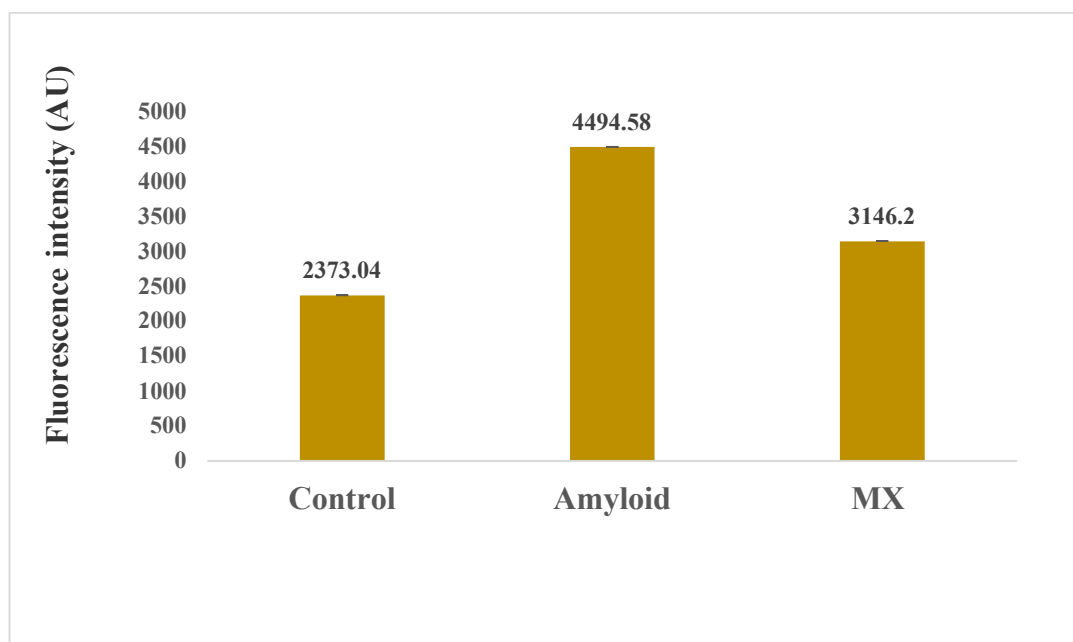


Fig. 29: Fluorescence observed for ROS study using DCFDA

ii. Indirect ELISA

An indirect ELISA offers a unique advantage over direct ELISA because it uses secondary antibody. It increases the sensitivity of the test. High sensitivity is crucial when detecting low levels of antigen in a sample. In general, indirect ELISA is used to determine the total antibody concentration in the sample. The accumulation of β – amyloid is a significant pathological characteristic of neurodegenerative diseases like Alzheimer's disease (AD).

Neuroprotective effect of methanolic extract of *M. xanthopus* was further substantiated by quantifying the expression of ChAt, Glutamate Receptor 2, Tau and alpha synuclein. IMR- 32 neuroblastoma cells were treated with previously calculated LC_{50} concentration of the extract ($61.34 \pm 0.32 \mu\text{g/mL}$) and subjected to indirect ELISA. The absorbance was measured and protein concentration was calculated for untreated control, amyloid treated cells and amyloid + MX treated cells. From this value, protein activity (**Fig. 30-33**) was calculated (OD value/protein concentration).

In case of almost all the proteins used, amyloid induced cells showed decline in the protein activity. While treating with the extract, protein activity gets recovered. This support the neuroprotective effect of the extract.

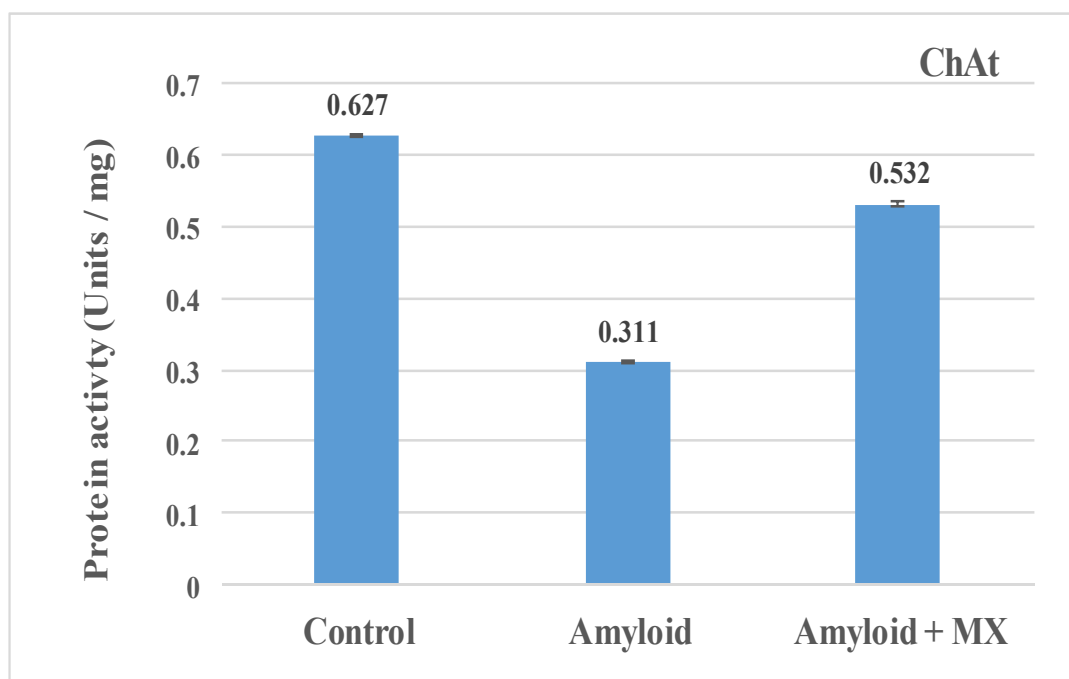


Fig. 30: Activity of protein (ChAt) using indirect ELISA

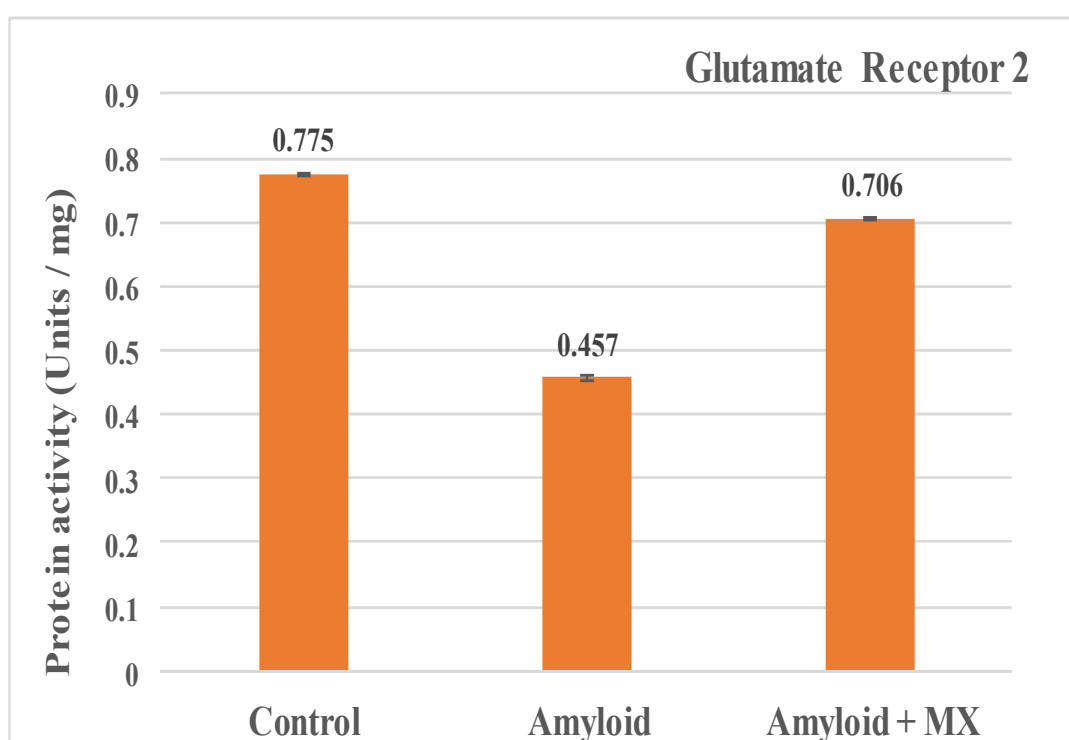


Fig. 31: Activity of protein (Glutamate Receptor 2) using indirect ELISA

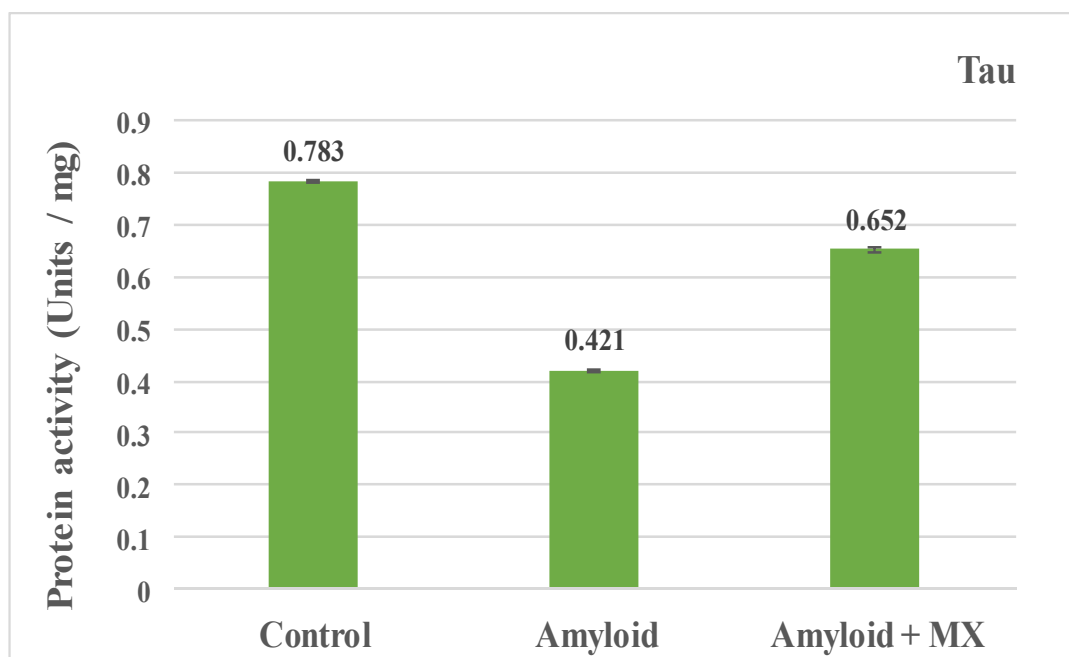


Fig. 32: Activity of protein (Tau) using indirect ELISA

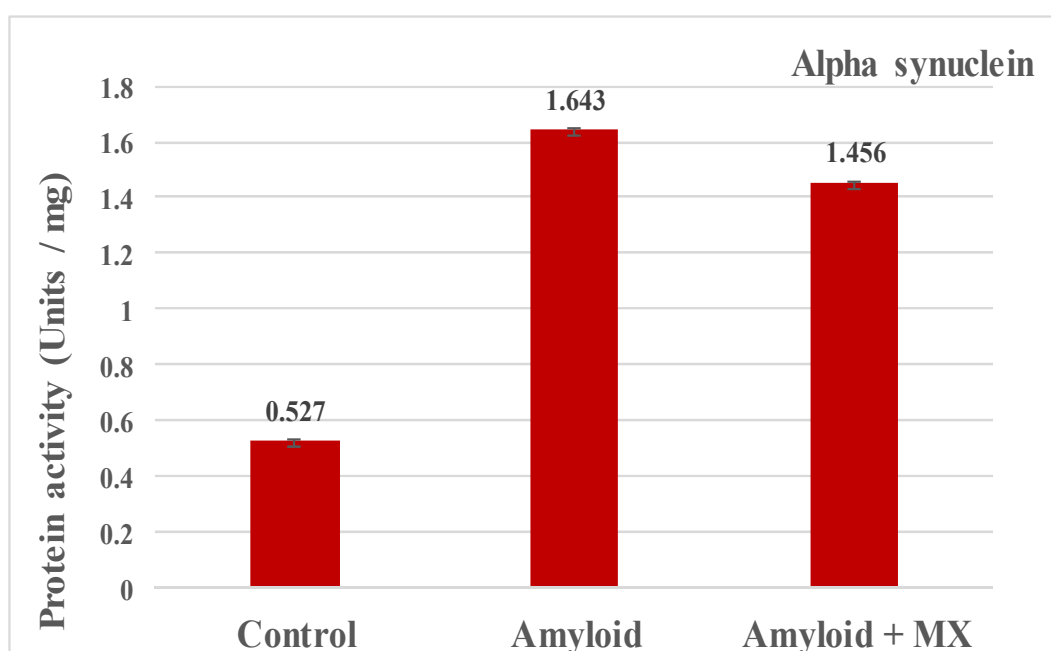


Fig. 33: Activity of protein (Alpha synuclein) using indirect ELISA

iii. Autophagy

When subjected to certain hostile conditions that threaten survival, such as when extracellular nutrients are limiting, eukaryotic cells employ a lysosome-mediated intracellular bulk degradation pathway for digesting their own cellular contents by a process referred to as autophagy. Under physiological conditions, autophagy plays a variety of important roles including maintenance of the amino acid pool during starvation, damaged protein and organelle turnover, prevention of neurodegeneration, tumour suppression, cellular differentiation, clearance of intracellular microbes and regulation of innate and adaptive immunity. Autophagy is considered to be a dynamic, multi-step process which can be regulated at several steps, in both a positive and negative manner. Autophagic activity is typically low under basal conditions, but can be markedly up-regulated, both in cultured cells and intact organisms, by a variety of physiological stimuli.

Population profile describe the characteristics of a cell population, while autophagy intensity reflects the extent of the autophagy process within those cells. Autophagy intensity is typically measured by observing the formation of autophagosomes (the vesicles that contain cellular waste) and the level of LC3 -II (a protein marker of autophagosomes). The amount of LC3-II reflects the number of autophagosomes. LC3 (Light chain 3) is a protein crucial for autophagy. Autophagy induction ratio is often measured as the ratio of GFP (Green Fluorescence Protein) LC3 to RFP (Red Fluorescence Protein) LC3 (**Fig. 34-42**). Low autophagy induction ratio suggests increased autophagy as GFP is degraded during autophagic flux while RFP – LC3 remains in the cytosol. Autophagy induction ratio in the cells treated with the extract of *M. xanthopus* was found (1.4) to be higher than that of cells treated with amyloid (1.0). This high ratio indicates that autophagy is blocked or not being effectively cleared. Thus, it is clear that *M. xanthopus* has the potential to trigger autophagy.

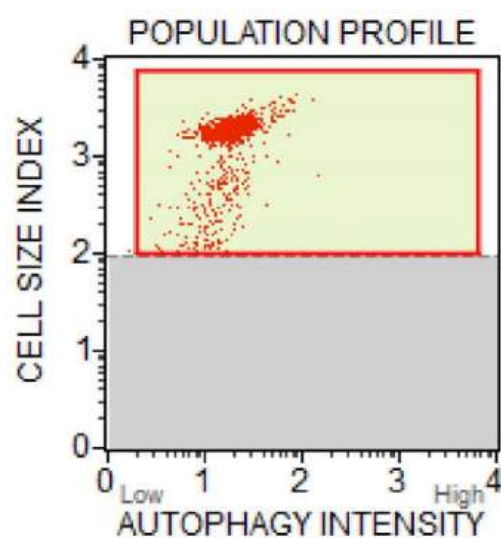


Fig. 34: Population profile of untreated control cells

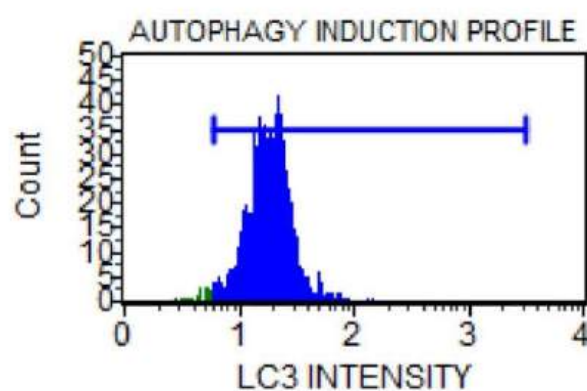


Fig. 35: Autophagy profile of untreated control cells

	Mean Autophagy Intensity	Autophagy Induction Ratio	Total No. of Cells	Sample Type
Autophagy (LC3 detection)	20.4	1.0	977	control

Fig. 36: Autophagy intensity in untreated control cells

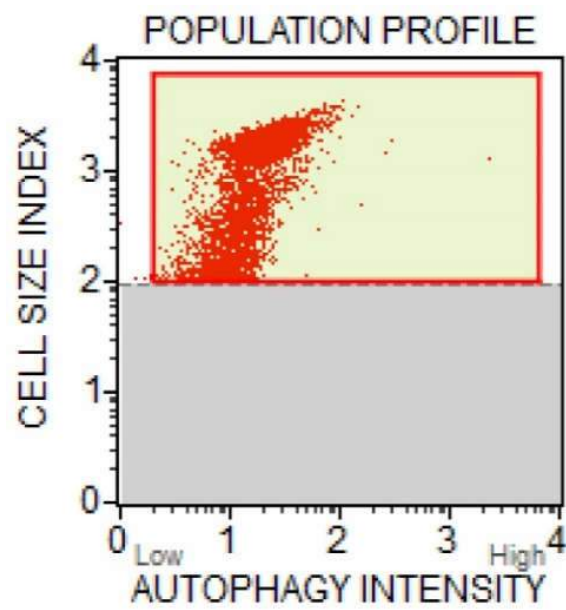


Fig. 37: Population profile of cells treated with Amyloid

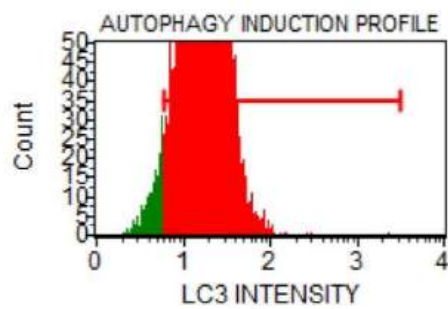


Fig. 38: Autophagy profile of cells treated with Amyloid

	Mean Autophagy Intensity	Autophagy Induction Ratio	Total No. of Cells	Sample Type
Autophagy (LC3 detection)	21.3	1.0	4756	target

Fig. 39: Autophagy intensity in cells treated with Amyloid

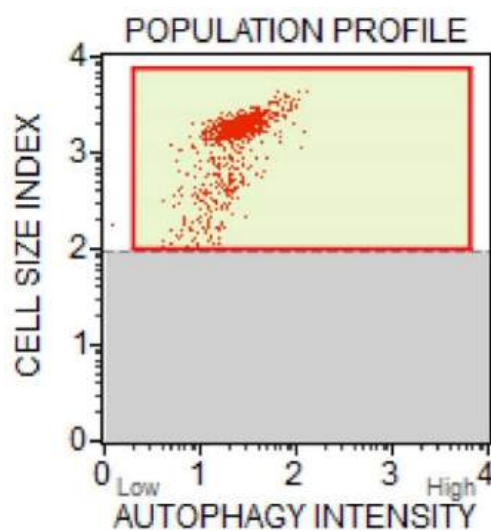


Fig. 40: Population profile of cells treated with sample

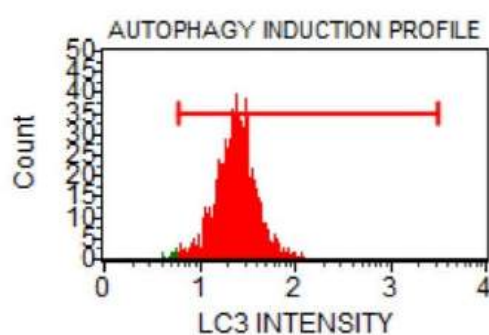


Fig. 41: Autophagy profile of cells treated with sample

	Mean Autophagy Intensity	Autophagy Induction Ratio	Total No. of Cells	Sample Type
Autophagy (LC3 detection)	27.6	1.4	986	target

Fig. 42: Autophagy intensity in cells treated with sample

iv. Annexin V- FITC apoptosis by flow cytometry

Apoptosis is a carefully regulated process of cell death that occurs as a normal part of development. Inappropriately regulated apoptosis is implicated in disease states, such as Alzheimer's disease and cancer. Apoptosis is distinguished from necrosis or accidental cell death by characteristic morphological and biochemical changes including compaction and fragmentation of the nuclear chromatin, shrinkage of the cytoplasm and loss of membrane asymmetry.

Annexin V- FITC is a fluorescent probe used to detect apoptosis, specifically identifying cells undergoing early apoptosis by binding to phosphatylserine (PS) exposed on the cell surface. PI (Propidium Iodide) is a nucleic acid dye which enter cells that have lost membrane integrity such as late apoptotic or necrotic cells. Thus, Annexin FITC is combined with (PI) to distinguish viable, early apoptotic and late apoptotic/necrotic cells. Annexin V is a calcium dependent phospholipid binding protein with a high affinity for PS. In healthy cells, PS is primarily found on the inner part of the cell membrane. During apoptosis, PS translocates to outer environment. Annexin V is labelled with FITC, a fluorescent dye that allows for the visualization of the binding of Annexin V to PS on the cell surface.

From the flow cytometry analysis, cells can be distinguished as follows:

- Viable cells: Negative for both Annexin V- FITC and PI
- Early apoptotic cells: Positive for Annexin V-FITC and negative for PI
- Late apoptotic cells: Positive for both Annexin V- FITC and PI
- Necrotic cells: Negative for Annexin V- FITC and positive for PI

After staining a cell population with FITC Annexin V and PI in the provided binding buffer, apoptotic cells show green fluorescence, dead cells show red fluorescence and live cells show little or no fluorescence (**Fig. 43-48**).

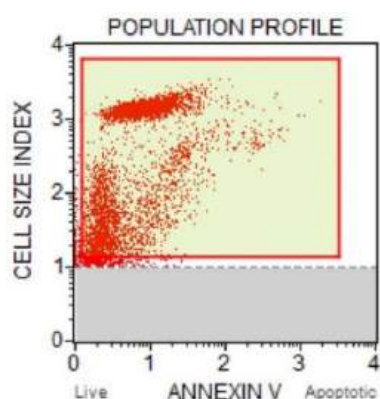


Fig. 43: Population profile of untreated cells (control)

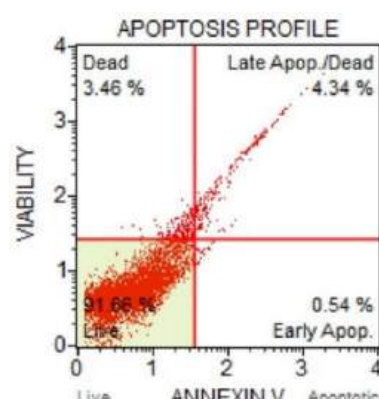


Fig. 44: Apoptosis profile of untreated cells (control)

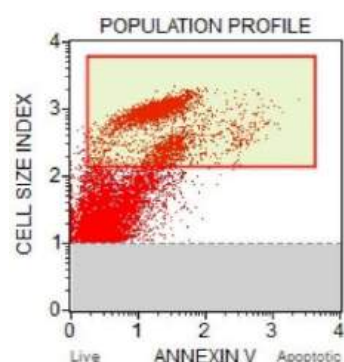


Fig. 45: Population profile of amyloid treated cells

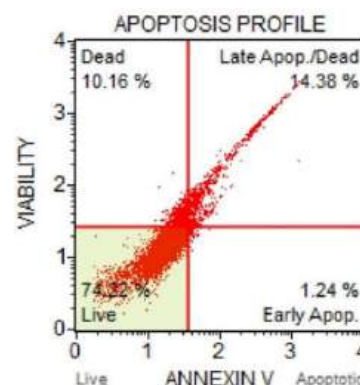


Fig. 46: Apoptosis profile of amyloid treated cells

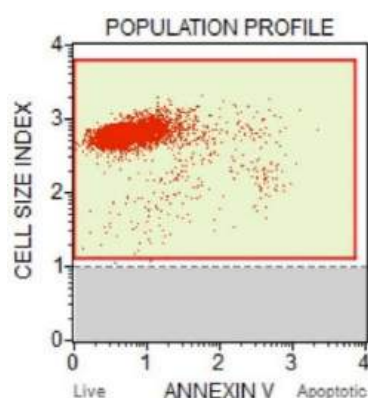


Fig. 47: Population profile of sample treated cells

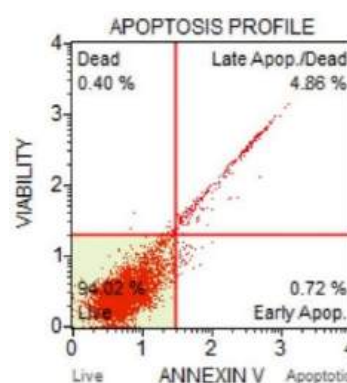


Fig. 48: Apoptosis profile of cells treated with sample

Table: 10: Distribution of cells in control, cells treated with amyloid and cells treated with sample

Cells	Cell concentration (cells /mL)			% gated		
	Control	Amyloid treated	Sample treated	Control	Amyloid treated	Sample treated
Live cells	2.10E+06	1.95E+06	6.18E+06	91.66	74.22	94.02
Early apoptotic	1.23E+04	3.25E+04	4.73E+04	0.54	1.24	0.72
Late apoptotic	9.92E+04	3.77E+05	3.20E+05	4.34	14.38	4.86
Debris / necrotic	7.91E+04	2.66E+05	2.63E+04	3.46	10.16	0.40
Total apoptotic	1.12E+05	4.10E+05	3.67E+05	4.88	15.62	5.58

v. Gene expression studies using qRT PCR

Gene expression and regulation are the basis of cell development and differentiation. qRT PCR (quantitative reverse transcription polymerase chain reaction) is a highly sensitive and accurate method for measuring gene expression levels. The ability of methanolic extract of *M. xanthopus* in neuroprotection was studied by analysing the expression of Parkin gene. The Parkin gene plays a crucial role in neuroprotection, particularly by promoting the clearance of amyloid, a hallmark of Alzheimer's disease (AD) and other neurodegenerative disorders. Parkin knock down led to accumulation of beta amyloid. In AD brain, Parkin was found to interact with beta amyloid and its levels were reduced. Thus, Parkin is cytoprotective, partially by increasing the removal of cellular amyloid through a proteasome dependent pathway. Mutations in Parkin cause autosomal recessive Parkinson's disease.

In the expression study of Parkin gene, control, amyloid treated cells and sample treated cells showed a change in expression fold as 1, -0.323 and 1.29 respectively (**Fig. 49**). Fungal extract has the potential for upregulation of Parkin's activity. Parkin can influence the processing of APP, the precursor protein for amyloid.

Parkin may directly participate in the degradation of beta amyloid, helping to clear them from the brain and reduce amyloid plaques.

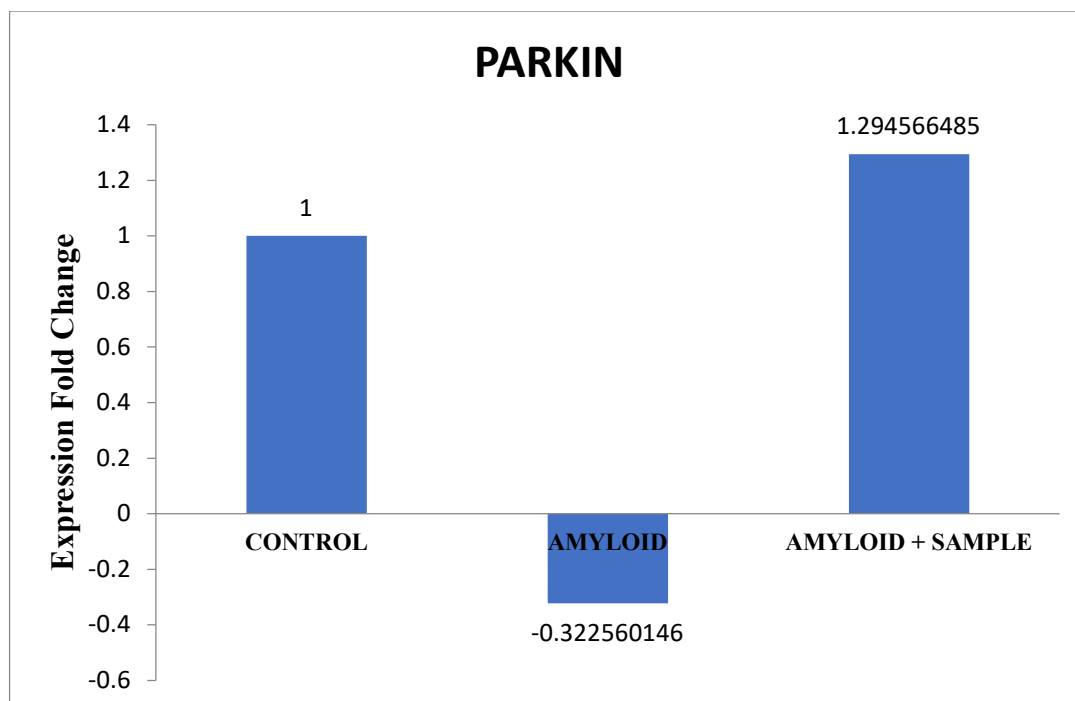


Fig. 49: Expression fold changes of Parkin gene in IMR 32 cell lines treated with amyloid and the sample



Discussion

DISCUSSION

Fungi and humans have a long history together, especially in terms of their chemical interactions. This strong connection was depicted by Alexander Flemming in 1928, who observed the efficiency of killing bacterium by a fungus called *Penicillium notatum* on a Petri dish inoculated with *Staphylococcus aureus* (Steffan et al., 2020). Fungi, a diverse group of organisms, have developed several communication and protection strategies, one of which involves the synthesis of a diverse group of bioactive compounds, also known as secondary metabolites (Kanerla et al., 2024; Elhamouly et al., 2022). These secondary metabolites are important for fungi for the interaction with the environment and for survival but are not required for reproduction of the fungi. The fungi have played an important role in shaping human society, which is evident from the discovery of penicillin (Moonjely, 2022). Due to the continuous investigations on fungal secondary metabolites today, several life-saving drugs are available in the market.

A good number of macrofungi produce large and conspicuous fruiting bodies. These macrofungi mainly belong to two orders, Polyporales and Agaricales, of class Basidiomycetes. Most of the polypores are wood inhabiting and rest are terrestrial. Beyond the ecological roles, polypores may have diverse industrial applications due to the capability of selective delignification (Wu et al., 2005). Many bioactive compounds which impart the medicinal polypore properties are still being isolated (Grienke, 2014). Bracket fungi are a complex group of species formed by different representatives of Basidiomycetes.

Indian mushroom industry is witnessing a fabulous change in the recent years with respect to its diversity, as a source for different types of chemicals, culinary properties and significant role in therapeutic field. Historically, medicinal mushrooms (especially Basidiomycetes) have been shown to have profound health promoting benefits and recent studies are now confirming their medical efficacy and identifying many of the bioactivities. Mushrooms are advantageous as source of bioactive compounds over plants because their fruiting body can be produced in shorter period

of time. Different wild mushrooms have been previously reported to have high antimicrobial and antioxidant activity. Mycochemical analysis from previous studies have shown that antioxidants found in mushrooms are mainly from phenolic compounds (phenolic acids and flavonoids), tocopherols, ascorbic acid and carotenoids (Ferreira et al., 2009).

Bracket fungi are one important group of mushroom family, a member of the division Basidiomycota with a woody and circular fruiting body called conks. A distinguishing factor of common mushroom and bracket fungi is the lack of a stipe, only a few of them have a short stipe. Otherwise, they grow directly from the wood on which they live (Fogel & Rogers, 2006). Bracket fungi are agents of root and wood destruction in the woody plants. Most of them are inedible and only a few bracket fungi species are edible. People consumed these fungi because of their nutritional and medicinal properties. *Ganoderma lucidum* (W. Curt. Fr.) P. Karst., is one of the examples of edible bracket fungi. Based on traditional knowledge, it is being used for the treatment of different ailments (Morris et al., 2017) and boosts the immune system (Wachtel-Galor et al., 2004).

Many polypore species are however, employed in pharmacology for the treatment of a wide range of ailments including bacterial and viral infections, allergies, neurological problems and cancer (Lindequist et al., 2005). Indeed, *Fomitopsis betulina* is reported to have been used as an antibacterial agent as long as 5300 years ago (Capasso, 1998) and has a long history of use in folk medicine. In modern day, its biotechnological potential has been highlighted recently with pharmacological studies indicating significant antimicrobial, anticancer, anti-inflammatory and neuroprotective activities (Pleszczyn'ska et al., 2017).

Studies conducted by Ao et al. (2016), reported that *M. xanthopus* is an inedible bracket fungus which grows in the fallen branches and twigs. It is distributed round the year. There is a report on the use of *M. xanthopus* to stop child from breast feeding (Chang & Miles, 2004; Das, 2009; Deshmukh, 2004).

Since ancient times, mushrooms were widely appreciated all over the world, particularly in China, Korea, Japan, Central and North American countries for their

nutritional value and medicinal properties. Mushrooms contain substantial amount of dietary fibers, as well. Modern scientific data has documented that mushrooms represent an unlimited source of bioactive molecules as an example of molecular diversity with recognized potential in drug discovery and development. Basidiomycetes mushrooms (phylum Basidiomycota) including agaric and bracket fungi are natural source of bioactive molecules and valuable enzymes with around 126 therapeutic effects (Wasser, 2002; Wasser, 2010; Badalyan, 2012). Therefore, they are considered as perspective organisms to develop different healthcare biotech-products. Basidiomycetes are the major class of fungi, able to grow at different environmental conditions and leads to the creation of a range of secondary metabolites. As opposed to ascomycetes fungi, basidiomycetes exhibit a diverse nature in their morphology and secondary metabolites.

PHASE I: MYCOCHEMICAL CHARACTERIZATION

i. Mycochemical characterization by qualitative & quantitative analysis, HR-LC/MS and GC/MS analysis

Mushrooms have a low fat, high protein content and a high content of several vitamins (B, C, D, K). In the present study, the presence of vitamin D (calciferol) is reported through HR-LC/MS in the extracts of *M. xanthopus*. GC/MS analysis conducted in both the species of *Microporus* reported to possess ergosterol. According to Phillips et al. (2011), ergosterol is the provitamin of ergocalciferol (Vitamin D2).

The main chemical constituent in the fruiting bodies of mushrooms falls primarily under the class of polysaccharide and also includes substantial amounts of fibers, other saccharides, terpenoids and proteins (Singdevsachan et al., 2016). A comprehensive study on various bioactive ingredients such as polysaccharides, terpenoids, steroids, phenolics and alkaloids isolated from mushrooms has been reported for their chemical and pharmacological properties (Öztürk et al., 2015). A diverse spectrum of chemical compounds with potential pharmacological activity has been isolated from the fruiting bodies of *Ganoderma* mushrooms: triterpenoids, polysaccharides, proteins, amino acids, alkaloids, steroids, fatty acids and enzymes (Becovic et al., 2014). Bracket fungi, also known as polypores, are known to produce

a wide variety of secondary metabolites, including alkaloids, terpenoids and steroids, which play important roles in their defence mechanisms and potential applications in medicine and other fields. These compounds are produced through complex biosynthetic pathways and can exhibit diverse biological activities. Present study revealed the presence of alkaloids, steroids and terpenoids in both species of *Microporus*. Saponins and glycosides are reported only in *M. xanthopus*. Qualitative analysis in both the species of *Microporus* showed negative results for flavonoids, phenols and tannins (**Table 4**).

Fungi are the rich source of bioactive secondary metabolites, many of which have been instrumental in transforming medicine and pharmaceuticals. Fungal metabolites have led to the discovery of several life-saving drugs, including antibiotics, anticancer agents, immunosuppressants and more.

Alkaloids are the nitrogen-containing compounds that are often derived from amino acids and exhibit a wide range of biological activities including antimicrobial and anticancer properties. Fungal alkaloids such as ergot alkaloids are well-known for their toxic and medicinal properties. Alkaloids are a major class of fungal secondary metabolites with several biological and therapeutic uses. Fungi synthesize these compounds through their secondary metabolism, which is not involved in their growth or reproduction. Alkaloids from fungi have shown significant promise in pharmaceutical applications. For example, verticillins, a group of epipolythiodioxopiperazine alkaloids, exhibit potent cytotoxicity against cancer cell lines and are being explored for their chemotherapeutic potential. These compounds probably function as specific inhibitors of histone methyltransferases, therefore affecting apoptosis and immune recognition (Jakubczyk & Dussart, 2020).

The wide range of activity demonstrated by fungal alkaloids, encompassing antibacterial, antifungal and immunosuppressive properties, highlights their promise as therapeutic agents. Their wide-ranging uses in medicine, particularly in Ayurvedic practices, emphasize the need for additional investigation and application (Roy et al., 2021).

Terpenes are one of the major fungal organic compounds. Terpenes demonstrate a wide range of biological actions, comprising antioxidative, anti-inflammatory, antiviral and antitumor activities. The aforementioned activities render them highly attractive contenders for pharmaceutical applications, including the development of anticancer medications and antibiotics. As a subset of terpenoids, diterpenes are known for their wide range of biological activities and applications in the fields of medicine, agriculture and personal care (Masyita et al., 2022).

Due to their low-fat content, mushrooms are low in calories (Muszynska et al., 2018). The total energetic value of mushrooms is between 250 and 350 calories/kg of fresh mushrooms (Panneerselvam et al., 2009; Sanchez, 2010). The dry matter of mushrooms comprises around 50% to 65% total carbohydrates, 19% to 35% proteins, and 2% to 6% fat (Rathore et al., 2017). While, the quantitative mycochemical screening resulted in the high amount of carbohydrate and minimal quantity of protein in the two species of *Microporus*. Comparatively, higher amount of carbohydrate in *M. affinis* substantiates its culinary property. In the present study, protein content was found to be minimum (0.21 mg/g for *M. affinis* and 0.15 mg/g for *M. xanthopus*) when compared with the total protein content of *Ganodema applanatum* (9.19 mg/g) and *G. lucidum* (16.29 mg/g) obtained in a study conducted by Mohammadifar et al. (2020). Edible mushroom comprises a maximum number of all essential amino acids (Hayes, 1976), with arginine, glutamic acid, and aspartic acid being the most abundant ones (Manzi et al., 1999). Thus, mushrooms can be considered as a great alternative to animal products (Dembitsky et al., 2010). Mycophagy or mushroom eating is inextricably linked to mushroom poisoning (Kumar & Kaviyarasan, 1976). Mushroom foraging involves a high risk of confusion, wherein the poisonous mushrooms are mistaken for their edible looks. Macroscopic characters cannot be the basis to differentiate poisonous mushrooms as it is the chemical nature of the mushroom which determines its edibility or toxicity.

Mushrooms are rich in essential nutrients with high contents of dietary fibre, significant contents of vitamins (B1, B2, B12, C, D and E), trace elements, a high quality of proteins including important content of essential amino acids. They may

however be limited in the cystine, methionine and sulphur-containing amino acids, carbohydrates and fats but with excellent important fatty acid content, low or no calories and cholesterol and antioxidants (Agrahar- Murugkar & Subbulakshmi, 2005; Heleno et al., 2010; Wani et al., 2010; Niazi & Ghafoor, 2021). Mushrooms also have bioactive nutraceuticals such as polysaccharides (Ruthes et al., 2016), phenolic compounds (Heleno et al., 2015a), glycoproteins (Kumar, 2015), ergosterols (Barreira et al., 2014), unsaturated fatty acids (Tel-Cayan et al., 2017) and tocopherols (Khatua et al., 2013). These mushrooms also contain four influential nutrients like ergothioneine, glutathione, vitamin D and selenium which serve to alleviate oxidative tension as well as possesses antioxidant property (CNN, 2018).

GC/MS analysis is used to identify and quantify volatile compounds in the fungal extract. GC/MS separates and identifies volatile compounds based on their boiling points and mass to charge ratio. By using GC/MS analysis, an array of chemicals was analysed in earlier studies conducted in a well explored bracket fungus called *Ganoderma* (Liu et al., 2023). GC/MS method can serve as an interesting tool for identification of active principles in extracts. It combines two analytical techniques to a single method of analysing mixtures of chemical compounds. Gas chromatography separates the components of the mixture and mass spectroscopy analyses each of the components separately (Bai et al., 2014). Gas chromatography coupled with mass spectrometry is a commonly used technique for the separation and identification of the components of complex volatile mixtures. GC is known for its high-resolution separation of structurally similar compounds. The use of electron ionization in mass spectrometry produces distinctive mass spectral fragmentation patterns enabling mass spectra of unknown compounds to be searched against libraries (Nguyen & Kimaru, 2014). So, in the present study, fungal extracts were subjected to GC/MS analysis which revealed a wide spectrum of chemicals. Regarding fats, mushrooms are rich in polyunsaturated, monounsaturated and saturated fatty acids such as palmitic, oleic and linoleic acids (Kavishree et al., 2008; Muszynska et al., 2018).

Mushrooms have a low fat, high protein content and a high content of several vitamins (B, C, D, K). In the present study, the presence of vitamin D (calciferol) is reported through HR-LC/MS in the extracts of *M. xanthopus* (**Table 5**). GC/MS analysis conducted in both the species of *Microporus* reported to possess ergosterol. According to Phillips et al. (2011), ergosterol is the provitamin of ergocalciferol (Vitamin D₂).

In the present study, fatty acids were found to be the leading compound, 72.66 % in *M. xanthopus* and 48.70% in *M. affinis*. Fatty acids like sativic acid, jalapinolic acid, decanoic acid, myristic acid, stearic acid, palmitic acid, palmitoleic acid, linoleic acid, oleic acid and linoceric acid are obtained through chromatographic methods in *M. affinis*. HR-LC/MS is another chromatographic method which is a valuable tool for studying bracket fungi, allowing researchers to explore their chemical composition, identify potentially bioactive compounds and gain insights into their metabolic processes. This powerful technique combines liquid chromatography for separating different compounds from a sample with mass spectrometry for accurately determining their masses. The ‘HR’ in HR-LC/MS stands for ‘high resolution’ which means the mass spectrometer can resolve very similar masses, providing more precise structural information. HR- LC/MS (**Table 5**) and GC/MS (**Table 7**) in *M. xanthopus* revealed the presence of fatty acids like decanoic acid, anandamide, caprylic acid, myristic acid, palmitic acid, palmitoleic acid, linoleic acid, margaric acid, stearic acid, oleic acid, behenic acid and lignoceric acid. The typical compounds of edible mushrooms can be divided into volatile and non-volatile components. Volatile components include alcohols, aldehydes, ketones and esters (Chao et al., 2008). While non-volatile components mainly consist of carbohydrate, polysaccharide, crude fibre, crude fat, mineral elements and amino acids (Quo et al., 2007).

In *M. xanthopus*, a total of 45 compounds were obtained through GC/MS analysis (**Table 7**). The compounds were grouped into 11 classes such as alkane, alkene, aldehyde, alcohol, ester, ether, fatty acid, fatty acid ester, ketone, organic compound and sterol. Among these, leading classes of compounds were fatty acids

(72.66 %), sterols (15.21 %) and fatty acid esters (10.05 %). Linoleic acid represents the compound with highest peak area (26.86 %). Other leading compounds are oleic acid (17.97 %), palmitic acid (15.81%), stellasterol (8.43 %), pentadecanoic acid (6.68 %), monoolein (6.45 %), ergosterol (5.6 %) etc.

In case of *M. affinis*, a total of 46 compounds were detected through GC/MS analysis (**Table 8**). As in *M. xanthopus*, here also compounds are grouped into 11 classes. Majority of classes are similar in both the species. Aldehydes, alkanes, alkenes, alcohols, esters, fatty acids, fatty acid esters, hexose sugar, ketones, sterols and terpenoids represent various classes of compounds. Fatty acids were found to be the leading class (48.70%). Other leading classes were fatty acid esters (28.38%), sterols (13.07%), alcohols (3.23%), ketones (2.80%) esters (1.59%) etc. Linoleic acid was found to be with highest peak area (26.35%) which is followed by palmitic acid (13.73%), 2-monopalmitin (11.73%), stellasterol (9.61%), linoleic acid methyl ester (8.53%), monoolein (5.93%), pentadecanoic acid (2.67%) and methyl palmitate (2.17%).

GC/MS analysis of *Microporus* revealed the presence of different fungal sterols like stigmasterol, stellasterol, ergosterol etc., which are already reported in various earlier studies and reported to possess potent antiproliferative, antitumor and anticancer activities (Chakraborty et al., 2023).

Farnesol, a sesquiterpene alcohol, which is a fungal quorum-sensing molecule (QSM) is produced by *Candida albicans*. It plays a significant role in fungal morphogenesis and biofilm formation. Farnesol also exhibits neuropharmacological properties and has been shown to have potential therapeutic effects (Jadhav & Karuppayil, 2020). Farnesol may influence these effects by interacting with the nervous system and potentially affecting neurotransmitter levels. The ability of farnesol to modulate fungal behaviour and its neuropharmacological effects make it a promising candidate for drug development. Fortunately, *M. affinis* is shown to possess the presence of farnesol, which was screened through GC/MS analysis in the present study.

Mostly the edible mushrooms contained polyunsaturated fatty acids and help to decrease the level of serum cholesterol. Tocopherols are the natural antioxidant compounds found in the lipidic fraction as they act as free radical scavenging peroxy components produced from several reactions. These antioxidants are protective against cardiovascular diseases, degenerative malfunctions and cancer. Linoleic acid, an essential fatty acid to humans, decreases triglyceride levels, arthritis, cardiovascular diseases and blood pressure (Hensley et al., 2004; Ferreira et al., 2009; Heleno et al., 2012; Reis et al., 2012). As discussed here, both species were shown to possess fatty acids as the leading class of compound. Linoleic acid represents the compound with highest peak area for both *M. xanthopus* and *M. affinis*.

Ergosterol is one of the major sterols produced by mushrooms and has shown important antioxidant properties (Guillamon et al., 2010). It also plays a major role in the prevention of cardiovascular diseases (Kalac, 2013). In the present study, GC/MS analysis revealed the presence of ergosterol in both the species of *M. affinis* and *M. xanthopus*. They are detected as tocopherols and considered as effective as well as novel natural antioxidants and have major biological activities for the protection against microbial and cardiovascular diseases and degenerative malfunctions (Heleno et al., 2012a, 2015b; Jaworska et al., 2015).

Polysaccharides are the best known and most potent mushroom-derived substances with antitumor and immunomodulating properties (Lorenzen & Anke, 1998; Mizuno et al., 1996; Borchers et al., 1999; Mizuno et al., 1999; Reshetnikov et al., 2001). In 1969, Ikekawa et al., published one of the first scientific reports on antitumor activities of essences obtained from fruiting bodies of mushrooms belonging to the family Polyporaceae and a few other families, manifested as host-mediated activity against grafted cancer – such as Sarcoma 180 – in animals (Ikekawa et al., 1982; Ikekawa et al., 1992). Soon thereafter the first three major drugs were developed from medicinal mushrooms. All three were polysaccharides, specifically β -glucans: krestin from cultured mycelial biomass of *Trametes versicolor* (Turkey tail mushroom), lentinan from fruiting bodies of *L. edodes*, and schizophyllan from the liquid cultured broth product of *Schizophyllum commune*. In the light of these, least

explored *Microporus* (polyporaceae) was screened for antitumor activities since it is rich in carbohydrate content. Mushrooms are rich sources of β -glucan, proteoglycan, lectin, phenolic compounds, flavonoids, polysaccharides, triterpenoids, dietary fibre, lentinan, schizophyllan, steroids, glycopeptides, terpenes, saponins, xanthenes, coumarins, alkaloids etc., and also have been used extensively in traditional medicine for curing various types of diseases such as antimicrobial, antiviral, anticancer, antitumor, anti-inflammatory, cardiovascular diseases, immunomodulating etc. (Wasser & Weis, 1999; Chihara, 1992; Wan et al., 2000; Bobek et al., 2001). Medicinal mushroom research has focused on discovery of compounds that can modulate positively or negatively the biologic response of immune cells. An earlier study on *M. affinis* has reported a new cadinane type sesquiterpenoid (Zhao et al., 2019).

Presence of pyranone in *M. affinis* revealed through GC/MS analysis is a hallmark to antiproliferative activities. This was discussed in detail in an earlier study of Chakraborty et al. (2023). Those compounds, which appear to stimulate the human immune response, are being sought for the treatment of cancer. The presence of some of these chemicals in *Microporus* acts as stepping stone for assessing its potential in anticancer activities.

There was a report on monoolein (Park et al., 2021), a lipid, which has demonstrated anti-cancer activity against the U937 leukemia cell line *in vitro*. Specifically, it has shown the ability to inhibit the proliferation of these cancer cells, likely through inducing apoptosis and cell cycle arrest. GC/MS analysis of *M. affinis* revealed the presence of this active compound in the present study. Study conducted by Park et al. 2021 suggests that monoolein can trigger programmed cell death in U937 cells, further contributing to its anti-cancer effect. The anti-cancer effects of monoolein may be linked to its ability to induce the production of ROS, which can damage cancer cells. The precise mechanisms by which monoolein exerts its effects on U937 cells are still being investigated, but evidence suggests that it is involved in ROS-dependent apoptosis and cell cycle arrest, along with potential mitochondrial dysfunction (Park et al., 2021).

GC/MS analysis of *M. xanthopus* in the present study revealed the presence of palmitoleic acid, a monosaturated fatty acid. It is reported to possess ability to prevent diabetes, cardiovascular diseases and have anti-thrombotic effect, which may reduce the risk of stroke (Hu et al., 2019).

Present study on *M. affinis* through HR-LC/MS analysis reported the presence of a bioactive compound called 16-Hydroxy-4-oxoretinoic acid. There is an earlier report on retinoic acid, a derivative of vitamin A, which shows promise in cancer treatment by inhibiting the growth and differentiation of various cancer cells. It can induce cell cycle arrest and apoptosis and also modulate signalling pathways involved in cancer progression (Chen et al., 2014).

In the present study, the presence of serotonin is reported through HR-LC/MS analysis in *M. xanthopus*. Serotonin is a neurotransmitter, that transmits signals between nerve cells and it also functions as a hormone. It plays a key role in regulating mood, sleep, appetite and other bodily functions. Most of the body's serotonin is found in the digestive tract and blood platelets, while the brain contains a smaller amount that is crucial for its various functions (Thébaud et al., 2018).

PHASE II: BIOACTIVITY SCREENING

a.) Free radical scavenging assays

The unstable molecules that damage cells are generally termed as free radicals. Free radical scavenging is crucial for maintaining cellular health and preventing disease. By nullifying harmful free radicals, antioxidants and other scavengers protect cells against oxidative stress which is a process linked to aging and various diseases. This protection is crucial for preventing cellular damage, maintaining proper physiological function and potentially reducing the risk of cancer and neurodegenerative diseases. The disturbance in equilibrium between free radical production and the body's ability to neutralize them is said to be an oxidative stress, which is implicated in many diseases. Free radical scavengers help to restore this balance and thereby reduce oxidative stress (Hatwalne, 2012).

Nitric oxide, DPPH, hydroxyl free radical and reducing power assays are the widely used free radical scavenging assays which are used to evaluate the antioxidant activity of a substance. These methods assess the ability of a compound to negate harmful free radicals (molecules with unpaired electrons that can damage cells). DPPH and hydroxyl radical assays assess the direct scavenging of free radicals, while nitric oxide and reducing power assays measure the ability to inhibit the formation or reaction of nitric oxide and to donate electrons respectively (Baliyan et al., 2022).

Formation of free radicals is a natural process associated with metabolism of cells. These free radicals can interact with macromolecules in cells like proteins, DNA and lipids, generating more reactive molecules as new radicals and lipid peroxides (Torel et al., 1986; Pryor et al., 1982; Gyamfi et al., 1999). These free radicals can damage the living system and are causative of many diseases such as cancer, coronary heart diseases and aging (Keys, 1995; Lipworth et al., 1997). Antioxidants are free radical scavengers and thus limit action of free radicals on cellular biomolecules (Halliwell, 1997; Hu, 2002). The results of the previous studies on antioxidant assays showed that *Ganoderma lucidum* (a well-studied member of Polyporaceae) occurring in south of India were found to possess *in vitro* antioxidant activities and scavenged free radicals (Lakshmi et al., 2008). In earlier studies conducted on *Ganoderma*, the antioxidant activity was measured using DPPH method to evaluate free radicals scavenging shown as IC₅₀. IC₅₀ indicates the extract concentration providing 50% inhibition of DPPH free radicals. The lower the IC₅₀, the higher the antioxidant power (Mohammadifar et al., 2020). In 2018, Gaylan et al. performed an antioxidant activity using DPPH scavenging assay in the ethanolic extract of *M. xanthopus* and revealed statistically comparable DPPH scavenging effects with the standard vitamin C. Previous reports on *M. xanthopus* showed it contains high amount of ascorbic acid and significant antioxidant activity (Tripathy et al., 2014). In an earlier study, *M. xanthopus* showed low level of DPPH scavenging activity (Liew et al., 2015). DPPH assay uses the stable free radical DPPH (with a characteristic purple colour). When an antioxidant is added, it donates a hydrogen atom to DPPH, resulting the purple colour to fade and the decline in absorbance at a specific wavelength is measured to quantify the scavenging activity.

According to Sowndhararajan & Kang (2013), hydroxyl radical assay measures the ability of a substance to scavenge hydroxyl radicals, which are highly reactive and causes significant cellular damage. The assay often involves producing hydroxyl radicals through the Fenton reaction (using Fe^{2+} and H_2O_2) and then assessing the antioxidant's ability to inhibit radical-mediated reactions like DNA damage or lipid peroxidation. In the present study, the extract of *M. affinis*, was found to be more potent in scavenging hydroxyl radicals when compared to the standard gallic acid used.

In nitric oxide scavenging assay, the ability of a compound to scavenge nitric oxide (NO) is evaluated. Nitric oxide can react with other molecules to form harmful species like peroxynitrite. Antioxidants can scavenge nitric oxide, which prevents the formation of these damaging by-products. While in reducing power assay, the ability of a substance to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) is measured. A higher reducing power indicates a greater ability to donate electrons, which is a characteristic of antioxidants (Chrocho et al., 2022). Present study evaluated the potential for nitric oxide scavenging (**Fig. 21**) and reducing power assay (**Fig. 22**), where *M. affinis* was found to be more effective than *M. xanthopus* in both assays. These, potential antioxidant activity of *M. affinis* might be due to various active chemicals. Present study is a direct evidence with the presence of strong antioxidant substance called maltol, screened through GC/MS analysis.

Oxidative damage is a major contributor to the aging process and is linked to diseases like Alzheimer's, Parkinson's and cancer. Free radical scavengers can help to mitigate these diseases.

b.) Cytotoxicity by *Allium cepa* assay

In an earlier study, there is a report on the activity of ergosterol obtained from *Fomitopsis pinicola* to induce ROS mediated apoptosis in colorectal cancer cell lines (Wang et al., 2014). Ergost-5-en-3-ol (Campesterol) and Stigmasta-5, 22-dien-3-ol (Stigmasterol) both are anti-cancerous phytosterols (Arora, 2017). Chemical profiling on *Microporus* revealed the presence of ergosterol which thus point out its potential in anticancer activities. Previous works have shown that, ergosterol exhibit some

degree of antitumor activities (Yazawa et al., 2000; Takaku et al., 2001) which will be a stepping stone for further studies in anticancer assays.

Clinical trials are costly in time and resources and consequently, efficiency of fungal extracts in preventing and treating cancer is still largely unproven. More and rigorous investigation is essential to explore this complex topic further. Nevertheless, there is some promising evidence, indicating that the consumption of some fungal extracts helps in protecting against some cancer types, especially breast and gastrointestinal cancers (Kim et al., 2002; Hara et al., 2003).

According to several previous studies (Klis & Hellingwerf, 2001; McIntosh et al., 2005; Schmid et al., 2001), β -glucans are the main polysaccharides that are found in mushrooms whereas 50% of the fungal cell wall mass is formed by β -glucans. For the industry, it is very essential due to their excretion into the cell growth medium and making their recovery possible. Its chemical characterization and purification are very simple. These β -glucans are highly responsible for the antioxidant, anticholesterolemic, anticancer, neuroprotective, immunomodulating activities of several edible mushrooms (Falch et al., 2000; Ishibashi et al., 2001; Kataoka et al., 2002; Khan et al., 2013). They are also essential in protection from cancer as well as infectious diseases and also recovered the patients from radiotherapy and chemotherapy (Manzi et al., 2001; Chen & Seviour, 2007). Consequently, reinforcing as well as activating the host immune system is the best strategy for inhibiting the cancer cells' growth (Daba & Ezeronye, 2003; Finimundy et al., 2013).

Furthermore, fungal metabolites play a significant role in the advancement of anticancer and immunomodulatory chemotherapy. These chemicals demonstrate several modes of action, such as triggering apoptosis in cancer cells, suppression of cell division and regulation of the immune system. Fungi synthesize wide range of secondary metabolites including amino acids, alkaloids, anthraquinones, and terpenes, that have notable anticancer effects (Behar et al., 2024; Evidente, 2024).

The induction of apoptosis in cancer cells by fungal metabolites is a vital mechanism underlying their anticancer action. The efficacy of these compounds in combating medication resistance has been especially observed in pancreatic cancer.

The metabolites exert their effects *via* diverse biosynthetic routes, selectively targeting molecular processes exclusive to cancer cells, thereby inducing cell cycle arrest and apoptosis (Giurini et al., 2024). Apoptosis is the process of programmed cell death and the most popular target of different anticancer therapies. Dysregulation of apoptosis signals in cancer promotes abnormal cell growth and tumorigenesis (Wong, 2011). The main objective of cancer treatments is the restoring of lost apoptotic function in cancer cells. The initiation of apoptosis in cells can be identified by morphological changes such as nuclear fragmentation, chromatin condensation, cell shrinkage and membrane blebbing (Elmore, 2007; Hassan et al., 2014). Similar morphological changes are observed for both species of *Microporus* in the present study.

In a previous study conducted on *Ganoderma*, it was reported that greater antioxidant activity will be a better option for the production of anticancer drugs (Mohammadifar et al., 2020). In the present study, *Microporus* has shown better antioxidant activities and thus it was screened for *in vitro* anticancer activities.

In earlier studies, polysaccharide from *Inonotus obliquus* has been shown to possess *in vivo* tumor growth inhibition via immunostimulation in colorectal and gastric cancers (Kim et al., 2006; Fan et al., 2012; Won et al., 2011; Kim et al., 2005). This correlates with the anticancer efficacy of *Microporus* since it possesses polysaccharides. Many of the purified mushroom polysaccharides have been in clinical use in Japan, China and Korea for many years, with no reports of any significant short term or long-term adverse effects. These compounds are not miracle drugs but can improve the quality of life of cancer patients and may offer increased survival rates for some types of cancer (Kidd, 2000).

The *Allium cepa* bioassay is a widely used method to assess the cytotoxic and genotoxic effects of various substances. It utilizes the rapid root growth of *Allium cepa* (common onion) as a sensitive indicator of cellular damage. This assay allows for the evaluation of several toxicity endpoints, such as chromosomal aberrations and mitotic index alterations (Wijeyaratne & Wadasinghe, 2019).

Root tip cells are examined under a microscope to determine the proportion of cells undergoing division (mitosis). The mitotic index and chromosomal aberrations are two key indicators used in cytogenetics to assess the effects of various factors on cell division. The mitotic index reflects the rate of cell division and a decrease in the mitotic index can indicate cytotoxic effects. The mitotic index is the proportion of cells undergoing mitosis within a population of cells. It is generally expressed as percentage and is the ratio of number of cells in mitosis to that of total number of cells in a field studied. Higher mitotic index might suggest increased cell division, potentially linked to cell damage or the presence of certain stimuli. Conversely, a lower mitotic index can indicate reduced cell proliferation due to toxicity or other factors inhibiting cell division (Nefic et al., 2013). Current study also revealed a reduction in mitotic index from lower concentration to higher concentration of the extracts of both *M. affinis* and *M. xanthopus*.

Structural or numerical changes in chromosomes are generally referred as chromosomal aberrations. These changes can involve breaks, deletions, rearrangements or changes in chromosome number. Chromosomal aberrations can be structural (morphological) or numerical (extra or missing of chromosomes). Normally, aberrations can arise from DNA damage caused by exposure to certain chemicals, radiation or other genotoxic agents (Nefic et al., 2013). In the present study, fungal extracts of both *M. affinis* and *M. xanthopus* possess increase in the percentage of chromosomal aberrations from lower concentrations to higher concentrations of the extract (**Fig. 23**).

There are some reports on cytotoxic studies on fungal extracts which resulted in various chromosomal aberrations (Aneesh & Thoppil, 2019; Aneesh & Thoppil, 2020). In the present study, cytotoxic screening on two species of *Microporus* revealed wide spectra of chromosomal aberrations, which are broadly classified into clastogenic and non-clastogenic aberrations (**Plates 4-12**). Chromosome stickiness, pulverization, nuclear lesions, nuclear erosion, nuclear extrusion, nuclear disintegration, nuclear budding, nuclear peak, giant cell formation, coagulated chromosomes, chromosome gap, chromosome bridges, chromosome fragmentation,

ring chromosomes, contorted chromosomes, hyperchromasia, cytomixis etc., are the important clastogenic aberrations observed. Non-clastogenic aberrations include hypoploid, hyperploid and polyploid conditions, vagrants, laggards, shift in microtubule organizing center (MTOC), ball metaphase, misorientation, binucleate cell, stellate metaphase, C-metaphase, diagonal arrangement of chromosomes, disturbed chromosomes, stathmo-anaphase, scattering of chromosomes, micronuclei, early movement of chromosomes, somatic pairing, tropokinesis, macro and micro cell formation, chained chromosomes, etc. The highlights of the present study include the induction of certain rare abnormalities (**Plate 12**) like somatic pairing in hyperploid cell, hyperchromasia at interphase, cytomixis at interphase, contorted strap cell showing disintegrated nucleus, cytostasis in a hypoploid cell, cytostasis in a polyploid cell, etc.

c.) Antiproliferative efficacy of *Microporus* using DLD1 cell line

Cancer fungotherapy is a promising scientific field, which deals with antitumor substances derived from mushrooms. It has been an integral part of the world traditional medicine since the antiquity (Xu et al., 2012a). Although the chemical composition of the mushroom is very complex which comprise nucleosides, steroids, fatty acids, alkaloids, proteins, peptides, amino acids, vitamins and inorganic elements (Niu et al., 2002), most studies have linked the antitumor effect of *Ganoderma lucidum* extracts to the presence of different polysaccharides and triterpenoids. During the past two decades more than 200 polysaccharides and 130 triterpene structures have been isolated from its fruiting bodies, spores and mycelia (Zhou et al., 2007). It is believed that these two classes of mushroom-derived compounds have completely different modes of anti-tumor action. Whereas triterpenoids directly suppress growth and invasive behavior of cancer cells, polysaccharides stimulate anticancer immune responses (Siva, 2006). The complex anticancer potential of mushrooms is mainly based on the different groups of characteristic compounds that interact with malignant tissues and cells through a wide range of biological processes. The features of fungal therapy for tumor treatment are usually studied by identifying the metabolism of cancer susceptible cells to fungi-

based bioactivities. There are several studies on the role of bracket fungi with antiproliferative effects. In a study (Blagodatski et al., 2018) a bracket fungus called *Trametes versicolor* (member of Polyporaceae) was found to possess significant potential in preventing the proliferation of breast cancer cell line (4T1) and prostate cancer cell line (DU145).

An abnormal cell growth that occurs in the colon or rectum is simply recognized as colorectal cancer (CRC). A non-cancerous cell growth known as a polyp, can develop in the mucosal layer of the colon or rectum and may develop into CRC. Less than 10% of polyps have a high potential to progress into invasive cancer over 10–20 years. CRC occurs predominantly in adults aged 50 and above (ACS, 2020). In 2020, CRC was recognized as the third most common cancer type worldwide and the second most common cause of cancer death. DLD1 cell line is a valuable and frequently used model for studying CRC. Thus, human colorectal cancer cell line such as DLD1 is used in the present study. Geographically, the occurrence of CRC is highest in Asian regions. The International Agency for Research on Cancer (IARC) estimated that the global burden of CRC will increase by 56% and CRC related mortality will increase by 69% between 2020 - 2040 (Honegger et al., 2013). Therefore, a strong research on the discovery of novel anticancer therapeutics is urgently required. Hence the present study was focussed on CRC cell lines using the fungal extracts of *M. xanthopus* and *M. affinis*.

Due to the presence of various active chemical constituents obtained through chromatographic methods, the emergence of various chromosome abnormalities obtained through cytotoxic assay in *Allium cepa* and also due to the potential antioxidant activities, fungal extract was subjected to antiproliferative assay. In the present study, antiproliferative effect of *Microporus* was elucidated by using MTT assay (**Fig. 26**) and Acridine Orange and Ethidium Bromide (AO/EtBr) double staining methods (**Plates 17 & 18**). Both MTT assay and AO/EtBr staining are used to study apoptosis, but they offer different insights. MTT assay measures cell viability by assessing mitochondrial dehydrogenase activity, which indirectly indicate cell survival and proliferation. AO/EtBr staining, on the other hand, directly visualizes

apoptotic and necrotic cells actually based on their DNA structure and membrane integrity. But the emission of fluorescence make it observe and distinguish clearly. MTT provides a quantitative aspect of cell viability, while AO/EtBr allows for qualitative assessment of cell death morphology. In the present study, cells with different morphology such as blebbing, shrinkage, rounding etc. are observed. MTT assay is being widely used to study apoptosis by observing the decrease in cell viability in response to apoptotic stimuli. It has been used to investigate the pro-apoptotic effects of chemotherapeutic drugs on cancer cells (Ariffin et al., 2015) In this method, the resulting formazan product is quantified and the percentage of cell viability is calculated relative to untreated controls. In the MTT assay, the LC₅₀ value (Lethal Concentration 50%) represents the concentration of a substance that causes 50% cell death, while antiproliferative effects refer to the inhibition of cell growth and division. A lower LC₅₀ value in the MTT assay typically indicates a stronger antiproliferative effect, meaning a smaller amount of the substance is needed to inhibit cell growth by 50%. A substance with low LC₅₀ in the MTT assay is more potent in causing cell death, which in turn indicates a stronger antiproliferative effect. Conversely, high LC₅₀ suggests that a larger concentration is required to induce the same level of cell death, which implies a weaker antiproliferative effect (Pinheiro et al., 2023). In the present study, *M. affinis* showed comparatively less LC₅₀ value ($171.76 \pm 0.43 \mu\text{g/mL}$) to that of *M. xanthopus* (with LC₅₀ value of $193.24 \pm 0.32 \mu\text{g/mL}$). From these data, it was obviously clear that *Microporus* can induce antiproliferation but not very effective in inhibiting the proliferation to a great extent. Reduction in cell viability noticed by MTT assay can indicate apoptosis, but it can also be due to other forms of cell death such as necrosis. Therefore, it is essential to use additional methods for the confirmation of degree of apoptosis.

This method seems to be more significant since it is easy to carry out, as it allows simultaneous analysis of multiple samples and also the quantitative data which gives accurate assessment of cytotoxicity and apoptosis. Still, it has some limitations like specificity to apoptotic cells, but not necrotic cells and there is chance for the interference of certain compounds, such as antioxidants and reducing agents, which

can interfere with MTT assay by reducing MTT to formazan, resulting in false-positive results.

In AO/EtBr method, AO enters all the cells and stains nucleic acids green. While, EtBr enters only cells with compromised membrane integrity such as late apoptotic and necrotic cells and it stains orange (**Plates 17 & 18**). The nuclei of cells undergone apoptosis has conjugation with green, although the nuclei come out and disintegrated. Late apoptotic and necrotic cells appear orange and red, respectively (Karami et al., 2012). The important advantage of AO/ EtBr method over MTT assay is that it gives a clear visual information about cell morphology and it allows distinguishing apoptotic and necrotic cells.

Many chemotherapeutics exert cytotoxicity and trigger cancer cell death by inducing apoptosis in cells. Apoptosis or programmed cell death is a cell suicide process that activates when their continuous survival is blocked (Gerl & Vaux, 2005). Morphological and biochemical changes such as chromatin condensation, nuclear fragmentation, cell shrinkage, membrane blebbing, DNA & protein breakdown and caspase activation can be observed in apoptotic cells. Cancer cells evade natural cell death as a result of genetic mutations acquired during transformation from normal cells to malignant cells. Disruption of the balance between pro-apoptotic and anti-apoptotic proteins, reduction in caspase function and impairment of death receptor signalling, all cause apoptosis resistance in cancer cells (Wong, 2011). Therefore, restoration of these functions by therapeutics can successfully prevent cancer progression. Furthermore, alterations that occur in cell cycle regulators at check points cause abnormal cell proliferation during various cancers (Kasibhatla & Tseng, 2003). Thus, the induction of G2/M phase arrest is another target of many anti-cancer agents. Controlling cell progression through the cell cycle by regulating related proteins or disrupting tubulin organization eventually leads cells to G2/M phase arrest and subsequent cell death (Choi & Yoo, 2012; Arora et al., 2009). Apoptosis is the process of programmed cell death and the most popular target of many anticancer therapies. Dysregulation of apoptosis signals in cancers promotes abnormal cell growth and tumorigenesis (Wong, 2011). Restoring the lost apoptotic function in cancer cells is

the main objective of much of the research that contribute into cancer treatments. The initiation of apoptosis in cells can be identified by morphological changes such as nuclear fragmentation, chromatin condensation, cell shrinkage and membrane blebbing (Elmore, 2007; Hassan et al., 2014).

Autophagy mainly involves the formation of an autophagosome by engulfing damaged organelles, fusion with lysosome and degradation of cellular debris by lysosomal hydrolase (Yang et al., 2011; Sun et al., 2013). Autophagy plays a paradoxical role in cancer progression. In the early stage of many cancers, autophagy acts as a tumor suppressor by protecting cell homeostasis. Conversely, autophagy promotes tumor growth in more advanced stages of cancer by increasing stress tolerance (Grácio et al., 2017). Many anticancer therapeutics are known to activate autophagy concurrently with apoptosis. This activation of autophagy can either promote or suppress cancer cell survival. Therefore, the role of autophagy is crucial in anticancer drug development (Sui et al., 2013).

d.) Preliminary neuroprotective study

There are several mental health disorders, including addiction disorders, mood disorders, impulse control issues, eating disorders, obsessive-compulsive disorders (OCD), anxiety disorders, psychotic disorders, post-traumatic stress disorders (PTSD) and personality disorders (Choudhari et al., 2024). Diagnostic and Statistical Manual of Mental Disorders 5 (DSM-5) 2013 states that “A mental disorder is a syndrome defined by a clinically significant disturbance in an individual’s cognition, emotion regulation or behaviour that reflects malfunction in the biological, psychological or developmental processes that underlies mental functioning in an individual.” According to WHO report, the prevalence of mental illness is increasing day by day across the world, with 20% of children and adolescents diagnosed with the disorder and suicide being the primary cause of death (Meade, et al., 2022). The foundation for the treatment of mental health disorders is combinatorial drug therapy that includes mood stabilizers, anti-psychoactive drugs and psychotherapeutic treatments. In many cases, patients suffering from mental illness frequently do not respond and are termed as “treatment resistant” (Howes et al., 2022). The drugs prescribed for the treatment

of mental illness exhibit side effects to patients, such as increased suicidal thoughts, declining physical health and difficulties in the workplace (Wolff et al., 2021; Stassen et al., 2022). To provide a good therapeutic drug in the market, scientists have brought up novel alternative options to treat mental illness. Fungal biologists have gathered attention for their potential to treat mental health disorders, using psilocybin from magic mushrooms, ergine from rye ergot fungus *Claviceps purpurea* etc. Bioactive compounds isolated from medicinal mushrooms are considered a new source of psychoactive drugs. Additionally, there is a growing interest in supporting medicinal mushroom companies for the development of novel therapeutic drugs. For this, the focus of attention will be based on bioactive compounds having low molecular weight, which can easily cross the blood-brain barrier (BBB) and should have similar structural features to available neurotransmitters (Meade et al., 2022).

Aging is a complex natural phenomenon that is frequently accompanied by the occurrence of several diseases, such as cognitive impairment, Alzheimer's and Parkinson's diseases and others. Memory decline is characteristic of aging and age-related neurodegenerative disorders, which result in a progressive loss of cognitive function, specifically in spatial memory (Zhang et al., 2007). Abundant evidence has pointed to an important role of oxidative stress during the pathogenesis of brain aging, age associated or neurodegenerative diseases (Reiter, 1995; Bowling & Beal, 1995; Perry et al., 2000). Indeed, with increasing age, accumulation of oxidant damaged cellular macromolecules, such as DNA, proteins and lipids of cell membranes takes place (Haripriya et al., 2005; Sohal, 2002). Brain is the most susceptible organ to oxidative damage with high oxygen demand, high level of unsaturated lipids, and relatively deficient in anti-oxidative defence mechanism. Thus, antioxidant assays may be an important area for managing neurodegenerative diseases.

The medicinal importance of *Hericium erinaceus* is known for its anti-cancer, anti-inflammatory, antimicrobial and anti-oxidative activities, but apart from this, neurotropic compounds present in the mushroom are generally used for the treatment of neuropsychiatric disorders. Erinacines and hericenones are the biologically occurring neurotrophic compounds that are found in the fruiting body and mycelium

of *H. erinaceus*. These drugs are used to treat Parkinson's and Alzheimer's disease because they influence nerve growth factor (NGF) and prevent amyloid beta (A β) aggregation. Presence of caffeic acid reported in an earlier study has been demonstrated to exert neuroprotective effect against amyloid-beta (A β) induced neurotoxicity *in vitro* (Vauzour et al., 2010). Know et al. (2010) have also suggested that caffeic acid exerts a neuroprotective effect against the oxidative injury in the brain caused by hydrogen peroxide and it also ameliorates cerebral ischemia (Zhou et al., 2007). Certain type of chemicals alone affects the nervous system of animals to cause the neurotoxicity and leads to promote the degeneration of neuroglial cells (Troshin, 2009). Earlier studies (Lee et al., 2019) reported that, edible-medicinal mushrooms can improve memory and cognitive functions and slow dementia and neurodegeneration in age-related neurodegenerative disorders, including Alzheimer's and Parkinson's. Mushrooms like *Hericium erinaceus*, *Ganoderma lucidum*, *Lignosus rhinocerotis*, *Pleurotus giganteus*, *Sarcodon scabrosus*, *Antrodia camphorata*, *Paxillus panuoides*, *Mycoleptodonoides aitchisonii* and other species contain bioactive secondary metabolites that exhibit neuroprotective effects through mechanisms like anti-acetylcholinesterase activity, neurite outgrowth stimulation, nerve growth factor synthesis, enhancement of mitochondrial functions, reduction of endoplasmic reticulum stress, as well as antioxidant and anti-inflammatory effects.

Human neurodegenerative diseases such as Alzheimer's, Huntington's and Parkinson's diseases are disrupting neuronal populations in adults worldwide. The discovery and development of neuroactive compounds from medicinal mushrooms with the potential to improve nerve functioning has been extensively studied. Mushrooms such as *Ganoderma spp.*, *Hericium erinaceus*, *Lignosus rhinocerotis* and *Pleurotus giganteus* have a long history of use in enhancing the peripheral nervous system. It has been demonstrated that neurodegenerative diseases mostly occur because of the disappearance of nerve growth factors. Therefore, scientists have been attempting for over 20 years to discover fungi-derived neuroactive components which are able to cross the blood–brain barrier and induce the production of nerve growth factors. Most of the potential neuroactive compounds which may aid in the prevention or therapy of neurodegenerative diseases have been discovered in *Hericium erinaceus*.

This medicinal mushroom is known to produce two unique terpenoid classes, namely hericenones and erinacines (Thongbai et al., 2015). In the present study, both the species were shown to possess terpenoids which supports neuroprotective activities of *Microporus*.

For the present study on neuroprotective effect, human neuroblastoma cell-line (IMR-32) was used and which has been widely used in various *in vitro* studies on Alzheimer's disease (Wang & Chang, 2020; Clementi et al., 2015). Neuroblastoma cells serve as an excellent *in vitro* model to test their effect of β -amyloid aggregation and release (Li et al., 2010b; Chaves et al., 2020). In the present study, human neuroblastoma cells were cultured and treated with amyloid- β to simulate the AD model.

In neuroprotective experiments, cell viability is often assessed using the MTT assay, which indicates the number of healthy, metabolically active cells surviving exposure to a neurotoxin or stressor (ie, amyloid β). Increased cell viability in the presence of a test compound suggests a neuroprotective effect, while decreased viability indicates toxicity (Boonyarat et al., 2023). In the present study, both the species of *Microporus* showed increase in the percentage of cell viability (**Fig. 28**). But among these, *M. xanthopus* developed a dose dependent increase in cell viability. In general, a test compound with increase in cell viability compared to a control group (or cells treated with a neurotoxin alone), suggests the compound has neuroprotective potential.

In neuroprotective experiments, AO/EtBr staining (**Plate 21**) helps to differentiate between live, apoptotic and necrotic cells. Acridine orange (AO) easily penetrates the live cells, intercalating with DNA and RNA, causing the nucleus and cytoplasm to emit fluorescent green colour. In early apoptosis, cells are characterized by green fluorescence, characterised by fragmented or condensed nuclei due to DNA degradation. As apoptosis progresses, the nuclear membrane becomes compromised and allowing ethidium bromide to enter. This results in orange/red fluorescence with chromatin condensation and fragmentation. Necrotic

cells stain orange with intact nuclear morphology (Robble et al., 2005). These observations clearly assess the neuroprotective effects of a substance.

PHASE III: DETAILED STUDY ON NEUROPROTECTION IN *M. XANTHOPUS*

In the present study, the extracts of *M. xanthopus* was found to be more effective in neuroprotective aspects. Thus, further studies are carried out only in *M. xanthopus*. The effects of treatments were assessed by using reactive oxygen species (ROS) levels, indirect ELISA, apoptosis flow cytometry, autophagy, qRT PCR

i. *In vitro* ROS measurement using DCFDA

Brain damage, depression and blurred vision are the symptoms of neurotoxicity (Asada et al., 2010). Neurotoxicity promoted by neurotoxins alter the normal functions of the nervous system due to the brain cell damage (Belyaeva et al., 2012; Pohl et al., 2011). Fungal secondary metabolites have shown promising neuroprotective effects, offering potential therapeutic avenues for neurodegenerative diseases. These metabolites, derived from various fungi exhibit diverse mechanisms of action including antioxidant activity, modulation of signalling pathways and inhibition of apoptosis. Several fungal secondary metabolites have neuroprotective effects. To date, several fungal metabolites have been used as neuroprotective drugs. These metabolites exhibit neuroprotection in two ways, one by oxidative stress reduction and another one by reactive oxygen species (ROS) scavenging. In oxidative stress reduction, the triterpenoid 2 α -hydroxy-inotodiol (2 α -HI) from the mushroom *Inonotus obliquus* has been shown to mitigate oxidative stress and apoptosis in SH-SY5Y cells. This suggests its potential as a dietary supplement for neurodegenerative diseases (Kou et al., 2022). Another method is ROS scavenging, and to date, several metabolites from fungi, *Penicillium* sp., have demonstrated neuroprotective effects in Parkinson's disease models by scavenging ROS, thereby protecting neuronal cells from oxidative damage (Yurchenko et al., 2018).

Reactive oxygen species (ROS) is a highly reactive and short-lived small molecule in the form of free radicals (Sies & Jones, 2020). ROS support many physiological functions in cells under balanced conditions. However, the over production of ROS can have a deleterious effect on cells and trigger oxidative stress. The generation of ROS can be triggered by both endogenous and exogenous sources such as mitochondrial transport chain leakage, high metabolic rate, environmental pollutants, radiation and the effect of drugs (Cosentino et al., 2019). ROS is responsible for activating many cellular signalling pathways that lead to cell survival or cell death including autophagy, apoptosis and necrosis.

Dysfunction of mitochondria is well known to generate reactive oxygen species (ROS). Increased ROS generation with compromised mitochondrial function ultimately affects neurons and accelerates neurodegenerative process (Reddy & Beal, 2005; Zeevalk, 2005). Reactive oxygen species (ROS) is a term that encompasses all highly reactive, oxygen containing molecules, including free radicals such as hydroxyl radical, the superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical and various lipid peroxides. All are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes and other small molecules, causing the cellular damage. ROS are generated by a number of pathways (Krishnamurthy & Wadhwani, 2012). Continuous synthesis of ROS promotes major alterations in sub cellular structural molecules such as proteins and DNA, lipid peroxidation (LPO) of polyunsaturated fatty acids and changes of cellular antioxidant system. In general, under the normal physiological condition of animal's low level of lipid peroxides occurred in all the tissues (Bashandy et al., 2011; Oda & El-Ashmawy, 2012). Different type of ROS species ($\bullet\text{OH}$, RO, ROO and NO) play a pivot role in the promotion of chemical-induced cellular damage in tissues and induce oxidative stress to cause neurodegenerative disorders (Nabavi et al., 2013). The brain cell damages are mediated by oxygen derived free radicals by altering the membranes through peroxidation of polyunsaturated fatty acids (Sankarsamipillai et al., 2010; Malini et al., 2011). The formation of oxidative stress is a serious imbalance between the synthesis of free radicals and anti-oxidant properties (Halliwell, 2011). As the cells progress into neurodegeneration, ROS increases. In the present study, increased ROS

(strong green fluorescence observed) was noticed for amyloid treated cells (**Plate 22**). The intensity of green fluorescence is directly proportional to the concentration of ROS. When the cells are treated with fungal extract, ROS get declined. This clearly support the recovery of neurodegenerated cells. During normal condition, a cell can maintain an equilibrium between ROS and antioxidant defence mechanism in an optimum level, but exposure to certain chemicals can promote ROS in enormous level and simultaneously inhibit the antioxidant defence mechanisms (Nordberg & Arner, 2001; Padma et al., 2011) which naturally lead to increased lipid peroxidation, protein degradation and finally cell death (Sorg et al., 1998).

ii. Indirect ELISA

An indirect ELISA (Enzyme-Linked Immunosorbent Assay) can be used for neuroprotective studies which helps to detect and quantify specific antibodies in a sample. This method is mainly useful to measure the levels of antibodies against disease-associated antigens in a sample, which provides an insight into the effectiveness of treatments and which is aimed at mitigating neurodegeneration.

An indirect ELISA uses two antibodies - a primary antibody that specifically recognizes and binds to the target antigen, followed by a labelled secondary antibody that detects the secondary antibody and forms primary-secondary antibody combination (Sakamoto et al., 2018). In this study, ChAt, Tau, Glutamate receptor-2 and Alpha synuclein was taken as primary antibodies. HRP conjugates was used as secondary antibody.

In indirect ELISA for neuroprotective studies, the intensity of the colour changes in the well indicates the amount of protein (antigen) present in the sample. It can then be used to assess neuroprotective effects. A stronger colour change (higher absorbance) typically suggests a higher concentration of the target protein, potentially indicating a positive neuroprotective effect. Conversely, a weaker colour change might suggest a lower concentration and potentially a lack of neuroprotection. The absorbance of each well is measured using a spectrophotometer. Higher the absorbance, stronger will be the colour change, which in turn suggests a greater amount of the target protein in the sample. In neuroprotective assays, a higher

concentration of the target protein, as indicated by a stronger colour change, might suggest that the treatment has a positive impact on the expression or release of that protein. This could be interpreted as a neuroprotective effect, as the protein might be involved in protecting neurons from damage (Sakamoto et al., 2018).

If the wells with the drug-treated cells show a significantly stronger colour change (higher absorbance) than the neurotoxin-only group, it suggests that the drug might be increasing BDNF (brain-derived neurotrophic factor, which is a protein essential for the survival and differentiation of neurons) levels, potentially contributing to neuroprotection (Dong et al., 2023). From this, it is obviously clear that in case of all the antibodies used, cells treated with amyloid and the extract shows remarkably stronger colour (protein activity) than that of cells treated with amyloid only (**Fig. 30 – 33**). Thus, the extract of *M. xanthopus* is effective in neuroprotection.

iii. Autophagy

Autophagy is a cellular process that removes damaged components and can play a neuroprotective role in the brain by clearing misfolded proteins and non-functional organelles. However, the role of autophagy in neuroprotection is complex and excessive autophagy can be detrimental. It is also implicated in neurodegenerative diseases where it can be both a protective and a destructive force. Autophagy removes aggregated proteins, such as those found in Alzheimer's disease and Parkinson's disease, which can be toxic to neurons. In case of Alzheimer's disease, autophagy is impaired in the later stages and contributing to the accumulation of amyloid plaques and neurofibrillary tangles. While during Parkinson's disease, autophagy dysfunction is linked to the accumulation of a protein aggregates called alpha-synuclein. Anyhow, maintaining a basal level of autophagy is crucial for neuronal health and disruptions to this process, which can lead to neurodegeneration. One striking function of autophagy is to promote cell survival either by eradicating the cells of damaged organelles or toxic pathogens or by regenerating metabolites for energy and growth during nutrient-deprivation (Kim et al., 2018). However, it is important to consider that excess and prolonged autophagy may also promote cell death by excessive self-digestion and degradation of essential cellular components (Wang et al., 2012).

Autophagy is an intracellular process responsible for the degradation and recycling of cytoplasmic components. It removes selectively the harmful cellular material and enables the cell to survive starvation by mobilizing nutrients via the bulk degradation of cytoplasmic components. While research over the last decades has led to the discovery of the key factors involved in autophagy, the pathway is not yet clearly understood (Toggler, 2017).

In autophagy research, the population profile refers to the distribution and characteristics of cells within a population, while the autophagy profile describes the state and activity of the autophagy pathway in those cells. Understanding about both population profile and autophagy profile is crucial for assessing autophagy's role in neuroprotection, as it helps to determine whether autophagy is beneficial or detrimental (Ray, 2020). A healthy population with balanced autophagy (both formation and degradation of autophagosomes like LC3-II) is often associated with neuroprotection. This helps to clear damaged proteins and organelles, preventing cellular dysfunction and cell death. Dysfunctional autophagy means reduced autophagosome formation or inefficient degradation, which can lead to the accumulation of toxic substances and finally contributing to neurodegeneration (Cherra & Chu, 2008). In Alzheimer's disease, autophagy is initially upregulated which helps to clear aggregated proteins, but it eventually becomes impaired and leading to the accumulation of harmful substances. It is possible to pinpoint the specific stages where autophagy dysfunction occurs and potentially develop therapies to restore its function by assessing the population and autophagy profiles.

Autophagy induction ratio can be measured by LC3-II turnover. This is a widely used and reliable method. It involves quantifying the conversion of LC3-I (Green fluorescent protein) to LC3-II (Red fluorescent protein). Low GFP/RFP ratio suggests increased autophagy as the GFP is degraded during autophagic flux. While RFP LC3 remains in the cytosol. High ratio indicates that autophagy is blocked or not being effectively cleared. Generally, the autophagy induction ratio is an important key for quantifying autophagy activity. Even though, there are different methods exist, measuring autophagic flux through techniques like LC3-II turnover represents the

most reliable approach for accurately assessing the dynamic nature of this essential cellular process.

In amyloid-treated cells, an increase in the autophagy induction ratio, often measured by the LC3-II/LC3-I ratio or autophagosome/autolysosome counts, suggests enhanced autophagy. This can be a protective mechanism against amyloid-induced toxicity by promoting the clearance of misfolded proteins and damaged organelles (Boland et al., 2008). In the context of amyloid-treated cells, increased autophagy can be a response to the cellular stress caused by amyloid accumulation. A common way to measure autophagy induction is by assessing the ratio of LC3-II to LC3-I, two proteins involved in autophagosome formation. An increased ratio indicates enhanced autophagosome formation and thus autophagy.

In the present study, the autophagy induction ratio for the cells treated with amyloid β is found to be 1.0 (**Fig. 39**). Guo et al. (2018) suggests that the amyloid treatment itself doesn't significantly alter autophagy levels or that the treatment may have both positive and negative effects on autophagy which balance out to a ratio of 1. From the present study, the autophagy induction ratio for the cells treated with the sample is observed as 1.4 (**Fig. 42**). This indicates that the extract is actively inducing autophagy. This increase in autophagy could be a beneficial effect, potentially leading to reduced amyloid accumulation, improved neuronal health and thus, neuroprotection (Wu et al., 2019). An autophagy induction ratio of 1 in amyloid-treated cells suggests that the amyloid treatment did not significantly alter autophagy levels, while a ratio of 1.4 of the sample indicates a 40% increase in autophagy compared to a control. In the context of neuroprotection, an increase in autophagy, particularly from the extract, could be a positive finding, potentially suggesting a protective mechanism against amyloid-induced damage (Yang et al., 2023).

iv. Annexin-V FITC apoptosis determination by flow cytometry

Apoptosis (programmed cell death), plays a complex and sometimes paradoxical role in neuroprotection. Apoptosis removes cells with damaged DNA, misfolded proteins (like amyloid plaques) or those that have become dysfunctional,

preventing the spread of damage and maintaining overall tissue health. Apoptosis is essential for usual brain development and function, it can also be a major contributor to neuronal death in neurodegenerative diseases. Modulating apoptosis pathways, either by inhibiting excessive cell death or promoting cell survival, is a key area of research in developing neuroprotective therapies. In neurodegenerative diseases like Alzheimer's, Parkinson's, and Huntington's, excessive apoptosis contributes to the loss of neurons and the progression of the disease. On the other hand, apoptosis can also serve as a protective mechanism by eliminating damaged or unwanted cells, thereby preventing further tissue damage and promoting tissue repair (Gachabayov et al., 2017).

Neuroprotection is crucial for preventing or slowing the progression of neurological disorders, such as Alzheimer's disease, Parkinson's disease and stroke. In this context, cells undergo specific changes, including the translocation of phosphatidylserine (PS) from the inner to the outer leaflet of the plasma membrane during apoptosis. Annexin V, a protein that binds to PS in a calcium-dependent manner is conjugated to FITC (fluorescein isothiocyanate), a fluorescent dye, which helps to detect this translocation. Phosphatidylserine translocation (from inner leaflet to outer leaflet of plasma membrane), annexin V FITC binding (with PS), detection of fluorescence (using flow cytometry) and detection of apoptotic or necrotic cells are the important areas of apoptosis in this scenario. In neuroprotection studies, Annexin V-FITC assays are used to assess the extent of neuronal apoptosis induced by neurotoxin called amyloid β and to evaluate the protective effects of potential therapeutic sample. By quantifying the number of apoptotic neurons, determination of the efficacy of neuroprotective strategies in reducing cell death is possible.

Propidium Iodide (PI) is often used in conjugation with Annexin V-FITC. PI can only enter cells with damaged membranes, which is characteristic of late apoptosis or necrosis (Kalda et al., 2000). Therefore, cells that are Annexin V-positive and PI-negative are typically in early apoptosis, while for late apoptosis or necrosis, cells will be positive for both FITC and PI.

In the present study, percentage gated for late apoptotic cells in amyloid-treated cells and sample treated cells are 14.38% and 4.86 % respectively (**Table 10**). From this data it is clear that, the difference in the reduction in late apoptotic cells due to the sample treatment is 9.52 % (14.38 % - 4.86 %). The sample treatment reduced late apoptotic cells by 9.52%, indicating a neuroprotective effect.

v. Gene expression studies using qRT PCR

Gene expression techniques are the methods used to study the process of gene expression, which involves reading genes to produce functional gene products, primarily proteins. These techniques can be broadly categorized into those that measure mRNA levels (transcription) and those that measure protein levels (translation). Gene expression is the process by which information from a gene is used in the synthesis of a functional gene product that enables it to produce end products like proteins or non-coding RNA. There are different techniques for quantifying the RNA transcripts (mRNA) or the resulting protein products. Northern blotting, qRT PCR, microarrays, Western blotting, etc. are the different methods for gene expression studies (Singh et al., 2018). Among these, qRT PCR (quantitative reverse transcription PCR) was used to assess the gene expression in the present study. This method first converts mRNA into cDNA (complementary DNA) through reverse transcription. Then, quantitative PCR is used to amplify and quantify the cDNA, providing a measure of the original mRNA levels. This method is considered as the gold standard for the study of changes in gene expression at the mRNA level in biomedical investigations (Guilder et al., 2008; Dheda et al., 2004).

Gene expression studies are significant for understanding the effectiveness of neuroprotection by revealing how genes influence neuronal survival and response to injury. Analysis of gene expression patterns, enables to identify genes that protect neurons from damage, as well as those that contribute to neurodegeneration. This knowledge can be employed to develop therapeutic strategies which enhance neuronal resilience and combat neurological diseases.

Studies have shown that Parkin protein is present in AD (Alzheimer's disease) brain lesions, specifically in amyloid plaques and blood vessels affected by amyloid

protein. Parkin expression is also seen to be increased in the astrocytes surrounding these lesions, which are cells that support and protect neurons. These findings suggest that Parkin might be involved in the pathological processes of AD, which potentially contribute to the accumulation of amyloid plaques and other abnormal protein deposits in the brain. Furthermore, some evidence suggests that Parkin might play an important role in the clearance of amyloid-beta ($A\beta$), a protein fragment that accumulates in the brains of people with AD (Takahashi & Mashima, 2022).

In neuroprotection studies, the Parkin gene and housekeeping genes, including actin, are frequently examined. Parkin, is a gene associated with Parkinson's disease and Alzheimer's disease, which plays a pivot role in maintaining mitochondrial quality through a process called mitophagy. House-keeping gene like actin, provides a baseline for gene expression, which is important for comparing Parkin's expression levels under different conditions in neuroprotection research (Alieva et al., 2021).

Currently the expression of Parkin gene is investigated. Parkin is an E3 ubiquitin ligase involved in the process of mitophagy (removal of damaged mitochondria). It helps to clear these dysfunctional organelles and preventing them from triggering cell death in neurons. According to Seirafi et al. (2015), the ability of Parkin gene to induce mitophagy is considered neuroprotective since it helps to maintain healthy mitochondrial populations, which are critical for the survival of neurons. House-keeping genes are genes that are essential for the basic cellular functions and are expressed at relatively constant levels in all the cells and tissues. Actin, GAPDH and β -tubulin are the common examples. Among these, current study was carried out by using Actin as the house keeping gene.

The significance of intracellular β -amyloid ($A\beta$) accumulation is increasingly recognized in Alzheimer's disease (AD). Parkin knock-down led to accumulation of $A\beta$. In AD brain, Parkin was found to interact with $A\beta$ and its levels were reduced. Thus, Parkin is cytoprotective, partially by increasing the removal of cellular $A\beta$ through a proteasome-dependent pathway. Previous study conducted by Rosen et al. (2010), demonstrated that Parkin expression facilitates the removal of intracellular β -amyloid. Conversely, the absence of Parkin activity tends to raise resting $A\beta$ levels. In the current study, expression of Parkin gene showed a change in expression fold as 1, -0.323 and 1.29 for control, amyloid treated cells and sample treated cells

respectively (**Fig. 49**). From this data, it is obviously clear that fungal extract has the potential for the upregulation of Parkin's activity. Parkin can influence the processing of the precursor protein for amyloid (APP). Parkin may directly participate in the degradation of beta amyloid, which in turn helps to clear them from the brain and thus reduce amyloid plaques.

In general, gene expression profiling is a powerful tool for understanding the molecular mechanisms of disease and other biological processes by revealing which genes are active and how their activity changes under different conditions.

Many modern pharmaceuticals trace their origin to plant-based sources, highlighting the significant contribution of herbal remedies to major pharmacopoeias. Ongoing research continues for herbal therapies to address numerous illnesses, particularly neurological disorders, where effective treatment options remain limited. Additionally, people consider herbal options due to their personal beliefs and perceived lesser toxic effects (Dhami, 2013; Zahn et al., 2019). Natural products and their isolated compounds are a significant source of drug discovery in the pursuit of more effective treatment for AD. But the present study opens a path for the exploitation of fungi, the biologically and biochemically diverse group of organisms.

Despite the promising medicinal and therapeutic potential of the mushrooms, the pharmacological applications are not commercialized sufficiently. More efforts are needed on the exploration of the therapeutic potential of medicinal mushrooms and in the field of drug development. Essential tasks include successful human-based clinical studies using high-quality mushroom-derived products for the treatment of diseases and establishing economical ways to produce these products under controlled conditions (Ito et al., 2020). Nowadays, in addition to conventional medicines, supplementary medicines are becoming more popular among patients to manage their distress during and after the treatment. Supplemental non-pharmacological treatments utilizing medicinal mushrooms have limited data on their cost and benefits. In order to explore and expand the knowledge in this field and facilitate the comprehensive assessment of mushrooms' therapeutic benefits, more research is needed in preclinical and clinical studies based on relevant data.

Even though, the neuroprotective potential of fungal secondary metabolites are promising, further research is needed to obtain an in-depth understanding of their

mechanisms and optimize their therapeutic applications. The diversity of fungal metabolites and their complex interactions with cellular pathways present both opportunities and challenges in developing effective neuroprotective agents.



Summary and Conclusion

SUMMARY AND CONCLUSION

Applied mycology is one of the most stimulating and rapidly evolving areas of the biological sciences. Fungal biology has great significance in the field of medicinal researches since it had led to the discovery of several wonder drugs. Fungi offer unique therapeutic compounds, especially antibiotics and immunosuppressants, that are not readily seen in plants. Fungi also produce diverse natural products with antitumor, antioxidant, anticancer, antimicrobial, anti-inflammatory, immunomodulatory, and neuroprotective properties, many of which have been led to the development of medicines or are being explored for potential health applications. Many fungal members are used for culinary and medicinal purposes. Among these, macro fungi (both gilled and bracket fungi) have more implications. Basidiomycete fungi play a pivotal role in the production of global edible mushroom and their wealth of metabolites, biosynthetic pathways and survival mechanisms are more highlighted as they have important applications in biotechnology and industry. Medicinal mushrooms especially those belonging to higher Basidiomycete groups are a reservoir of bioactive compounds with multiple therapeutic properties. Polyporaceae, the most important poroid family under the class Basidiomycetes, which has been widely explored especially for the genera like *Agaricus*, *Auricularia*, *Phellinus*, *Ganoderma*, *Pleurotus*, *Trametes*, *Lentinus*, *Fomitopsis* etc., due to their biologically active secondary metabolites. *Microporus* is a least explored genus of bracket fungi in the family Polyporaceae. As of January 2017, Index Fungorum accepts 12 species in *Microporus*. The principal aim of the present study was to explore the mycochemical and bioactive potential of two species of *Microporus* such as *M. affinis* and *M. xanthopus*. The presence of potential mycochemicals was validated by both qualitative and quantitative methods. Because of the presence of some active mycochemicals, antioxidant, cytotoxic, antiproliferative and neuroprotective studies were carried out in detail. Special emphasis was given to the extended neuroprotective studies of *M. xanthopus* which was confirmed by different modern techniques like ROS measurement using DCFDA,

Annexin V -FITC apoptosis using flow cytometry, autophagy, indirect ELISA and gene expression studies using qRT PCR.

Chemical characterization in the two species of *Microporus* was carried out and revealed in four sections, viz., preliminary qualitative mycochemical screening, quantitative estimation, identification of non-volatile chemicals through HR-LC/MS analysis and determination of volatile chemical constituents through GC/MS analysis. The secondary metabolites are an excellent source of new medicinal compounds.

Methanolic extracts of both the species revealed the presence of major chemical compounds such as terpenoids, steroids and alkaloids. In addition, *M. xanthopus* was found to possess glycosides and saponins. Quantitative analyses of four different classes of compounds like alkaloids, terpenoids, carbohydrates and proteins were conducted. The alkaloid content was quantified using the standard compound caffeine. *M. xanthopus* showed the maximum alkaloid content than *M. affinis*. The total terpenoids were quantified by using the standard compound lupeol. Both the species showed comparatively high amount of terpenoids. The total protein estimation was done by Lowry's method. Bovine Serum Albumin (BSA) was used as the standard. Both the species showed a minimum amount of protein. Total carbohydrate was estimated by using the standard glucose. Both the species showed high amount of carbohydrates. Carbohydrate and protein contents were higher in *M. affinis* than that of *M. xanthopus*. HR-LC/MS analysis in *M. xanthopus*, revealed the presence of 31 compounds. The compounds obtained were grouped into 16 classes. These are alkyl amines, amines, amino glycosides, dipeptides, ethers, fatty acids, glycosides, monoamine neurotransmitters, organic compounds, peptides, phospholipids, sphingolipids, steroids, terpenes, diterpenes and vitamins. In case of *M. affinis*, 9 compounds were revealed. These are belonging to 6 classes. Carboxylic acids, fatty acids, fatty acid esters, organic compounds, steroids and terpenes are the various classes. GC/MS analysis of both the fungal extracts had shown to possess more than forty compounds under 11 classes. Both the species are reported to possess fatty acids as the predominant class of compounds with the leading compound being linoleic acid in GC/MS. Different types of fungal sterols with a wide spectrum of bioactivities have also been reported.

Free radicals are unstable molecules that are made during normal cell metabolism. Free radicals can disrupt normal cellular processes. By removing them, free radical scavengers ensure cells to function optimally. So, the fungal extracts were subjected to free radical scavenging assays like DPPH, hydroxyl radicals, nitric oxide and reducing power. An IC_{50} value represents the concentration of a substance needed to scavenge 50% of free radicals. Therefore, the lower the IC_{50} value, the greater will be the antioxidant activity. In this scenario, *M. affinis* was found to be more potent than *M. xanthopus* even though, both the species are having antioxidant potential. In effect, free radical scavenging is a critical part of the body's defence system against damage and diseases. By neutralizing harmful molecules, antioxidants and other scavengers maintain cellular health and promote overall well-being. Oxidative damage has a key role in aging process and is linked to diseases like Cancer, Alzheimer's and Parkinson's. Free radical scavengers can help to mitigate these effects. So, the study was carried out on cytotoxic, antiproliferative and neuroprotective areas.

Cytotoxicity is the ability of a cell to induce damage or induce cell death. Both mitotic index and chromosomal aberrations are the important parameters of cytotoxic studies. An increased frequency of chromosomal aberrations indicates DNA damage, whereas a decreased mitotic index often suggests cytotoxicity. In the present study, a dose dependent reduction in mitotic index due to the activity of fungal extracts was noticed in *Allium cepa* assay, which is a clear indication of cytotoxic potential of fungal extracts. The extracts were also reported to induce an increase in the percentage of chromosomal aberrations. In general, the decline in mitotic index can be correlated with an increase in the frequency of chromosomal aberrations, suggesting that cellular damage is affecting cell division. Even though it is having cytotoxic potential, there may be some agents that induce DNA damage indirectly. Therefore, proper test design including control groups and avoiding excessive cytotoxicity is crucial to avoid misleading results.

Indian traditional system of medicine has been the focus of cancer research recently, as the natural drugs are eco-friendly and safe. Cancer is a dreadful disease,

which is characterized by uncontrolled cell growth. It is a multistep disease involving various factors. The synthetic drugs which are in use clinically have not been completely succeeded in fulfilling the expectations, because of the extensive cost of their production. There is a constant need for the development of cost-effective novel drugs. The chemotherapeutic anticancer compounds can trigger a chain of cellular responses that influence cell proliferation and tumorigenesis. Apoptosis is the much-studied cellular response. In the present study, fungal extract was tested *in vitro* for antiproliferation on DLD1 cell line (colorectal cancer) using MTT assay. Comparatively less LC₅₀ value of *M. affinis* indicates its higher cytotoxic potential towards DLD1 cell line than that of *M. xanthopus*. It was compared with the normal cell line L929. Apoptotic characters are observed by analysing the morphology of treated cells. The degree of apoptosis was confirmed by AO/EtBr double staining method. This method seems to be more significant since it gives conspicuous visual information about the cells and distinguishing apoptotic and necrotic cells.

The presence of terpenoids in *Microporus* supports neuroprotective activities. Hence the fungal extracts were subjected to the *in vitro* study of neuroprotective efficacy on IMR-32 (human neuroblastoma cell line). Cell viability was assessed by using MTT assay. Neurodegeneration was induced by using β amyloid. In the present study, both the species was found to possess an increase in the percentage of cell viability. This increase in the viability percentage indirectly depicts its neuroprotective potential. But a dose dependent increase in cell viability was noticed in *M. xanthopus* only. Apoptosis determination using AO/EtBr method clearly revealed the neuroprotective efficacy of *M. xanthopus*. So further validations on neuroprotection was carried out in *M. xanthopus* only.

Reactive Oxygen Species (ROS) is a highly reactive short-lived molecule in the form of a free radical. Normally, ROS production will be high in cells that undergo neurodegeneration. In the present study, amyloid treated cells were found to possess increased ROS level. From this study it was clear that the increased ROS level could be controlled by using fungal extract on amyloid treated cells. It indirectly supports the recovery of cells with neurodegeneration. Neuroprotective potential was also

substantiated by using an indirect ELISA. In this study, ChAt, Glutamate Receptor 2, Tau and alpha synuclein was taken as the primary antibodies. HRP conjugate was used as the secondary antibody. A strong protein activity was observed in case of all the antibodies used in the cells treated with amyloid and the extract, which in turn indicates its neuroprotective efficacy.

A healthy population with balanced autophagy is often associated with neuroprotection. This helps to eradicate the damaged proteins and thereby preventing cellular dysfunction and cell death. In this study, cells treated with sample showed an increase in the autophagy induction ratio than the cells treated with amyloid. In the context of neuroprotection, an increase in autophagy especially from the extract, could be a positive finding and thus, potentially suggesting a protective mechanism against amyloid induced damage.

One of the important areas of neuroprotection is the detection of apoptotic or necrotic cells. For this, Annexin V- FITC assay was used to evaluate the extent of neuronal apoptosis induced by amyloid β . In this study, it was clear that the sample treatment was able to reduce the late apoptotic cells, which indicates its potential role in neuroprotection.

Gene expression studies are significant for a thorough understanding about the effectiveness of neuroprotection by revealing how the genes influence neuronal survival. Parkin is a protein present in those individuals with AD (Alzheimer's disease). Parkin might play an important role in the clearance of β amyloid which is accumulated in the brain of people with AD. In the present study, expression of Parkin gene was examined by comparing a house keeping gene called Actin. From this study it was clear that the extract of *Microporus xanthopus* has the potential for the upregulation of Parkin's activity. In general, gene expression profiling forms the powerful tool for understanding the molecular mechanisms of disease and other biological process by revealing which genes are active and how their activity changes under different conditions.

According to the Alzheimer's Association 2016, AD is the most prevalent neurological disorder affecting 50 million people throughout the world.

Neurodegenerative disorders are a major health concern in geriatrics, affecting nearly 15% of the global population. These are characterised by progressive neuronal damage with loss of neuronal function in the central nervous system (CNS), leading to cognitive decline. Abnormal protein aggregation activating inflammatory pathways is a characteristic feature observed in most prototypical neurodegenerative diseases, such as Alzheimer's disease (AD), amyotrophic lateral sclerosis, Parkinson's disease etc.

Present study proved that the increased cell viability, decreased apoptosis and lower ROS levels are good for neuroprotection. While these findings support the neuroprotective role of *M. xanthopus*, future studies are inevitable to investigate the bioactivity, standardization and clinical translation of its active secondary metabolites to completely unlock their therapeutic potential.

Deliverables:

- ❖ Several potential mycochemicals were revealed from the fungal extract of both the species of *Microporus* (*M. affinis* and *M. xanthopus*).
- ❖ Antioxidant potential of fungal extracts was found to be moderately significant.
- ❖ Screening of cytotoxicity of fungal extracts was demonstrated using *Allium cepa* root tip assay.
- ❖ The antiproliferative potential of *M. affinis* and *M. xanthopus* on DLD1 cell line was examined *in vitro* using MTT assay and apoptosis determination using AO/EtBr staining method.
- ❖ Neuroprotective efficacy of *M. affinis* and *M. xanthopus* was assessed on IMR-32 cell line using MTT and AO/ EtBr method.
- ❖ Potential role of *M. xanthopus* in neuroprotection was validated by a number of modern techniques like autophagy, annexin V-FITC apoptosis, ROS scavenging method using DCFDA, indirect ELISA and by gene expression analysis using qRT PCR.

Recommendations:

- ✓ Isolation of species specific mycoconstituents from *M. affinis* and *M. xanthopus*.
- ✓ Validation of antiproliferative activity *in vivo* on both species.
- ✓ *In vivo* studies on neuroprotection using the extracts of *M. affinis* and *M. xanthopus*.
- ✓ Extended study on neuroprotection to substantiate the neuroprotective efficacy of *M. xanthopus*.



References

REFERENCES

- Abate, D., & Abraham, W. R. (1994). Antimicrobial metabolites from *Lentinus crinitus*. *The Journal of Antibiotics*, 47(11), 1348-1350.
- Abou-Ghali, M., & Stiban, J. (2015). Regulation of ceramide channel formation and disassembly: Insights on the initiation of apoptosis. *Saudi journal of biological sciences*, 22(6), 760-772.
- Acharya, K., Acharya, S. N., & Thomas, J. E. (2007). Medicinal properties of mushroom. *Advances in medicinal plant research*, 215-36.
- Acharya, K., Chatterjee, S., Biswas, G., Chatterjee, A., & Saha, G. K. (2012). Hepatoprotective effect of a wild edible mushroom on carbon tetrachloride-induced hepatotoxicity in mice. *International Journal of Pharmacy and Pharmaceutical Sciences*, 4(3), 285-288.
- ACS (2020). Colorectal Cancer Facts & Figures 2020–2022. *American Cancer Society*. 66, 48.
- Adinew, G. M., Taka, E., Mendonca, P., Messeha, S. S., & Soliman, K. F. (2021). The anticancer effects of flavonoids through miRNAs modulations in triple-negative breast cancer. *Nutrients*, 13(4), 1212. <https://doi.org/10.3390/nu13041212>
- Adrio, J. L., & Demain, A. L. (2003). Fungal biotechnology. *International Microbiology*, 6(3), 191-199. <https://doi.org/0.1007/s10123-003-0133-0>
- Aggarwal, P., Sharma, P., Sharma, S., & Aggarwal, J. (2012). Antioxidant mushrooms: A review. *International Research Journal of Pharmacy*, 3(6), 65-70.
- Agrahar-Murugkar, D., & Subbulakshmi, G. J. F. C. (2005). Nutritional value of edible wild mushrooms collected from the Khasi hills of Meghalaya. *Food Chemistry*, 89(4), 599-603. <https://doi.org/10.1016/j.foodchem.2004.03.042>
- Akihisa, T., Mizushima, Y., Ukiya, M., Oshikubo, M., Kondo, S., Kimura, Y., Suzuki, T., & Tai, T. (2004). Dehydrotrametenonic acid and dehydroeburiconic acid from *Poria cocos* and their inhibitory effects on eukaryotic DNA polymerase α and β . *Bioscience, Biotechnology, and Biochemistry*, 68(2), 448-450. <https://doi.org/10.1271/bbb.68.448>
- Akutsu, M., Kano, Y., Tsunoda, S., Suzuki, K., Yazawa, Y., & Miura, Y. (1995). Schedule-dependent interaction between paclitaxel and doxorubicin in human cancer cell lines *in vitro*. *European Journal of Cancer*, 31(13-14), 2341-2346. [https://doi.org/10.1016/0959-8049\(95\)00448-3](https://doi.org/10.1016/0959-8049(95)00448-3)
- Alexopoulos, C. J., Mims, C. W., & Blackwell, M. (1996). *Introductory mycology* (No. Ed. 4, pp. x+-869).
- Alieva, A. K., Filatova, E. V., Rudenok, M. M., Slominsky, P. A., & Shadrina, M. I. (2021). Housekeeping genes for Parkinson's disease in humans and mice. *Cells*, 10(9), 1-11. <https://doi.org/10.3390/cells10092252>

References

- Ames, B. N. (1983). Dietary carcinogens and anticarcinogens: Oxygen radicals and degenerative diseases. *Science*, 221(4617), 1256-1264. <https://doi.org/10.1126/science.6351251>
- Ames, B. N., Shigenaga, M. K., & Hagen, T. M. (1993). Oxidants, antioxidants, and the degenerative diseases of aging. *Proceedings of the National Academy of Sciences*, 90(17), 7915-7922.
- Anand, P., Kunnumakara, A. B., Sundaram, C., Harikumar, K. B., Tharakan, S. T., Lai, O. S., Sung, B., & Aggarwal, B. B. (2008). Cancer is a preventable disease that requires major lifestyle changes. *Pharmaceutical Research*, 25(9), 2097-2116. <https://doi.org/10.1007/s11095-008-9661-9>
- Aneesh, S., & Thoppil, J. E. (2019). Study on cytotoxicity and chemical profiling of *Hexagonia tenuis* (Hook.) Fr., a potential bracket fungus (Polyporaceae). *International Journal of Pharmacy and Biological Science*, 9(3), 822-831.
- Aneesh, S., & Thoppil, J. E. (2020). Screening of bioactive components by GC-MS analysis and the study on *In vitro* anticancer efficacy of *Microporus affinis* (Blume & T. Nees) Kunt. *International Research Journal of Pharmacy*, 11(12), 31-34. <http://dx.doi.org/10.7897/2230-8407.1112104>
- Anusiya, G., Gowthama Prabu, U., Yamini, N. V., Sivarajasekar, N., Rambabu, K., Bharath, G., & Banat, F. (2021). A review of the therapeutic and biological effects of edible and wild mushrooms. *Bioengineered*, 12(2), 11239-11268.
- Ao, T., Deb, C. R., & Khruomo, N. K. (2016). Wild edible mushrooms of Nagaland, India: A potential food resource. *Journal of Experimental Biology and Agricultural Sciences*, 4, 59-65.
- Apak, R., Güçlü, K., Demirata, B., Özyürek, M., Çelik, S. E., Bektaşoğlu, B., & Özyurt, D. (2007). Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. *Molecules*, 12(7), 1496-1547. <https://doi.org/10.3390/12071496>
- Appleton, R. E., Jan, J. E., & Kroeger, P. D. (1988). *Laetiporus sulphureus* causing visual hallucinations and ataxia in a child. *CMAJ: Canadian Medical Association Journal*, 139(1), 48-49.
- Ariffin, F., Zubaidi, A. A., Yasin, M. M., & Ishak, R. (2015). Management of pulmonary tuberculosis in health clinics in the Gombak district: How are we doing so far? *Malaysian Family Physician*, 10(1), 26-33.
- Arora, I., Sharma, M., & Tollefsbol, T. O. (2017). Combinatorial epigenetics impact of polyphenols and phytochemicals in cancer prevention and therapy. *International journal of molecular sciences*, 20(18), 4567.
- Arora, R., Yates, C., Gary, B. D., McClellan, S., Tan, M., Xi, Y., Reed, E., Piazza, G. A., Owen, L. B., & Dean-Colomb, W. (2014). Panepoxydone targets NF-kB and FOXM1 to inhibit proliferation, induce apoptosis and reverse epithelial to mesenchymal transition in breast cancer. *PLoS One*, 9(6), 1-12. <https://doi.org/10.1371/journal.pone.0098370>

- Arora, S., Wang, X. I., Keenan, S. M., Andaya, C., Zhang, Q., Peng, Y., & Welsh, W. J. (2009). Novel microtubule polymerization inhibitor with potent antiproliferative and antitumor activity. *Cancer research*, 69(5), 1910-1915. <https://doi.org/10.1158/0008-5472.CAN-08-0877>
- Asada, K., Toyota, K., Nishimura, T., Ikeda, J. I., & Hori, K. (2010). Accumulation and mobility of zinc in soil amended with different levels of pig-manure compost. *Journal of Environmental Science and Health Part B*, 45(4), 285-292.
- Awad, A. B., Chen, Y. C., Fink, C. S., & Hennessey, T. (1996). beta-Sitosterol inhibits HT-29 human colon cancer cell growth and alters membrane lipids. *Anticancer Research*, 16(5A), 2797-2804.
- Ayeka, P. A. (2018). Potential of mushroom compounds as immunomodulators in cancer immunotherapy: A review. *Evidence-based Complementary and Alternative Medicine*, 2018(1), 7271509.
- Aziz, T., Duru, M. E., Mercan, N., Kivrak, I., & Gezer, K. (2007). Antioxidant and antimicrobial activities of *Laetiporus sulphureus* (Bull.) Murrill. *Food Chemistry*, 101(1), 267-273.
- Badalyan, S. M. (2012). Edible Ectomycorrhizal Mushrooms. In: Edible Ectomycorrhizal Mushrooms. A. Zambonelli & G. Bonito (Eds), Soil Biology (pp: 317-334). Springer-Verlag. ISBN: 978-3-642-33822-9
- Badalzadeh, R., Mokhtari, B., & Yavari, R. (2015). Contribution of apoptosis in myocardial reperfusion injury and loss of cardioprotection in diabetes mellitus. *The Journal of Physiological Sciences*, 65(3), 201-215.
- Bai, S., Seasotiya, L., Malik, A., Bharti, P., & Dalal, S. (2014). GC-MS analysis of chloroform extract of *Acacia nilotica* L. leaves. *Journal of Pharmacognosy and Phytochemistry*, 2(6), 79-82.
- Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R. P., & Chang, C. M. (2022). Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. *Molecules*, 27(4), 1-19. <https://doi.org/10.3390/molecules27041326>
- Barreira, J. C., Oliveira, M. B. P., & Ferreira, I. C. (2014). Development of a novel methodology for the analysis of ergosterol in mushrooms. *Food Analytical Methods*, 7(1), 217-223. <https://doi.org/10.1007/s12161-013-9621-9>
- Barros, L., Baptista, P., & Ferreira, I. C. (2007). Effect of *Lactarius piperatus* fruiting body maturity stage on antioxidant activity measured by several biochemical assays. *Food and Chemical Toxicology*, 45(9), 1731-1737. <https://doi.org/10.1016/j.fct.2007.03.006>
- Bashandy, S. A., Alhazza, I. M., El-Desoky, G. E., & Al-Othman, Z. A. (2011). Hepatoprotective and hypolipidemic effects of *Spirulina platensis* in rats administered mercuric chloride. *African Journal of Pharmacy Pharmacology*, 5(2), 175-182.
- Baskar, A. A., Ignacimuthu, S., Paulraj, G. M., & Al Numair, K. S. (2010). Chemopreventive potential of β -sitosterol in experimental colon cancer model-an *in vitro* and *in vivo*

- study. *BMC Complementary and Alternative medicine*, 10(1), 1-10. <https://doi.org/10.1186/1472-6882-10-24>
- Becovic, M., Stage, M., Boh, B., Plankl, M., Wraber, B., & Kreft, E. S. (2014). Advances in cultivation of medicinal fungi biomass and pharmaceutical compounds in bioreactors, Proceedings of the 8th International Conference on Mushroom Biology and Mushroom Products (ICMBMP8), Yugantar Prakashan Pvt. Ltd, New Delhi.
- Behar, N., Sharma, S., Parihar, R., Dubey, S. K., Mehta, S., & Pandey, V. (2024). Role of fungal metabolites in pharmaceuticals, human health, and agriculture. In *Fungal Secondary Metabolites* (pp. 519-535). Elsevier. doi: 10.1016/B978-0-323-95241-5.00005-8
- Belyaeva, E. A., Sokolova, T. V., Emelyanova, L. V., & Zakharova, I. O. (2012). Mitochondrial electron transport chain in heavy metal-induced neurotoxicity: Effects of cadmium, mercury, and copper. *The Scientific World Journal*, 2012(1), 136063.
- Biswas, G., Chatterjee, S., & Acharya, K. (2012). Chemopreventive activity of the ethanolic extract of *Astraeus hygrometricus* (pers.) morg. on Ehrlich's ascites carcinoma cells. *Digest Journal of Nanomaterials & Biostructures (DJNB)*, 7(1), 185 - 191.
- Blagodatski, A., Yatsunskaya, M., Mikhailova, V., Tiasto, V., Kagansky, A., & Katanaev, V. L. (2018). Medicinal mushrooms as an attractive new source of natural compounds for future cancer therapy. *Oncotarget*, 9(49), 29259-29274.
- Blokhina, O., Virolainen, E., & Fagerstedt, K. V. (2003). Antioxidants, oxidative damage and oxygen deprivation stress: A review. *Annals of Botany*, 91(2), 179-194. <https://doi.org/10.1093/aob/mcf118>
- Bobek, P., Nosalova, V., & Cerna, S. (2001). Effect of pleuran (β -glucan from *Pleurotus ostreatus*) in diet or drinking fluid on colitis in rats. *Food/Nahrung*, 45(5), 360-363. [https://doi.org/10.1002/1521-3803\(20011001\)45:5%3C360::AID-FOOD360%3E3.0.CO;2-C](https://doi.org/10.1002/1521-3803(20011001)45:5%3C360::AID-FOOD360%3E3.0.CO;2-C)
- Boland, B., Kumar, A., Lee, S., Platt, F. M., Wegiel, J., Yu, W. H., & Nixon, R. A. (2008). Autophagy induction and autophagosome clearance in neurons: Relationship to autophagic pathology in Alzheimer's disease. *Journal of Neuroscience*, 28(27), 6926-6937. doi: 10.1523/JNEUROSCI.0800-08.2008.
- Boonyarat, C., Tantiwatcharakunthon, M., Takomthong, P., Yenjai, C., Hayakawa, Y., Dejkriengkraikul, P., & Waiwut, P. (2023). Neuroprotective and anticancer effects of 7-Methoxyheptaphylline via the TAK1 pathway. *Oncology Reports*, 49(1), 15.
- Borchers, A. T., Stern, J. S., Hackman, R. M., Keen, C. L., & Eric, M. (1999). Mushrooms, tumors, and immunity. *Proceedings of the Society for Experimental Biology and Medicine*, 221(4): 281-293. <https://doi.org/10.3181/00379727-221-44412>
- Borkar, P., Doshi, A., & Navathe, S. (2015). Mushroom diversity of Konkan region of Maharashtra, India. *Journal of Threatened Taxa*, 7(10), 7625-7640. <http://dx.doi.org/10.11609/JoTT.o4283.7625-40>
- Bowling, A. C., & Beal, M. F. (1995). Bioenergetic and oxidative stress in neurodegenerative diseases. *Life Sciences*, 56(14), 1151-1171. [https://doi.org/10.1016/0024-3205\(95\)00055-B](https://doi.org/10.1016/0024-3205(95)00055-B)

- Brandes, M. S., & Gray, N. E. (2020). NRF2 as a therapeutic target in neurodegenerative diseases. *ASN Neuro*, 12, 1-23. <http://dx.doi.org/10.1177/1759091419899782>
- Branen, A. L. (1975). Toxicology and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. *Journal of the American Oil Chemists' Society*, 52(2), 59-63. <https://doi.org/10.1007/BF02901825>
- Brown, R. C., Lockwood, A. H., & Sonawane, B. R. (2005). Neurodegenerative diseases: An overview of environmental risk factors. *Environmental Health Perspectives*, 113(9), 1250-1256. <https://doi.org/10.1289/ehp.7567>
- Brussel, J. P. V., van Steenbrugge, G. J., Romijn, J. C., Schröder, F. H., & Mickisch, G. H. J. (1999). Chemosensitivity of prostate cancer cell lines and expression of multidrug resistance-related proteins. *European Journal of Cancer*, 35(4), 664-671. [https://doi.org/10.1016/S0959-8049\(98\)00435-3](https://doi.org/10.1016/S0959-8049(98)00435-3)
- Budihardjo, I., Oliver, H., Lutter, M., Luo, X., & Wang, X. (1999). Biochemical pathways of caspase activation during apoptosis. *Annual Review of Cell and Developmental Biology*, 15(1), 269-290.
- Butler, M. S. (2004). The role of natural product chemistry in drug discovery. *Journal of Natural Products*, 67(12), 2141-2153.
- Cai, J., Ma, H., Huang, F., Zhu, D., Bi, J., Ke, Y., & Zhang, T. (2013). Correlation of Bevacizumab-induced hypertension and outcomes of metastatic colorectal cancer patients treated with Bevacizumab: A systematic review and meta-analysis. *World Journal of Surgical Oncology*, 11(1), 1-8. <https://doi.org/10.1186/1477-7819-11-306>
- Caltagirone, S., Rossi, C., Poggi, A., Ranelletti, F. O., Natali, P. G., Brunetti, M., Aiello, F. B., & Piantelli, M. (2000). Flavonoids apigenin and quercetin inhibit melanoma growth and metastatic potential. *International Journal of Cancer*, 87(4), 595-600. [https://doi.org/10.1002/1097-0215\(20000815\)87:4%3C595::AID-IJC21%3E3.0.CO;2-5](https://doi.org/10.1002/1097-0215(20000815)87:4%3C595::AID-IJC21%3E3.0.CO;2-5)
- Capasso, L. (1998). 5300 years ago, the Ice Man used natural laxatives and antibiotics. *The Lancet*, 352(9143), 1864.
- Chaitanya, G. V., Alexander, J. S., & Babu, P. P. (2010). PARP-1 cleavage fragments: Signatures of cell-death proteases in neurodegeneration. *Cell Communication and Signaling*, 8(1), 1-11. <https://doi.org/10.1186/1478-811X-8-31>
- Chakraborty, S., Majumder, S., Ghosh, A., & Bhattacharya, M. (2023). Endemic and endangered ethno-herbal medicinal climber of Darjeeling hills (*Edgaria darjeelingensis* CB Clarke) is a treasure of anti-cancer molecules: A study on GC/MS analysis and probable biosynthetic pathways. *Indian Journal of Traditional Knowledge*, 22(2), 371-380. <https://doi.org/10.56042/ijtk.v22i2.38484>
- Chang, H. L., Chao, G. R., Chen, C. C., & Mau, J. L. (2001). Non-volatile taste components of *Agaricus blazei*, *Antrodia camphorata* and *Cordyceps militaris* mycelia. *Food chemistry*, 74(2), 203-207.
- Chang, R. (1996). Functional properties of edible mushrooms. *Nutrition Reviews*, 54(11), S91-S93.

- Chang, S. T., & Buswell, J. A. (1999). *Ganoderma lucidum* (Curt.: Fr.) P. karst. (Aphyllphoromycetideae)— A mushrooming medicinal mushroom. *International Journal of Medicinal Mushrooms*, 1(2), 139-46.
- Chang, S. T., & Miles, P. G. (1993). Mushrooms: trends in production and technological development. *Genetic Engineering and Biotechnology Monitor*, 41(42), 73-81.
- Chang, S., & Miles, G. P. (2004). Mushrooms: Cultivation, nutritional value, medicinal effects and environmental impact (p. 436). Boca Raton, FL: CRC Press. <http://dx.doi.org/10.1201/9780203492086>
- Chang, S., & Miles, G. P. (2004). Mushrooms: Cultivation, Nutritional Value, Medicinal Effects and Environmental Impact (p. 436). Boca Raton, FL: CRC Press. <http://dx.doi.org/10.1201/9780203492086>
- Chatterjee, S., Biswas, G., Chandra, S., Saha, G. K., & Acharya, K. (2013). Apoptogenic effects of *Tricholoma giganteum* on Ehrlich's ascites carcinoma cell. *Bioprocess and Biosystems Engineering*, 36(1), 101-107. <https://doi.org/10.1007/s00449-012-0765-6>
- Chatterjee, S., Saha, G. K., & Acharya, K. (2011). Antioxidant activities of extracts obtained by different fractionation from *Tricholoma giganteum* basidiocarps. *Pharmacology*, 3, 88-97.
- Chaudhari, K. S., Dhapkas, M. P., Kumar, A., & Ingle, R. G. (2024). Mental disorders—a serious global concern that needs to address. *International Journal of Pharmaceutical Quality Assurance*, 15(02), 973-978. <https://doi.org/10.25258/ijpqa.15.2.66>.
- Chaves, S., Resta, S., Rinaldo, F., Costa, M., Josselin, R., Gwizdala, K., & Santos, M. A. (2020). Design, synthesis, and *in vitro* evaluation of hydroxybenzimidazole-donepezil analogues as multitarget-directed ligands for the treatment of Alzheimer's disease. *Molecules*, 25(4), 985.
- Cheeseman, K. H., & Slater, T. F. (1993). Free radicals in medicine. *British Medical Bulletin*, 49(3), 479-724. <https://doi.org/10.1093/oxfordjournals.bmb.a072625>
- Chen, G. F., Xu, T. H., Yan, Y., Zhou, Y. R., Jiang, Y., Melcher, K., & Xu, H. E. (2017). Amyloid beta: Structure, biology and structure-based therapeutic development. *Acta Pharmacologica Sinica*, 38(9), 1205-1235. <https://doi.org/10.1038/aps.2017.28>
- Chen, H. H., Zhou, H. J., & Fang, X. (2003). Inhibition of human cancer cell line growth and human umbilical vein endothelial cell angiogenesis by artemisinin derivatives *in vitro*. *Pharmacological Research*, 48(3), 231-236. [https://doi.org/10.1016/S1043-6618\(03\)00107-5](https://doi.org/10.1016/S1043-6618(03)00107-5)
- Chen, H., Ju, Y., Li, J., & Yu, M. (2012). Antioxidant activities of polysaccharides from *Lentinus edodes* and their significance for disease prevention. *International Journal of Biological Macromolecules*, 50(1), 214-218. <https://doi.org/10.1016/j.ijbiomac.2011.10.027>
- Chen, J., & Seviour, R. (2007). Medicinal importance of fungal β -(1 $\rightarrow$ 3), (1 $\rightarrow$ 6)-glucans. *Mycological Research*, 111(6), 635-652. <https://doi.org/10.1016/j.mycres.2007.02.011>

- Chen, M. C., Hsu, S. L., Lin, H., & Yang, T. Y. (2014). Retinoic acid and cancer treatment. *Biomedicine*, 4(4), 1-6. <https://doi.org/10.7603/s40681-014-0022-1>
- Chen, T. R., Dorotinsky, C. S., McGuire, L. J., Macy, M. L., & Hay, R. J. (1995). DLD-1 and HCT-15 cell lines derived separately from colorectal carcinomas have totally different chromosome changes but the same genetic origin. *Cancer Genetics and Cytogenetics*, 81(2), 103-108. [https://doi.org/10.1016/0165-4608\(94\)00225-Z](https://doi.org/10.1016/0165-4608(94)00225-Z)
- Chen, W., Zhao, Z., & Li, Y. (2011). Simultaneous increase of mycelial biomass and intracellular polysaccharide from *Fomes fomentarius* and its biological function of gastric cancer intervention. *Carbohydrate Polymers*, 85(2), 369-375. <https://doi.org/10.1016/j.carbpol.2011.02.035>
- Chen, X., Hu, Z. P., Yang, X. X., Huang, M., Gao, Y., Tang, W., ... & Zhou, S. F. (2006). Monitoring of immune responses to a herbal immuno-modulator in patients with advanced colorectal cancer. *International Immunopharmacology*, 6(3), 499-508.
- Cheng, C. R., Yue, Q. X., Wu, Z. Y., Song, X. Y., Tao, S. J., Wu, X. H., Xu, P. P., Liu, X., Guan, S. H., & Guo, D. A. (2010). Cytotoxic triterpenoids from *Ganoderma lucidum*. *Phytochemistry*, 71(13), 1579-1585. <https://doi.org/10.1016/j.phytochem.2010.06.005>
- Cheng, J. J., Lin, C. Y., Lur, H. S., Chen, H. P., & Lu, M. K. (2008). Properties and biological functions of polysaccharides and ethanolic extracts isolated from medicinal fungus, *Fomitopsis pinicola*. *Process Biochemistry*, 43(8), 829-834. <https://doi.org/10.1016/j.procbio.2008.03.005>
- Chepkirui, C., Yuyama, K. T., Wanga, L. A., Decock, C., Matasyoh, J. C., Abraham, W. R., & Stadler, M. (2018). Microporenic acids A–G, biofilm inhibitors, and antimicrobial agents from the *Basidiomycete* *Microporus* species. *Journal of Natural Products*, 81(4), 778-784. <http://dx.doi.org/10.1021/acs.jnatprod.7b00764>
- Cherra, S. J., & Chu, C. T. (2008). Autophagy in neuroprotection and neurodegeneration: A question of balance. *Future Neurology*, 3(3), 309-323. doi: 10.2217/14796708.3.3.309.
- Chihara, G. (1992). Immunopharmacology of Lentinan, a polysaccharide isolated from *Lentinus edodes*: Its application as a host defence potentiator. *International Journal of Oriental Medicine*, 17, 57-77. <https://doi.org/10.1271/bbb.66.1052>
- Cho, I. H., Namgung, H. J., Choi, H. K., & Kim, Y. S. (2008). Volatiles and key odorants in the pileus and stipe of pine-mushroom (*Tricholoma matsutake* Sing.). *Food Chemistry*, 106(1), 71-76. <https://doi.org/10.1016/j.foodchem.2007.05.047>
- Choi, Y. H., & Yoo, Y. H. (2012). Taxol-induced growth arrest and apoptosis is associated with the upregulation of the Cdk inhibitor, p21WAF1/CIP1, in human breast cancer cells. *Oncology Reports*, 28(6), 2163-2169. <https://doi.org/10.3892/or.2012.2060>
- Chroho, M., Bouymajane, A., Aazza, M., Oulad, E. M. Y., Cacciola, F., Mondello, L., Zair, T., & Bouissane, L. (2022). Determination of the phenolic profile, and evaluation of biological activities of hydroethanolic extract from aerial parts of *Origanum compactum* from Morocco. *Molecules*, 27(16), 1-12. <https://doi.org/10.3390/molecules27165189>

References

- Clementi, M. E., Tringali, G., Triggiani, D., & Giardina, B. (2015). *Aloe arborescens* extract protects IMR-32 cells against alzheimer amyloid beta peptide via inhibition of radical peroxide production. *Natural Product Communications*, 10(11), 1993-1995.
- CNN Health. (2018). The humble mushroom is the newest superfood. *NCBI*. <https://www.ncbi.nlm.nih.gov/search/research-news/10634/>
- Cobley, J. N., Fiorello, M. L., & Bailey, D. M. (2018). 13 reasons why the brain is susceptible to oxidative stress. *Redox Biology*, 15, 490-503. <https://doi.org/10.1016/j.redox.2018.01.008>
- Coletto, B.M.A., Mondino, P. 1991. Antibiotic activity in Basidiomycetes: V. Antibiotic activity of mycelia and cultural filtrates. *Allionia* (Turin), 30, 61– 64.
- Coletto, M. A. B. (1992). Antibiotic activity in Basidiomycetes. VI. Antibiotic activity of mycelia and cultural filtrates.
- Cordell, G. A. (1993). Separation, structure elucidation and bioassay of cytotoxic natural products. *Bioactive natural products: Detection, isolation, and structure determination*, 197.
- Cosentino, G., Plantamura, I., Cataldo, A., & Iorio, M. V. (2019). MicroRNA and oxidative stress interplay in the context of breast cancer pathogenesis. *International Journal of Molecular Sciences*, 20(20), 5143. <https://doi.org/10.3390/ijms20205143>
- Cozzi, R., Ricordy, R., Aglitti, T., Gatta, V., Perticone, P., & De Salvia, R. (1997). Ascorbic acid and beta-carotene as modulators of oxidative damage. *Carcinogenesis*, 18(1), 223-228. <https://doi.org/10.1093/carcin/18.1.223>
- Csernus, V., Schally, A. V., & Groot, K. (1999). Antagonistic analogs of growth hormone releasing hormone (GHRH) inhibit cyclic AMP production of human cancer cell lines *in vitro*. *Peptides*, 20(7), 843-850. [https://doi.org/10.1016/S0196-9781\(99\)00071-6](https://doi.org/10.1016/S0196-9781(99)00071-6)
- Daba, A. S., & Ezeronye, O. U. (2003). Anti-cancer effect of polysaccharides isolated from higher basidiomycetes mushrooms. *African Journal of Biotechnology*, 2(12), 672-678.
- Das, K. (2009). Mushrooms of Sikkim I: Barsey Rhododendron Sanctuary Sikkim: Sikkim State Biodiversity Board and Botanical Survey of India.
- Das, K., & Roychoudhury, A. (2014). Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Frontiers in environmental science*, 2, 53.
- De Silva, D. D., Rapior, S., Fons, F., Bahkali, A. H., & Hyde, K. D. (2012). Medicinal mushrooms in supportive cancer therapies: An approach to anti-cancer effects and putative mechanisms of action. *Fungal Diversity*, 55(1), 1-35. <https://doi.org/10.1007/s13225-012-0151-3>
- Decker, E. A. (1997). Phenolics: prooxidants or antioxidants? *Nutrition Reviews*, 55(11), 396-398. <https://doi.org/10.1111/j.1753-4887.1997.tb01580.x>
- Dembitsky, V. M., Terent'ev, A. O., & Levitsky, D. O. (2010). Amino and fatty acids of wild edible mushrooms of the genus *Boletus*. *Records of Natural Products*, 4(4), 218.

- Deshmukh, S. K. (2004). Biodiversity of tropical Basidiomycetes as sources of novel secondary metabolites. In P. C. Jain (Ed.), *Microbiology and biotechnology for sustainable development* (pp. 121-140). New Delhi: CBS Publishers and Distributors.
- Devasagayam, T. P. A., Tilak, J. C., Bloor, K. K., Sane, K. S., Ghaskadbi, S. S., & Lele, R. D. (2004). Free radicals and antioxidants in human health: Current status and future prospects. *Journal of the Association of Physicians of India*, 52, 794-804.
- Dhami, N. (2013). Trends in Pharmacognosy: A modern science of natural medicines. *Journal of Herbal Medicine*, 3(4), 123-131. doi: 10.1016/j.hermed.2013.06.001
- Dheda, K., Huggett, J. F., Bustin, S. A., Johnson, M. A., Rook, G., & Zumla, A. (2004). Validation of housekeeping genes for normalizing RNA expression in real-time PCR. *Biotechniques*, 37(1), 112-119. <https://doi.org/10.2144/04371RR03>
- DiSabato, D. J., Quan, N., & Godbout, J. P. (2016). Neuroinflammation: The devil is in the details. *Journal of Neurochemistry*, 139, 136-153. <https://doi.org/10.1111/jnc.13607>
- Dong, N., Wang, Z., Sun, Q., Chen, X., Zhang, H., Zheng, J., Zhang, X., Qiu, Y., Li, Z., Li, B., Liu, K., Shao, D., Wei, J., Sun, J., & Ma, Z. (2023). Establishment and application of an indirect ELISA for the detection of antibodies to *Porcine Streptococcus* based on a recombinant GMD protein. *Animals*, 13(4), 719. doi: 10.3390/ani13040719.
- Drag, M., Surowiak, P., Drag-Zalesinska, M., Dietel, M., Lage, H., & Oleksyszyn, J. (2009). Comparison of the cytotoxic effects of birch bark extract, betulin and betulinic acid towards human gastric carcinoma and pancreatic carcinoma drug-sensitive and drug-resistant cell lines. *Molecules*, 14(4), 1639-1651. <https://doi.org/10.3390/molecules14041639>
- Duan, W., Li, X., Shi, J., Guo, Y., Li, Z., & Li, C. (2010). Mutant TAR DNA-binding protein-43 induces oxidative injury in motor neuron-like cell. *Neuroscience*, 169(4), 1621-1629. <https://doi.org/10.1016/j.neuroscience.2010.06.018>
- Dziezak, J. D. (1986). Preservatives: Antioxidants. The ultimate answer to oxidation. *Food Technology*, 40(9), 94-102.
- Ekor, M. (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*, 4, 1-10. <https://doi.org/10.3389/fphar.2013.00177>
- El Sohaimy, S. A. (2012). Functional foods and nutraceuticals-modern approach to food science. *World Applied Sciences Journal*, 20(5), 691-708. <https://doi.org/10.5829/idosi.wasj.2012.20.05.66119>
- Elbatrawy, E. N., Ghonimy, E. A., Alassar, M. M., & Wu, F. S. (2015). Medicinal mushroom extracts possess differential antioxidant activity and cytotoxicity to cancer cells. *International Journal of Medicinal Mushrooms*, 17(5), 471-479. <http://dx.doi.org/10.1615/IntJMedMushrooms.v17.i5.70>
- Elhamouly, N. A., Hewedy, O. A., Zaitoon, A., Miraples, A., Elshorbagy, O. T., Hussien, S., El-Tahan, A., & Peng, D. (2022). The hidden power of secondary metabolites in plant-fungi interactions and sustainable phytoremediation. *Frontiers in Plant Science*, 13, 1-21. <https://doi.org/10.3389/fpls.2022.1044896>

- Elmastas, M., Isildak, O., Turkecul, I., & Temur, N. (2007). Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. *Journal of Food Composition and Analysis*, 20(3-4), 337-345. <https://doi.org/10.1016/j.jfca.2006.07.003>
- Elmore, S. (2007). Apoptosis: A review of programmed cell death. *Toxicologic Pathology*, 35(4), 495-516. <https://doi.org/10.1080/01926230701320337>
- Evan, G. I., & Vousden, K. H. (2001). Proliferation, cell cycle and apoptosis in cancer. *Nature*, 411(6835), 342-348. <https://doi.org/10.1038/35077213>
- Evidente, A. (2024). Advances on anticancer fungal metabolites: Sources, chemical and biological activities in the last decade (2012–2023). *Natural Products and Bioprospecting*, 14(1), 3-42. <https://doi.org/10.1007/s13659-024-00452-0>
- Falch, B. H., Espevik, T., Ryan, L., & Stokke, B. T. (2000). The cytokine stimulating activity of (1→3)-β-D-glucans is dependent on the triple helix conformation. *Carbohydrate Research*, 329(3), 587-596. [https://doi.org/10.1016/S0008-6215\(00\)00222-6](https://doi.org/10.1016/S0008-6215(00)00222-6)
- Fan, L., Ding, S., Ai, L., & Deng, K. (2012). Antitumor and immunomodulatory activity of water-soluble polysaccharide from *Inonotus obliquus*. *Carbohydrate Polymers*, 90(2), 870-874. <https://doi.org/10.1016/j.carbpol.2012.06.013>
- Farah, M., Parhar, K., Moussavi, M., Eivemark, S., & Salh, B. (2003). 5, 6-Dichloro-ribifuranosylbenzimidazole-and apigenin-induced sensitization of colon cancer cells to TNF-α-mediated apoptosis. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 285(5), G919-G928. <https://doi.org/10.1152/ajpgi.00205.2003>
- Feeney, M. J., Miller, A. M., & Roupas, P. (2014). Mushrooms—Biologically distinct and nutritionally unique: Exploring a “third food kingdom”. *Nutrition today*, 49(6), 301-307. <https://doi.org/10.1097/NT.0000000000000063>
- Feng, B. X., Song, W. X., Qun, D., Jinian, F., & Yu, L. X. (2002). Structural features of immunologically active polysaccharides from *Ganoderma lucidum*. *Phytochemistry*, 59, 175-181.
- Ferreira, I. C., Baptista, P., Vilas-Boas, M., & Barros, L. (2007). Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity. *Food Chemistry*, 100(4), 1511-1516. <https://doi.org/10.1016/j.foodchem.2005.11.043>
- Ferreira, I. C., Barros, L., & Abreu, R. (2009). Antioxidants in wild mushrooms. *Current Medicinal Chemistry*, 16(12), 1543-1560. <https://doi.org/10.2174/092986709787909587>
- Fields, C. R., Bengoa-Vergniory, N., & Wade-Martins, R. (2019). Targeting alpha-synuclein as a therapy for Parkinson's disease. *Frontiers in molecular neuroscience*, 12, 299.
- Finimundy, T. C., Gambato, G., Fontana, R., Camassola, M., Salvador, M., Moura, S., Henriques, J. A. P., Dillon, A. J. P., & Roesch-Ely, M. (2013). Aqueous extracts of *Lentinula edodes* and *Pleurotus sajor-caju* exhibit high antioxidant capability and promising *in vitro* antitumor activity. *Nutrition Research*, 33(1), 76-84. <https://doi.org/10.1016/j.nutres.2012.11.005>

- Fogel, R. & Rogers, P. (2006). "Shelf fungi." Fun Facts About Fungi. Utah State University Intermountain Herbarium.
- Fraga, C. G., Shigenaga, M. K., Park, J. W., Degan, P., & Ames, B. N. (1990). Oxidative damage to DNA during aging: 8-hydroxy-2'-deoxyguanosine in rat organ DNA and urine. *Proceedings of the National Academy of Sciences*, 87(12), 4533-4537.
- Frankel, E. N. (1997). Nutritional benefits of flavonoids. In *Food factors for cancer prevention* (pp. 613-616). Tokyo: Springer Japan.
- Franks, L. M. (1997). Prostatic cancer: Future prospects for diagnosis and screening. *British Journal of Urology*, 79, 107-108. <https://doi.org/10.1111/j.1464-410x.1997.tb00811.x>
- Fukamachi, Y., Karasaki, Y., Sugiura, T., Itoh, H., Abe, T., Yamamura, K., & Higashi, K. (1998). Zinc suppresses apoptosis of U937 cells induced by hydrogen peroxide through an increase of the Bcl-2/Bax ratio. *Biochemical and Biophysical Research Communications*, 246(2), 364-369. <https://doi.org/10.1006/bbrc.1998.8621>
- Gachabayov, M., Babyshin, V., Durymanov, O., & Neronov, D. (2017). Surgical scales: Primary closure versus gastric resection for perforated gastric ulcer-A surgical debate. *Nigerian Journal of Surgery*, 23(1), 1-4. doi: 10.4103/1117-6806.199959.
- Gams, W. (2002). Dictionary of the fungi Ainsworth & Bisby's Dictionary of the Fungi. Edited by PM Kirk, PF Cannon, JC David & JA Stalpers. Oct. 2001. CABI Publishing, Wallingford. Pp. 655. ISBN 0 85199 377 X. Price£ 49.95. *Mycological Research*, 106(4), 507-511.
- Gardner, M. J., Jones, L. M., Catterall, J. B., & Turner, G. A. (1995). Expression of cell adhesion molecules on ovarian tumour cell lines and mesothelial cells, in relation to ovarian cancer metastasis. *Cancer letters*, 91(2), 229-234.
- Gaylan, C. M., Estebal, J. C., Tantengco, O. A. G., & Ragragio, E. M. (2018). Anti-staphylococcal and antioxidant properties of crude ethanolic extracts of macrofungi collected from the Philippines. *Pharmacognosy Journal*, 10(1), 106-109. <http://dx.doi.org/10.5530/pj.2018.1.19>
- Gençcelep, H., Uzun, Y., Tunçtürk, Y., & Demirel, K. (2009). Determination of mineral contents of wild-grown edible mushrooms. *Food Chemistry*, 113(4), 1033-1036. <https://doi.org/10.1016/j.foodchem.2008.08.058>
- Gerl, R., & Vaux, D. L. (2005). Apoptosis in the development and treatment of cancer. *Carcinogenesis*, 26(2), 263-270. <https://doi.org/10.1093/carcin/bgh283>
- Ghorai, S., Banik, S. P., Verma, D., Chowdhury, S., Mukherjee, S., & Khowala, S. (2009). Fungal biotechnology in food and feed processing. *Food Research International*, 42(5-6), 577-587. <https://doi.org/10.1016/j.foodres.2009.02.019>
- Giavasis, I. (2014). Bioactive fungal polysaccharides as potential functional ingredients in food and nutraceuticals. *Current Opinion in Biotechnology*, 26, 162-173. <https://doi.org/10.1016/j.copbio.2014.01.010>
- Gilbert, F. A., & Robinson, R. F. (1957). Food from fungi. *Economic Botany*, 11, 126-145. <https://doi.org/10.1007/BF02985303>

- Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, 48(12), 909-930. <https://doi.org/10.1016/j.plaphy.2010.08.016>
- Girometta, C. (2019). Antimicrobial properties of *Fomitopsis officinalis* in the light of its bioactive metabolites: A review. *Mycology*, 10(1), 32-39. <https://doi.org/10.1080/21501203.2018.1536680>
- Giurini, E. F., Godla, A., & Gupta, K. H. (2024). Redefining bioactive small molecules from microbial metabolites as revolutionary anticancer agents. *Cancer Gene Therapy*, 31(2), 187-206. <https://doi.org/10.1038/s41417-023-00715-x>
- Grácio, D., Magro, F., Lima, R. T., & Máximo, V. (2017). An overview on the role of autophagy in cancer therapy. *Hematology and Medical Oncology*, 2(1), 1-4.
- Grienke, U., Zöll, M., Peintner, U., & Rollinger, J. M. (2014). European medicinal polypores—A modern view on traditional uses. *Journal of Ethnopharmacology*, 154(3), 564-583.
- Grygansky, A. P., Moldavan, M., & Kolotushkina, O. V. (2001). *Hericium erinaceus* (Bull.: Fr.) Pers. extract effect on nerve cells. *International Journal of Medicinal Mushrooms*, 3(2-3), 1-1. <https://doi.org/10.1615/IntJMedMushr.v3.i2-3.690>
- Grygansky, A. P., Moldavan, M., & Kolotushkina, O. V. (2001). *Hericium erinaceus* (Bull.: Fr.) Pers. extract effect on nerve cells. *International Journal of Medicinal Mushrooms*, 3(2-3).
- Gryglewski, R. J., Korbut, R., Robak, J., & Świąż, J. (1987). On the mechanism of antithrombotic action of flavonoids. *Biochemical Pharmacology*, 36(3), 317-322. [https://doi.org/10.1016/0006-2952\(87\)90288-7](https://doi.org/10.1016/0006-2952(87)90288-7)
- Guilder, H. D. V., Vrana, K. E., & Freeman, W. M. (2008). Twenty-five years of quantitative PCR for gene expression analysis. *Biotechniques*, 44(5), 619-626. <https://doi.org/10.2144/000112776>
- Guillamón, E., García-Lafuente, A., Lozano, M., Rostagno, M. A., Villares, A., & Martínez, J. A. (2010). Edible mushrooms: Role in the prevention of cardiovascular diseases. *Fitoterapia*, 81(7), 715-723. <https://doi.org/10.1016/j.fitote.2010.06.005>
- Guo, C., Sun, L., Chen, X., & Zhang, D. (2014). Oxidative stress, mitochondrial damage and neurodegenerative diseases. *Neural Regeneration Research*, 8(21), 2003-2014. <https://doi.org/10.3969/j.issn.1673-5374.2013.21.009>
- Guo, F., Liu, X., Cai, H., & Le, W. (2018). Autophagy in neurodegenerative diseases: Pathogenesis and therapy. *Brain pathology*, 28(1), 3-13. doi: 10.1111/bpa.12545.
- Guo, L. Q., Lin, J. Y., & Lin, J. F. (2007). Non-volatile components of several novel species of edible fungi in China. *Food Chemistry*, 100(2), 643-649. <https://doi.org/10.1016/j.foodchem.2005.09.087>
- Gyamfi, M. A., Yonamine, M., & Aniya, Y. (1999). Free-radical scavenging action of medicinal herbs from Ghana: *Thonningia sanguinea* on experimentally-induced liver injuries. *General Pharmacology: The Vascular System*, 32(6), 661-667. [https://doi.org/10.1016/S0306-3623\(98\)00238-9](https://doi.org/10.1016/S0306-3623(98)00238-9)

References

- Hall, I. (1977). Species and mycorrhizal infections of New Zealand endogonaceae. *Transactions of The British Mycological Society*, 68 (3), 341–356. [https://doi.org/10.1016/S0007-1536\(77\)80186-1](https://doi.org/10.1016/S0007-1536(77)80186-1)
- Halliwell, B. (1996). Antioxidants in human health and disease. *Annual Review of Nutrition*, 16(1), 33-50. <https://doi.org/10.1146/annurev.nu.16.070196.000341>
- Halliwell, B. (2011). Free radicals and antioxidants—quo vadis? *Trends in Pharmacological Sciences*, 32(3), 125-130.
- Halliwell, B. 1997. In: Oxygen Radicals and Disease Process. Thomas, C. E. and Kalyanaraman, B. eds. pp 1-14, Hardwood Academic Publishers, The Netherlands.
- Halliwell, B., & Gutteridge, J. (1984). Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochemical Journal*, 219(1), 1-4. <https://doi.org/10.1042/bj2190001>
- Halliwell, B., & Gutteridge, J. M. (2015). *Free radicals in biology and medicine*. Oxford university press. U. K.
- Hara, M., Hanaoka, T., Kobayashi, M., Otani, T., Adachi, H. Y., Montani, A., Natsukawa, S., Shaura, K., Koizumi, Y., Kasuga, Y., Matsuzawa, T., Ikekawa, T., Sasaki, S., & Tsugane, S. (2003). Cruciferous vegetables, mushrooms, and gastrointestinal cancer risks in a multicenter, hospital-based case-control study in Japan. *Nutrition and Cancer*, 46(2), 138-147. https://doi.org/10.1207/S15327914NC4602_06
- Harborne, A. J. (1998). *Phytochemical methods a guide to modern techniques of plant analysis*. springer science & business media.
- Haripriya, D., Sangeetha, P., Kanchana, A., Balu, M., & Panneerselvam, C. (2005). Modulation of age-associated oxidative DNA damage in rat brain cerebral cortex, striatum and hippocampus by L-carnitine. *Experimental Gerontology*, 40(3), 129-135. <https://doi.org/10.1016/j.exger.2004.10.006>
- Harman, D. (1993). Free radical involvement in aging: Pathophysiology and the therapeutic implications. *Drugs & Aging*, 3(1), 60-80. <https://doi.org/10.2165/00002512-199303010-00006>
- Hasima, N., & Ozpolat, B. (2014). Regulation of autophagy by polyphenolic compounds as a potential therapeutic strategy for cancer. *Cell Death & Disease*, 5(11), 1-13. <https://doi.org/10.1038/cddis.2014.467>
- Hassan, M., Watari, H., AbuAlmaaty, A., Ohba, Y., & Sakuragi, N. (2014). Apoptosis and molecular targeting therapy in cancer. *BioMed Research International*, 2014(1), 1-23. <https://doi.org/10.1155/2020/2451249>
- Hatwalne, M. S. (2012). Free radical scavengers in anaesthesiology and critical care. *Indian Journal of Anaesthesia*, 56(3), 227-233. <https://doi.org/10.4103/0019-5049.98760>
- Hayes, W. (1976). The food value of the cultivated mushroom and its importance to the mushroom industry. *Mushroom Journal*, 40, 104–110.
- Hayes, W. A. (1978). Edible mushrooms. *The Biology and Cultivation of Edible Mushrooms*, 191.

References

- He, X., Wang, X., Fang, J., Chang, Y., Ning, N., Guo, H., Huang, L., Huang, X., & Zhao, Z. (2017). Structures, biological activities, and industrial applications of the polysaccharides from *Hericium erinaceus* (Lion's Mane) mushroom: A review. *International Journal of Biological Macromolecules*, 97, 228-237. <https://doi.org/10.1016/j.ijbiomac.2017.01.040>
- Heleno, S. A., Barros, L., Martins, A., Morales, P., Fernandez-Ruiz, V., Glamoclija, J., Sokovic, M., & Ferreira, I. C. (2015b). Nutritional value, bioactive compounds, antimicrobial activity and bioaccessibility studies with wild edible mushrooms. *LWT-Food Science and Technology*, 63(2), 799-806. <https://doi.org/10.1016/j.lwt.2015.04.028>
- Heleno, S. A., Barros, L., Martins, A., Queiroz, M. J. R., Santos-Buelga, C., & Ferreira, I. C. (2012). Phenolic, polysaccharidic, and lipidic fractions of mushrooms from Northeastern Portugal: Chemical compounds with antioxidant properties. *Journal of Agricultural and Food Chemistry*, 60(18), 4634-4640. <https://doi.org/dx.doi.org/10.1021/jf300739m>
- Heleno, S. A., Barros, L., Sousa, M. J., Martins, A., & Ferreira, I. C. (2010). Tocopherols composition of Portuguese wild mushrooms with antioxidant capacity. *Food Chemistry*, 119(4), 1443-1450. <https://doi.org/10.1016/j.foodchem.2009.09.025>
- Heleno, S. A., Martins, A., Queiroz, M. J. R., & Ferreira, I. C. (2015a). Bioactivity of phenolic acids: Metabolites versus parent compounds: A review. *Food Chemistry*, 173, 501-513. <https://doi.org/10.1016/j.foodchem.2014.10.057>
- Hensley, K., Benaksas, E. J., Bolli, R., Comp, P., Grammas, P., Hamdheydari, L., Mou, S., Pye, Q. N., Stoddard, M. F., Wallis, G., Williamson, K. S., West, M., Wechter, W. J., & Floyd, R. A. (2004). New perspectives on vitamin E: γ -tocopherol and carboxyethylhydroxychroman metabolites in biology and medicine. *Free Radical Biology and Medicine*, 36(1), 1-15. <https://doi.org/10.1016/j.freeradbiomed.2003.10.009>
- Hibbett, D. D., Bauer, R., Binder, M., Giachini, A. J., Hosaka, K., Justo, A., Larsson, E., Larsson, K. H., Lawrey, J. D., Miettinen, O., Nagy, L., Nilsson, R. H., Weiss, M., & Thorn, R. G. (2014). Agaricomycetes. In D. J. McLaughlin & J. W. Spatafora (Eds.), *The mycota: Vol. VII, part A. Systematics and evolution* (pp. 373–429). Springer-Verlag.
- Hisaka, T., Yano, H., Ogasawara, S., Momosaki, S., Nishida, N., Takemoto, Y., ... & Kojiro, M. (2004). Interferon- α Con1 suppresses proliferation of liver cancer cell lines *in vitro* and *in vivo*. *Journal of Hepatology*, 41(5), 782-789. <https://doi.org/10.1016/j.jhep.2004.07.012>
- Hleba, L., Vuković, N., Petrová, J., & Kačániová, M. (2014). Antimicrobial activity of crude methanolic extracts from *Ganoderma lucidum* and *Trametes versicolor*. *Scientific Papers Animal Science and Biotechnologies*, 47(2), 89-89.
- Honegger, R., Axe, L., & Edwards, D. (2013). Bacterial epibionts and endolichenic actinobacteria and fungi in the Lower Devonian lichen *Chlorolichenomycites salopensis*. *Fungal Biology*, 117(7-8), 512-518. <https://doi.org/10.1016/j.funbio.2013.05.003>

- Howes, O. D., Thase, M. E., & Pillinger, T. (2022). Treatment resistance in psychiatry: State of the art and new directions. *Molecular Psychiatry*, 27(1), 58-72. <https://doi.org/10.1038/s41380-021-01200-3>
- Hu, F. B. (2002). Dietary pattern analysis: A new direction in nutritional epidemiology. *Current Opinion in Lipidology*, 13(1), 3-9.
- Hu, W., Fitzgerald, M., Topp, B., Alam, M., & O'Hare, T. J. (2019). A review of biological functions, health benefits, and possible de novo biosynthetic pathway of palmitoleic acid in macadamia nuts. *Journal of Functional Foods*, 62, 1-12. <https://doi.org/10.1016/j.jff.2019.103520>
- Ikekawa, T., Ikeda, Y., Yoshioka, Y., Nakanishi, K., & Yamazaki, E. (1982). Studies on antitumor polysaccharides of *Flammulina velutipes* (Curt. ex Fr.) Sing. II. The structure of EA3 and further purification of EA5. *Journal of Pharmacobio-dynamics*, 5(8), 576-581.
- Ikekawa, T., Saitoh, H., Feng, W., Zhang, H., Li, L., & Matsuzawa, T. (1992). Antitumor activity of *Hypsizigus marmoreus*. I. Antitumor activity of extracts and polysaccharides. *Chemical and Pharmaceutical Bulletin*, 40(7), 1954-1957.
- Ikekawa, T., Uehara, N., Maeda, Y., Nakanishi, M., & Fukuoka, F. (1969). Antitumor activity of aqueous extracts of edible mushrooms. *Cancer Research*, 29(3), 734-735.
- Ishibashi, K. I., Dogasaki, C., Iriki, T., Motoi, M., Kurone, Y. I., Miura, N. N., Adachi, Y., & Ohno, N. (2005). Anti- β -glucan antibody in bovine sera. *International Journal of Medicinal Mushrooms*, 7(4), 513-524.
- Ishibashi, K. I., Miura, N. N., Adachi, Y., Ohno, N., & Yadomae, T. (2001). Relationship between solubility of grifolan, a fungal 1, 3- β -D-glucan, and production of tumor necrosis factor by macrophages *in vitro*. *Bioscience, Biotechnology, and Biochemistry*, 65(9), 1993-2000. <https://doi.org/10.1271/bbb.65.1993>
- Ito, T., Kobayashi, T., Egusa, C., Maeda, T., Abe, N., Okubo, Y., Tsuboi, R., & Niitsuma, T. (2020). A case of food allergy due to three different mushroom species. *Allergology International*, 69(1), 152-153. <https://doi.org/10.1016/j.alit.2019.08.003>
- Iwalokun, B. A., Usen, U. A., Otunba, A. A., & Olukoya, D. K. (2007). Comparative phytochemical evaluation, antimicrobial and antioxidant properties of *Pleurotus ostreatus*. *African Journal of Biotechnology*, 6(15), 1732-1739.
- Jadhav, A. K., & Karuppayil, S. M. (2020). Recent advances in application of fungi and fungal metabolites: Biotechnological interventions and futuristic approaches, chapter 7 -Farnesol: From perfumery to quorum sensing. *New and Future Developments in Microbial Biotechnology and Bioengineering*, Elsevier. pp: 71-77. <http://dx.doi.org/10.1016/B978-0-12-821008-6.00007-4>
- Jakubczyk, D., & Dussart, F. (2020). Selected fungal natural products with antimicrobial properties. *Molecules*, 25(4), 911. doi: 10.3390/molecules25040911.
- Jaworska, G., Pogoń, K., Bernaś, E., & Duda-Chodak, A. (2015). Nutraceuticals and antioxidant activity of prepared for consumption commercial mushrooms *Agaricus bisporus* and *Pleurotus ostreatus*. *Journal of Food Quality*, 38(2), 111-122. <https://doi.org/10.1111/jfq.12132>

- Johnston, N. (2005). Medicinal mushroom cuts off prostate cancer cells' blood supply. *Drug Discovery Today*, 10(23-24), 1584.
- Jong, S. C., & Donovick, R. (1989). Antitumor and antiviral substances from fungi. *Advances in Applied Microbiology*, 34, 183-262. [https://doi.org/10.1016/S0065-2164\(08\)70319-8](https://doi.org/10.1016/S0065-2164(08)70319-8)
- Jordan, M. (1995). Evidence of severe allergic reactions to *Laetiporus sulphureus*. *Mycologist Journal*, 9, 157-158.
- Kalač, P. (2013). A review of chemical composition and nutritional value of wild-growing and cultivated mushrooms. *Journal of the Science of Food and Agriculture*, 93(2), 209-218. <https://doi.org/10.1002/jsfa.5960>
- Kalda, A., Kaasik, A., Vassiljev, V., Pokk, P., & Zharkovsky, A. (2000). Neuroprotective action of group I metabotropic glutamate receptor agonists against oxygen–glucose deprivation-induced neuronal death. *Brain Research*, 853(2), 370-373.
- Kalra, E. K. (2003). Nutraceutical-definition and introduction. *AAPS*, 5(3), 27-28. <https://doi.org/10.1208/ps050325>
- Kamal-Eldin, A., & Appelqvist, L. Å. (1996). The chemistry and antioxidant properties of tocopherols and tocotrienols. *Lipids*, 31(7), 671-701. <https://aocs.onlinelibrary.wiley.com/doi/10.1007/BF02522884>
- Kaneria, M., Rakholiya, K., Bavaliya, K. R., Pandya, M. H., Sipai, T. N., Vadher, S. A., Patel, M., Yadav, V. K., Solanki, R., Patel, S., Sahoo, D. K., & Patel, A. (2024). Untargeted metabolomics-based identification of bioactive compounds from *Mangifera indica* L. seed extracts in drug discovery through molecular docking and assessment of their anticancer potential. *Journal of the Science of Food and Agriculture*, 104(10), 5907-5920. <https://doi.org/10.1002/jsfa.13421>
- Kang, J. H., Jang, J. E., Mishra, S. K., Lee, H. J., Nho, C. W., Shin, D., Jin, M., Kim, K., Choi, C., & Oh, S. H. (2015). Ergosterol peroxide from Chaga mushroom (*Inonotus obliquus*) exhibits anti-cancer activity by down-regulation of the β -catenin pathway in colorectal cancer. *Journal of Ethnopharmacology*, 173, 303-312. <https://doi.org/10.1016/j.jep.2015.07.030>
- Karami, L., Majd, A., Mehrabian, S., Nabiuni, M., Salehi, M., & Irian, S. (2012). Antimutagenic and anticancer effects of *Avicennia marina* leaf extract on *Salmonella typhimurium* TA100 bacterium and human promyelocytic leukaemia HL-60 cells. *Science Asia*, 38(4), 349-355.
- Kasibhatla, S., & Tseng, B. (2003). Why target apoptosis in cancer treatment? *Molecular Cancer Therapeutics*, 2(6), 573-580.
- Kataoka, K., Muta, T., Yamazaki, S., & Takeshige, K. (2002). Activation of macrophages by linear (1 $\rightarrow$ 3)- β -d-glucans: Implications for the recognition of fungi by innate immunity. *Journal of Biological Chemistry*, 277(39), 36825-36831. <https://doi.org/10.1074/jbc.M206756200>
- Kaur, G., Kalia, A., & Sodhi, H. S. (2018). Selenium biofortification of *Pleurotus* species and its effect on yield, phytochemical profiles, and protein chemistry of fruiting bodies. *Journal of Food Biochemistry*, 42(2), 1-7. <https://doi.org/10.1111/jfbc.12467>

References

- Kavishree, S., Hemavathy, J., Lokesh, B. R., Shashirekha, M. N., & Rajarathnam, S. (2008). Fat and fatty acids of Indian edible mushrooms. *Food Chemistry*, 106(2), 597-602. doi: <https://doi.org/10.1016/j.foodchem.2007.06.018>
- Kawakami, Y., Nagai, N., Ohama, K., Zeki, K., Yoshida, Y., Kuroda, E., & Yamashita, U. (2000). Macrophage-colony stimulating factor inhibits the growth of human ovarian cancer cells *in vitro*. *European Journal of Cancer*, 36(15), 1991-1997. [https://doi.org/10.1016/S0959-8049\(00\)00282-3](https://doi.org/10.1016/S0959-8049(00)00282-3)
- Kessler, J. H., Mullauer, F. B., de Roo, G. M., & Medema, J. P. (2007). Broad *in vitro* efficacy of plant-derived betulinic acid against cell lines derived from the most prevalent human cancer types. *Cancer Letters*, 251(1), 132-145. <https://doi.org/10.1016/j.canlet.2006.11.003>
- Keypour, S., Riahi, H., Moradali, M. F., & Rafati, H. (2008). Investigation of the antibacterial activity of a chloroform extract of Lingzhi or Reishi medicinal mushroom, *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst. (Aphyllphoromycetideae), from Iran. *International Journal of Medicinal Mushrooms*, 10(4), 345-349. <https://doi.org/10.1615/IntJMedMushr.v10.i4.70>
- Keys, A. (1995). Mediterranean diet and public health: personal reflections. *The American Journal of Clinical Nutrition*, 61(6), 1321S-1323S. <https://doi.org/10.1093/ajcn/61.6.1321S>
- Khan, A., Ikram, M., Hahm, J. R., & Kim, M. O. (2020). Antioxidant and anti-inflammatory effects of *Citrus* flavonoid hesperetin: Special focus on neurological disorders. *Antioxidants*, 9(7), 1-15. <https://doi.org/doi:10.3390/antiox9070609>
- Khan, M. A., Tania, M., Liu, R., & Rahman, M. M. (2013). *Heridium erinaceus*: An edible mushroom with medicinal values. *Journal of Complementary and Integrative Medicine*, 10(1), 253-258. <https://doi.org/10.1515/jcim-2013-0001>
- Khastini, R. O., Wahyuni, I. & Saraswati, I. (2018). Ethnomycology of bracket fungi in Baduy tribe Indonesia. *Journal of Biology & Biology Education*, 10(2), 423-431.
- Khatua, S., Paul, S., & Acharya, K. (2013). Mushroom as the potential source of new generation of antioxidant: A review. *Research Journal of Pharmacy and Technology*, 6(5), 496-505.
- Kim, B. K., Cho, H. Y., Kim, J. S., Kim, H. W., & Choi, E. C. (1993). Studies on constituents of higher fungi of Korea (LXVIII)-Antitumor components of the cultured mycelia of *Gonoderma lucidum*. *Korean Journal of Pharmacognosy*, 24(3), 203-212.
- Kim, H. J., Chang, W. K., Kim, M. K., Lee, S. S., & Choi, B. Y. (2002). Dietary factors and gastric cancer in Korea: A case-control study. *International Journal of Cancer*, 97(4), 531-535. <https://doi.org/10.1002/ijc.10111>
- Kim, K. A., Shin, D., Kim, J. H., Shin, Y. J., Rajanikant, G. K., Majid, A., Baek, S., & Bae, O. N. (2018). Role of autophagy in endothelial damage and blood-brain barrier disruption in ischemic stroke. *Stroke*, 49(6), 1571-1579.
- Kim, M. Y., Seguin, P., Ahn, J. K., Kim, J. J., Chun, S. C., Kim, E. H., & Chung, I. M. (2008). Phenolic compound concentration and antioxidant activities of edible and medicinal

- mushrooms from Korea. *Journal of Agricultural and Food Chemistry*, 56(16), 7265-7270.
- Kim, S. H., Jakhar, R., & Kang, S. C. (2015). Apoptotic properties of polysaccharide isolated from fruiting bodies of medicinal mushroom *Fomes fomentarius* in human lung carcinoma cell line. *Saudi Journal of Biological Sciences*, 22(4), 484-490. <https://doi.org/10.1016/j.sjbs.2014.11.022>
- Kim, Y. O., Han, S. B., Lee, H. W., Ahn, H. J., Yoon, Y. D., Jung, J. K., Kim, H. M., & Shin, C. S. (2005). Immuno-stimulating effect of the endo-polysaccharide produced by submerged culture of *Inonotus obliquus*. *Life Sciences*, 77(19), 2438-2456. <https://doi.org/10.1016/j.lfs.2005.02.023>.
- Kim, Y. O., Park, H. W., Kim, J. H., Lee, J. Y., Moon, S. H., & Shin, C. S. (2006). Anti-cancer effect and structural characterization of endo-polysaccharide from cultivated mycelia of *Inonotus obliquus*. *Life Sciences*, 79(1), 72-80. <https://doi.org/10.1016/j.lfs.2005.12.047>.
- Kishida, S., Yamamoto, H., Ikeda, S., Kishida, M., Sakamoto, I., Koyama, S., & Kikuchi, A. (1998). Axin, a negative regulator of the Wnt signaling pathway, directly interacts with adenomatous polyposis coli and regulates the stabilization of β -catenin. *Journal of Biological Chemistry*, 273(18), 10823-10826.
- Klis, F. M., de Groot, P., & Hellingwerf, K. (2001). Molecular organization of the cell wall of *Candida albicans*. *Medical Mycology*, 39(1), 1-8. <https://doi.org/10.1080/mmy.39.1.1.8-0>.
- Kobayashi, T., Ruan, S., Clodi, K., Kliche, K. O., Shiku, H., Andreeff, M., & Zhang, W. (1998). Overexpression of Bax gene sensitizes K562 erythroleukemia cells to apoptosis induced by selective chemotherapeutic agents. *Oncogene*, 16(12), 1587-1591. <https://doi.org/10.1038/sj.onc.1201681>
- Koleva, I. I., Van Beek, T. A., Linssen, J. P., Groot, A. D., & Evstatieva, L. N. (2002). Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques*, 13(1), 8-17. <https://doi.org/10.1002/pca.611>
- Kou, R. W., Xia, B., Han, R., Li, Z. Q., Yang, J. R., Yin, X., Gao, Y., & Gao, J. M. (2022). Neuroprotective effects of a new triterpenoid from edible mushroom on oxidative stress and apoptosis through the BDNF/TrkB/ERK/CREB and Nrf2 signalling pathway *in vitro* and *in vivo*. *Food & Function*, 13(23), 12121-12134. <https://doi.org/10.1039/d2fo02854a>
- Krishnamurthy, P., & Wadhwani, A. (2012). Antioxidant enzymes and human health. *Antioxidant Enzyme*, 1, 3-18.
- Król, S. K., Kielbus, M., Rivero-Müller, A., & Stepulak, A. (2015). Comprehensive review on betulin as a potent anticancer agent. *BioMed Research International*, 2015(1), 1-11. <http://dx.doi.org/10.1155/2015/584189>
- Kumar, K. (2015). Role of edible mushrooms as functional foods—A Review. *South Asian Journal of Food Technology and Environment*, 1(3&4), 211-218. <https://doi.org/10.46370/sajfte.2015.v01i03and04.02>

References

- Kumar, M., & Kaviyarasan, V. (1976). Few common poisonous mushrooms of Kolli hills, South India. *Mycotaxon*, 3(3), 363.
- Kumaran, A., & Karunakaran, R. J. (2006). Nitric oxide radical scavenging active components from *Phyllanthus emblica* L. *Plant Foods for Human Nutrition*, 61(1), 1-5.
- Kurutas, E. B. (2015). The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: Current state. *Nutrition Journal*, 15(1), 1-22. <https://doi.org/10.1186/s12937-016-0186-5>
- Kwon, S. H., Lee, H. K., Kim, J. A., Hong, S. I., Kim, H. C., Jo, T. H., Park, Y. I., Lee, C. K., Kim, Y. B., Lee, S. Y., & Jang, C. G. (2010). Neuroprotective effects of chlorogenic acid on scopolamine-induced amnesia via anti-acetylcholinesterase and anti-oxidative activities in mice. *European journal of pharmacology*, 649(1-3), 210-217.
- Lakhanpal, T. N., & Rana, M. (2005). Medicinal and nutraceutical genetic resources of mushrooms. *Plant Genetic Resources*, 3(2), 288-303. <https://doi.org/10.1079/PGR200581>
- Lakshmi, B., Ajith, T. A., Jose, N., & Janardhanan, K. K. (2006). Antimutagenic activity of methanolic extract of *Ganoderma lucidum* and its effect on hepatic damage caused by benzo [a] pyrene. *Journal of Ethnopharmacology*, 107(2), 297-303.
- Lakshmi, B., Tilak, J. C., Adhikari, S., Devasagayam, T. P. A., & Janardhanan, K. K. (2008). Evaluation of antioxidant activity of selected Indian mushrooms. *Pharmaceutical Biology*, 42(3), 179-185. <https://doi.org/10.1080/13880200490514023>
- Lam, K. S. (2007). New aspects of natural products in drug discovery. *Trends in Microbiology*, 15(6), 279-289. <http://dx.doi.org/10.1016/j.tim.2007.04.001>
- Lee, I. K., Kim, Y. S., Jang, Y. W., Jung, J. Y., & Yun, B. S. (2007a). New antioxidant polyphenols from the medicinal mushroom *Inonotus obliquus*. *Bioorganic & Medicinal Chemistry Letters*, 17(24), 6678-6681.
- Lee, T. J., Kim, E. J., Kim, S., Jung, E. M., Park, J. W., Jeong, S. H., Park, S. E., Yoo, Y. H., & Kwon, T. K. (2006). Caspase-dependent and caspase-independent apoptosis induced by evodiamine in human leukemic U937 cells. *Molecular cancer therapeutics*, 5(9), 2398-2407. <https://doi.org/10.1158/1535-7163.MCT-06-0167>
- Lee, T. K. W., Castilho, A., Cheung, V. C. H., Tang, K. H., Ma, S., & Ng, I. O. L. (2011). Lupeol targets liver tumor-initiating cells through phosphatase and tensin homolog modulation. *Hepatology*, 53(1), 160-170. <https://doi.org/10.1002/hep.24000>
- Lee, T. K., Poon, R. T., Wo, J. Y., Ma, S., Guan, X. Y., Myers, J. N., Altevogt, P., & Yuen, A. P. (2007b). Lupeol suppresses cisplatin-induced nuclear factor-κB activation in head and neck squamous cell carcinoma and inhibits local invasion and nodal metastasis in an orthotopic nude mouse model. *Cancer Research*, 67(18), 8800-8809. <https://doi.org/10.1158/0008-5472.CAN-07-0801>
- Lee, W., Fujihashi, A., Govindarajulu, M., Ramesh, S., Deruiter, J., Majrashi, M., Almaghrabi, M., Nadar, R. M., Moore, T., Agrawal, D. C., & Dhanasekaran, M. (2019). Role of mushrooms in neurodegenerative diseases. *Medicinal Mushrooms: Recent Progress in Research and Development*, 223-249.

- Leeser, O., 1987. Lehrbuch der Homöopathie. 1973 Karl F. Haug Verlag, Heidelberg.
- Lemieszek, M., & Rzeski, W. (2012). Anticancer properties of polysaccharides isolated from fungi of the Basidiomycetes class. *Contemporary Oncology/Współczesna Onkologia*, 16(4), 285-289. <https://doi.org/10.5114/wo.2012.30055>
- Li, J. W. H., & Vederas, J. C. (2009). Drug discovery and natural products: *End of an era or an endless frontier*. *Science*, 325(5937), 161-165.
- Li, Q., Wu, D., Zhang, L., & Zhang, Y. (2010b). Effects of galantamine on β -amyloid release and beta-site cleaving enzyme 1 expression in differentiated human neuroblastoma SH-SY5Y cells. *Experimental Gerontology*, 45(11), 842-847.
- Li, W., Khor, T. O., Xu, C., Shen, G., Jeong, W. S., Yu, S., & Kong, A. N. (2008). Activation of Nrf2-antioxidant signaling attenuates NF κ B-inflammatory response and elicits apoptosis. *Biochemical Pharmacology*, 76(11), 1485-1489. <https://doi.org/10.1016/j.bcp.2008.07.017>
- Li, Y. L., Gan, G. P., Zhang, H. Z., Wu, H. Z., Li, C. L., Huang, Y. P., & Liu, J. W. (2007). A flavonoid glycoside isolated from Smilax china L. rhizome in vitro anticancer effects on human cancer cell lines. *Journal of ethnopharmacology*, 113(1), 115-124.
- Li, Y., Cozzi, P. J., Qu, C. F., Zhang, D. Y., Rizvi, S. M. A., Raja, C., & Allen, B. J. (2004). Cytotoxicity of human prostate cancer cell lines *in vitro* and induction of apoptosis using ²¹³Bi-Herceptin α -conjugate. *Cancer Letters*, 205(2), 161-171. <https://doi.org/10.1016/j.canlet.2003.10.035>
- Li, Y., He, K., Huang, Y., Zheng, D., Gao, C., Cui, L., & Jin, Y. H. (2010a). Betulin induces mitochondrial cytochrome C release associated apoptosis in human cancer cells. *Molecular Carcinogenesis*, 49(7), 630-640. <https://doi.org/10.1002/mc.20638>
- Liew, G. M., Khong, H. Y., Kutoi, C. J., & Sayok, A. K. (2015). Phytochemical screening, antimicrobial and antioxidant activities of selected fungi from Mount Singai, Sarawak, Malaysia. *International Journal of Research Studies in Biosciences*, 3(1), 191-197.
- Lin, C. H., Chang, C. Y., Lee, K. R., Lin, H. J., Lin, W. C., Chen, T. H., & Wan, L. (2016). Cold-water extracts of *Grifola frondosa* and its purified active fraction inhibit hepatocellular carcinoma *in vitro* and *in vivo*. *Experimental Biology and Medicine*, 241(13), 1374-1385. <https://doi.org/10.1177/1535370216640149>
- Lin, J. Y., Chen, M. L., Chiang, B. L., & Lin, B. F. (2006). *Ganoderma tsugae* supplementation alleviates bronchoalveolar inflammation in an airway sensitization and challenge mouse model. *International Immunopharmacology*, 6(2), 241-251.
- Lindequist, U., Niedermeyer, T. H., & Jülich, W. D. (2005). The pharmacological potential of mushrooms. *Evidence-Based Complementary and Alternative Medicine*, 2(3), 285-299. doi:10.1093/ecam/neh107
- Lipworth, L., Martínez, M. E., Angell, J., Hsieh, C. C., & Trichopoulos, D. (1997). Olive oil and human cancer: An assessment of the evidence. *Preventive Medicine*, 26(2), 181-190.

References

- Liu, B., Zhong, M., Lun, Y., Wang, X., Sun, W., Li, X., Ning, A., Cao, J., Zhang, W., Liu, L., & Huang, M. (2012). A novel apoptosis correlated molecule: Expression and characterization of protein latcripin-1 from *Lentinula edodes* C91-3. *International Journal of Molecular Sciences*, 13(5), 6246-6265. <https://doi.org/10.3390/ijms13056246>
- Liu, H. M., Cheng, J., Wang, X. Y., Jiang, Y., Ni, J., Zhang, Y., & Wang, W. (2023). Structure identification of *Ganoderma lucidum* spore polysaccharides and their antitumor activity in vivo. *Molecules*, 29(10), 2348.
- Liu, J., Jia, L., Kan, J., & Jin, C. H. (2013). *In vitro* and *in vivo* antioxidant activity of ethanolic extract of white button mushroom (*Agaricus bisporus*). *Food and Chemical Toxicology*, 51, 310-316.
- Liu, P. F., Hu, Y. C., Kang, B. H., Tseng, Y. K., Wu, P. C., Liang, C. C., Hou, Y. Y., Fu, T. Y., Liou, H. H., Hsieh, I. C., Ger, L. P., & Shu, C. W. (2017). Expression levels of cleaved caspase-3 and caspase-3 in tumorigenesis and prognosis of oral tongue squamous cell carcinoma. *PLoS One*, 12(7), 1-14. <https://doi.org/10.1371/journal.pone.0180620>
- Liu, X. H., Connolly, J. M., & Rose, D. P. (1996). The 12-lipoxygenase gene-transfected MCF-7 human breast cancer cell line exhibits estrogen-independent, but estrogen and omega-6 fatty acid-stimulated proliferation *in vitro*, and enhanced growth in athymic nude mice. *Cancer Letters*, 109(1-2), 223-230. [https://doi.org/10.1016/S0304-3835\(96\)04462-X](https://doi.org/10.1016/S0304-3835(96)04462-X)
- Lorenzen, K., & Anke, T. (1998). Basidiomycetes as a source for new bioactive natural products. *Current Organic Chemistry*, 2(4), 329-364.
- Malini, I. P., Kanchana, G., & Rajadurai, M. (2011). Antiperoxidative and antioxidant effect of ellagic acid on normal and streptozotocin induced diabetes in albino wistarrats. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 2(3), 24-34.
- Manikandan, K. (2011). Nutritional and Medicinal Values of Mushrooms. In M. Singh, B. Vijay, S. Kamal, & G. C. Wakchaure (Eds.), *Mushrooms Cultivation, Marketing and Consumption* (pp. 11-14). Solan: Directorate of Mushroom Research.
- Manzi, P., Aguzzi, A., & Pizzoferrato, L. (2001). Nutritional value of mushrooms widely consumed in Italy. *Food Chemistry*, 73(3), 321-325. [https://doi.org/10.1016/S0308-8146\(00\)00304-6](https://doi.org/10.1016/S0308-8146(00)00304-6)
- Manzi, P., Gambelli, L., Marconi, S., Vivanti, V., & Pizzoferrato, L. (1999). Nutrients in edible mushrooms: an inter-species comparative study. *Food Chemistry*, 65(4), 477-482. [https://doi.org/10.1016/S0308-8146\(98\)00212-X](https://doi.org/10.1016/S0308-8146(98)00212-X)
- Martin, S. J., & Green, D. R. (1995). Protease activation during apoptosis: death by a thousand cuts? *Cell*, 82(3), 349-352.
- Masyita, A., Sari, R. M., Astuti, A. D., Yasir, B., Rumata, N. R., Emran, T. B., Nainu, F., & Simal-Gandara, J. (2022). Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives. *Food Chemistry: X*, 13, 1-14. <https://doi.org/10.1016/j.fochx.2022.100217>

- Mau, J. L., Lin, H. C., & Song, S. F. (2002). Antioxidant properties of several specialty mushrooms. *Food Research International*, 35(6), 519-526. [https://doi.org/10.1016/S0963-9969\(01\)00150-8](https://doi.org/10.1016/S0963-9969(01)00150-8)
- McIntosh, M., Stone, B. A., & Stanisich, V. A. (2005). Curdlan and other bacterial (1→3)-β-D-glucans. *Applied Microbiology and Biotechnology*, 68(2), 163-173. <https://doi.org/10.1007/s00253-005-1959-5>
- Meade, E., Hehir, S., Rowan, N., & Garvey, M. (2022). Mycotherapy: Potential of fungal bioactives for the treatment of mental health disorders and morbidities of chronic pain. *Journal of Fungi*, 8(3), 290. <https://doi.org/10.3390/jof8030290>
- Meek, P. D., McKeithan, E. K., & Schumock, G. T. (1998). Economic considerations in Alzheimer's disease. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, 18(2P2), 68-73. <https://doi.org/10.1002/j.1875-9114.1998.tb03880.x>
- Menaga, D., Rajakumar, S., & Ayyasamy, P. M. (2013). Free radical scavenging activity of methanolic extract of *Pleurotus florida* mushroom. *International Journal of Pharmacy and Pharmaceutical Sciences*, 5(4), 601-606.
- Miles, P. G., & Chang, S. T. (1997). *Mushroom biology: concise basics and current developments*. World Scientific.
- Miyazawa, M., Iwahashi, M., Ojima, T., Katsuda, M., Nakamura, M., Nakamori, M., Ueda, K., Naka, K., Hayata, K., Lida, T., & Yamaue, H. (2011). Dendritic cells adenovirally-transduced with full-length mesothelin cDNA elicit mesothelin-specific cytotoxicity against pancreatic cancer cell lines *in vitro*. *Cancer Letters*, 305(1), 32-39. <https://doi.org/10.1016/j.canlet.2011.02.013>
- Mizuno, M., Minato, K. I., Ito, H., Kawade, M., Terai, H., & Tsuchida, H. (1999). Anti-tumor polysaccharide from the mycelium of liquid-cultured *Agaricus blazei* Mill. *Biochemistry and Molecular Biology International*, 47(4), 707-714. <https://doi.org/10.1080/15216549900201773>
- Mizuno, T. (1999). The extraction and development of antitumor-active polysaccharides from medicinal mushrooms in Japan. *International Journal of Medicinal Mushrooms*, 1(1), 9-29.
- Mizuno, T., Saito, H., Nishitoba, T., & Kawagishi, H. (1995). Antitumor-active substances from mushrooms. *Food Reviews International*, 11(1), 23-61. <https://doi.org/10.1080/87559129509541018>
- Mizuno, T., Yokohui, P., Kinoshita, T., Zhuang, C., Ito, H., & Mayuzumi, Y. (1996). Antitumor activity and chemical modification of polysaccharides from niohshimeji mushroom, *Tricholma giganteum*. *Bioscience, Biotechnology, and Biochemistry*, 60(1), 30-33. <https://doi.org/10.1271/bbb.60.30>
- Mohammadifar, S., Fallahi Gharaghoz, S., Asef Shayan, M. R., & Vaziri, A. (2020). Comparison between antioxidant activity and bioactive compounds of *Ganoderma applanatum* (Pers.) Pat. and *Ganoderma lucidum* (Curt.) P. Karst from Iran. *Iranian Journal of Plant Physiology*, 11(1), 3417-3424.
- Moldavan MG, Grodzinskaya AA, Wasser SP, Storozhuk VM, Grygansky AP. (2002). Neurotropic effect of the edible and medicinal mushroom *Lentinus edodes* (Berk.)

References

- Sing. extracts on the neurons of hippocampal slices in rats. *International Journal of Medicinal Mushrooms*, 4(1), 43–48. <https://doi.org/10.1615/IntJMedMushr.v4.i1.50>
- Moldavan MG, Grygansky AP, Kirchoff B. *Hericium erinaceus* (Bull.: Fr.) Pers. extracts effect on the neuron's impulse activity in stratum pyramidale of zone CA1 hippocampal slices in rats. Third International Conference on Mushroom Biology & Mushroom Products; 1999a Oct 12–16; Sydney, Australia.
- Moldavan, M., Grodzinskaya, A. A., & Storozhuk, V. M. (2000a). Activation of hippocampal pyramidal neurons N-methyl-D-aspartate receptors and M-cholinoreceptors during the application of *Amanita* (Pers.: Fr.) Hook. species extracts. *International Journal of Medicinal Mushrooms*, 2(1), 55–61. <https://doi.org/10.1615/IntJMedMushr.v2.i1.60>
- Moldavan, M., Grodzinskaya, A. A., Solomko, E. F., & Storozhuk, V. M. (2001b). Higher Basidiomycetes mushroom extract effects on nerve cell spike activity *in vitro*. *International Journal of Medicinal Mushrooms*, 3(2-3), 183–183. <https://doi.org/10.1615/IntJMedMushr.v3.i2-3.1000>
- Moldavan, M., Grodzinskaya, A. A., Storozhuk, V. M., & Grygansky, A. P. (2001a). Neurotropic effect of the edible and medicinal mushroom *Lentinus edodes* (Berk.) Sing. extracts on hippocampal slices in rats. *International Journal of Medicinal Mushrooms*, 3(2-3), 1–1. <https://doi.org/10.1615/IntJMedMushr.v3.i2-3.690>
- Moldavan, M., Grodzinskaya, A. A., Wasser, S. P., & Storozhuk, V. M. (1999b). Effects of *Amanita* species extracts on neuron responses in the rat hippocampal stratum pyramidale (CA1 region) *in vitro*. *International Journal of Medicinal Mushrooms*, 1(4), 337–344. <https://doi.org/10.1615/IntJMedMushr.v1.i4.60>
- Moldavan, M., Grodzinskaya, A. A., Wasser, S. P., & Storozhuk, V. M. (1999a). Effects of higher Basidiomycetes mushrooms on the hippocampal slices in rats. *International Journal of Medicinal Mushrooms*, 1(2), 173–80. <https://doi.org/10.1615/IntJMedMushrooms.v1.i2.70>
- Moldavan, M., Solomko, E. F., Grodzinskaya, A. A., Storozhuk, V. M., & Lomberg, M. L. (2000b). Neurotropic effect of extracts from the hallucinogenic mushroom *Psilocybe cubensis* (Earle) Sing. (Agaricomycetidae). *In vitro* studies. *International Journal of Medicinal Mushrooms*, 2(4), 329–338. <https://doi.org/10.1615/IntJMedMushr.v2.i4.130>
- Mondal, A., Banerjee, D., Majumder, R., Maity, T. K., & Khowala, S. (2016). Evaluation of *in vitro* antioxidant, anticancer and *in vivo* antitumour activity of *Termitomyces clypeatus* MTCC 5091. *Pharmaceutical Biology*, 54(11), 2536–2546. <https://doi.org/10.3109/13880209.2016.1168854>
- Moonjely, S. (2022). Fungi: Essential Elements in the Ecosystems. In: Frías-De-León, M. G., Brunner-Mendoza, C., Reyes-Montes, M.R. & Duarte-Escalante, E. (eds), *The Impact of Climate Change on Fungal Diseases*. (pp: 19–35) Fungal Biology. Springer, Cham. <https://doi.org/10.1007/978-3-030-89664-5-2>
- Moore, D., & Chiu, S. W. (2001). Fungal products as food. Bio-exploitation of filamentous fungi. *Fungal Diversity Press*, Hong Kong, 223–251.

- Morris, H. J., Llauradó, G., Beltrán, Y., Lebeque, Y., Bermúdez, R. C., García, N., Gaime-Perraud, I., & Moukha, S. (2016). The use of mushrooms in the development of functional foods, drugs, and nutraceuticals. In: I. C. F. R. Ferreira, P. Morales and L. Barros (eds), *Wild plants, mushrooms and nuts: Functional food properties and applications*, (pp:123-157). John Wiley & Sons, Ltd. <https://doi.org/10.1002/9781118944653>
- Morrissey, C., O'Neill, A., Spengler, B., Christoffel, V., Fitzpatrick, J. M., & Watson, R. W. G. (2005). Apigenin drives the production of reactive oxygen species and initiates a mitochondrial mediated cell death pathway in prostate epithelial cells. *The Prostate*, 63(2), 131-142. <https://doi.org/10.1002/pros.20167>
- Mothana, R. A., Jansen, R., Jülich, W. D., & Lindequist, U. (2000). Ganomycins A and B, new antimicrobial farnesyl hydroquinones from the basidiomycete *Ganoderma pfeifferi*. *Journal of Natural Products*, 63(3), 416-418.
- Mullauer, F. B., Kessler, J. H., & Medema, J. P. (2009). Betulin is a potent anti-tumor agent that is enhanced by cholesterol. *PloS One*, 4(4), 1-8. <https://doi.org/10.1371/journal.pone.0005361>
- Muller, F. L., Liu, Y., & Van Remmen, H. (2004). Complex III releases superoxide to both sides of the inner mitochondrial membrane. *Journal of Biological Chemistry*, 279(47), 49064-49073. <https://doi.org/10.1074/jbc.M407715200>
- Murugesan, S. (2017). Sustainable food security: edible and medicinal mushroom. In *Sustainable Agriculture towards Food Security* (pp. 185-196). Singapore: Springer Singapore. https://doi.org/10.1007/978-981-10-6647-4_11
- Muszyńska, B., Grzywacz-Kisielewska, A., Kała, K., & Gdula-Argasińska, J. (2018). Anti-inflammatory properties of edible mushrooms: A review. *Food Chemistry*, 243, 373-381. <http://dx.doi.org/10.1016/j.foodchem.2017.09.149>
- Nabavi, S. F., Nabavi, S. M., Habtemariam, S., Moghaddam, A. H., Sureda, A., Jafari, M., & Latifi, A. M. (2013). Hepatoprotective effect of gallic acid isolated from *Peltiphyllum peltatum* against sodium fluoride-induced oxidative stress. *Industrial Crops and Products*, 44, 50-55.
- Nefic, H., Musanovic, J., Metovic, A., & Kurteshi, K. (2013). Chromosomal and nuclear alterations in root tip cells of *Allium cepa* L. induced by alprazolam. *Medical Archives*, 67(6), 388-392. <https://doi.org/10.5455/medarh.2013.67.388-392>
- Nguyen, H. P., Kimaru, I. W. (2014). GC/MS analysis of an herbal medicinal remedy to identify potential toxic compounds. *Advances in Pharmaceutical Analysis*, 12(4), 14-18.
- Niazi, A. R., & Ghafoor, A. (2021). Different ways to exploit mushrooms: A review. *All Life*, 14(1), 450-460.
- Nimmo, J. T., Kelly, L., Verma, A., Carare, R. O., Nicoll, J. A., & Dodart, J. C. (2021). Amyloid- β and α -synuclein immunotherapy: From experimental studies to clinical trials. *Frontiers in Neuroscience*, 15, 1-25. <https://doi.org/10.3389/fnins.2021.733857>

- Niu, J. F., Fang, Z., Wang, H. J., & Wang, G. C. (2002). The research advance on the effective constituents of *Ganoderma* spp. *Journal of Agricultural University Hebei*, 25, 51-54.
- Nordberg, J., & Arnér, E. S. (2001). Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free Radical Biology and Medicine*, 31(11), 1287-1312.
- Nowell, P. C. (1986). Mechanisms of tumor progression. *Cancer Res*, 46(5), 2203-2207.
- Oda, S. S., & El-Ashmawy, I. M. (2012). Protective effect of silymarin on mercury-induced acute nephro-hepatotoxicity in rats. *Global Veterinaria*, 9(4), 376-383.
- Oh, J. Y., Baek, Y. M., Kim, S. W., Hwang, H. J., Hwang, H. S., Lee, S. H., & Yun, J. W. (2008). Apoptosis of human hepatocarcinoma (HepG2) and neuroblastoma (SKN-SH) cells induced by polysaccharides-peptide complexes produced by submerged mycelial culture of an entomopathogenic fungus *Cordyceps sphecocephala*. *Journal of Microbiology and Biotechnology*, 18(3), 512-519.
- Oh, S. H., Choi, J. E., & Lim, S. C. (2006). Protection of betulin against cadmium-induced apoptosis in hepatoma cells. *Toxicology*, 220(1), 1-12. <https://doi.org/10.1016/j.tox.2005.08.025>
- Okan, O. T., Yıldız, S., Yılmaz, A., Barutçıyan, J., & Deniz, I. (2013). Wild edible mushrooms having an important potential in East Black Sea Region. In *International Caucasian forestry symposium* (pp. 24-26). Artvin, Turkey.
- Oliveira, J. S. D., Bezerra, D. P., de Freitas, C. D. T., Marinho Filho, J. D. B., de Moraes, M. O., Pessoa, C., Costa-Lotufo, L. V., & Ramos, M. V. (2007). *In vitro* cytotoxicity against different human cancer cell lines of laticifer proteins of *Calotropis procera* (Ait.) R. Br. *Toxicology In Vitro*, 21(8), 1563-1573. <https://doi.org/10.1016/j.tiv.2007.05.007>
- Oltval, Z. N., Milliman, C. L., & Korsmeyer, S. J. (1993). Bcl-2 heterodimerizes *in vivo* with a conserved homolog, Bax, that accelerates programmed cell death. *Cell*, 74(4), 609-619.
- Otsubo, A., Bhawal, U. K., Nomura, Y., Mitani, Y., Ozawa, K., Kuniyasu, H., & Sugiyama, M. (2007). UCN-01 (7-hydroxystaurosporine) induces apoptosis and G1 arrest of both primary and metastatic oral cancer cell lines *in vitro*. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, 103(3), 391-397.
- Ouyang, F., Wang, G., Guo, W., Zhang, Y., Xiang, W., & Zhao, M. (2013). AKT signaling and mitochondrial pathways are involved in mushroom polysaccharide-induced apoptosis and G1 or S phase arrest in human hepatoma cells. *Food Chemistry*, 138(4), 2130-2139. <https://doi.org/10.1016/j.foodchem.2012.10.047>
- Öztürk, C., Kaşık, G., DOĞAN, H. H., & Aktaş, S. (2003). Macrofungi of Alanya district. *Turkish Journal of Botany*, 27(4), 303-312.
- Öztürk, M., Tel-Çayan, G., Muhammad, A., Terzioğlu, P., & Duru, M. E. (2015). Mushrooms: a source of exciting bioactive compounds. *Studies in natural products chemistry*, 45, 363-456. <http://dx.doi.org/10.1016/B978-0-444-63473-3.00001-0>

References

- Padma, V. V. (2015). An overview of targeted cancer therapy. *BioMedicine*, 5(4), 1-6. <https://doi.org/10.7603/s40681-015-0019-4>
- Padma, V. V., Sowmya, P., Felix, T. A., Baskaran, R., & Poornima, P. (2011). Protective effect of gallic acid against lindane induced toxicity in experimental rats. *Food and Chemical Toxicology*, 49(4), 991-998.
- Pandey, K. B., & Rizvi, S. I. (2010). Markers of oxidative stress in erythrocytes and plasma during aging in humans. *Oxidative medicine and cellular longevity*, 3(1), 2-12.
- Pankavec, S., Hanc, A., Baralkiewicz, D., Dryzalowska, A., Zhang, J., & Falandysz, J. (2019). Mineral constituents of conserved white button mushrooms: similarities and differences. *Roczniki Państwowego Zakładu Higieny*, 70(1), 15–25. <https://doi.org/10.32394/rpzh.2019.0051>
- Panneerselvam, A., Nadimuthu, N., Ambikapathy, V., Murugan, R., & Gopinathan, M. (2009). Hand book on edible mushroom cultivation. *KP Print. Thanjavur, I*, 71-99.
- Park, C., Jeong, J. W., Han, M. H., Lee, H., Kim, G. Y., Jin, S., Park, J. H., Kwon, H. J., Kim, B. W., & Choi, Y. H. (2021). The anti-cancer effect of betulinic acid in u937 human leukemia cells is mediated through ROS-dependent cell cycle arrest and apoptosis. *Animal Cells and Systems*, 25(2), 119-127. <https://doi.org/10.1080/19768354.2021.1915380>
- Park, C., Moon, D. O., Rhu, C. H., Choi, B. T., Lee, W. H., Kim, G. Y., & Choi, Y. H. (2007). β -Sitosterol induces anti-proliferation and apoptosis in human leukemic U937 cells through activation of caspase-3 and induction of Bax/Bcl-2 ratio. *Biological and Pharmaceutical Bulletin*, 30(7), 1317-1323. <https://doi.org/10.1248/bpb.30.1317>
- Parkash, V., & Sharma, A. (2016). *In vitro* efficacy of bracket fungi for their potential antimicrobial activity. *The Journal of Microbiology, Biotechnology and Food Sciences*, 6(2), 818-812. <https://doi.org/10.15414/jmbfs.2016.6.2.818-822>
- Pastor, N., Weinstein, H., Jamison, E., & Brenowitz, M. (2000). A detailed interpretation of OH radical footprints in a TBP-DNA complex reveals the role of dynamics in the mechanism of sequence-specific binding. *Journal of Molecular Biology*, 304(1), 55-68. <https://doi.org/10.1006/jmbi.2000.4173>
- Patel, F., & Mandal, P. (2019). Neurodegenerative diseases and their therapeutic approaches. *Neurons Dendrites and Axons*.
- Paterson, R. R. M. (2006). Ganoderma—a therapeutic fungal biofactory. *Phytochemistry*, 67(18), 1985-2001. <https://doi.org/10.1016/j.phytochem.2006.07.004>
- Peralta, R. M., da Silva, B. P., Côrrea, R. C. G., Kato, C. G., Seixas, F. A. V., & Bracht, A. (2017). Enzymes from Basidiomycetes—Peculiar and efficient tools for biotechnology. In: *Biotechnology of microbial enzymes* (pp. 119-149). Academic Press.
- Perry, G., Nunomura, A., Hirai, K., Takeda, A., Aliev, G., & Smith, M. A. (2000). Oxidative damage in Alzheimer's disease: The metabolic dimension. *International Journal of Developmental Neuroscience*, 18(4-5), 417-421. [https://doi.org/10.1016/S0736-5748\(00\)00006-X](https://doi.org/10.1016/S0736-5748(00)00006-X)

- Petrelli, F., Borgonovo, K., Cabiddu, M., Lonati, V., & Barni, S. (2012). Relationship between skin rash and outcome in non-small-cell lung cancer patients treated with anti-EGFR tyrosine kinase inhibitors: a literature-based meta-analysis of 24 trials. *Lung Cancer*, 78(1), 8-15. <https://doi.org/10.1016/j.lungcan.2012.06.009>
- Petrova, R. D. (2012). New scientific approaches to cancer treatment: Can Medicinal Mushrooms defeat the curse of the century? *International journal of medicinal mushrooms*, 14(1), 1-20. <https://doi.org/10.1615/IntJMedMushr.v14.i1.10>
- Phan, C. W., David, P., & Sabaratnam, V. (2017). Edible and medicinal mushrooms: Emerging brain food for the mitigation of neurodegenerative diseases. *Journal of Medicinal Food*, 20(1), 1–10. <https://doi.org/10.1089/jmf.2016.3740>
- Phillips, K. M., Ruggio, D. M., Horst, R. L., Minor, B., Simon, R. R., Feeney, M. J., Byrdwell, W. C., & Haytowitz, D. B. (2011). Vitamin D and sterol composition of 10 types of mushrooms from retail suppliers in the United States. *Journal of Agricultural and Food Chemistry*, 59(14), 7841-7853. [dx.doi.org/10.1021/jf104246z](https://doi.org/10.1021/jf104246z)
- Pinheiro, A. C., Nunes, I. J., Ferreira, W. V., Tomasini, P. P., Trindade, C., Martins, C. C., Wilhelm, E. A., Oliboni, R. S., Netz, P. A., Stieler, R., Casagrande, O. L., & Saffi, J. (2023). Antioxidant and anticancer potential of the new Cu (II) complexes bearing imine-phenolate ligands with pendant amine N-donor groups. *Pharmaceutics*, 15(2), 376.
- Place, A. E., Jin Huh, S., & Polyak, K. (2011). The microenvironment in breast cancer progression: Biology and implications for treatment. *Breast Cancer Research*, 13(6), 227. <https://doi.org/10.1186/bcr2912>
- Pleszczyńska, M., Lemieszek, M. K., Siwulski, M., Wiater, A., Rzeski, W., & Szczodrak, J. (2017). *Fomitopsis betulina* (formerly *Piptoporus betulinus*): The Iceman's polypore fungus with modern biotechnological potential. *World Journal of Microbiology and Biotechnology*, 33(5), 83. <https://doi.org/10.1007/s11274-017-2247-0>
- Pohl, H. R., Roney, N., & Abadin, H. G. (2011). Metal ions affecting the neurological system. *Metal Ions in Life Sciences*, 8, 247–262.
- Polkowski, K., Popiołkiewicz, J., Krzeczyński, P., Ramza, J., Pucko, W., Zegrocka-Stendel, O., Boryski, J., Skierski, J. S., Mazurek, A. P., & Gryniewicz, G. (2004). Cytostatic and cytotoxic activity of synthetic genistein glycosides against human cancer cell lines. *Cancer Letters*, 203(1), 59-69. <https://doi.org/10.1016/j.canlet.2003.08.023>
- Prusiner, S. B. (2012). A unifying role for prions in neurodegenerative diseases. *Science*, 336(6088), 1511-1513. <https://doi.org/10.1126/science.1222951>
- Pryor, W. A., Lightsey, J. W., & Prier, D. G. (1982). The production of free radicals *in vivo* from the action of xenobiotics: the initiation of auto-oxidation of polyunsaturated fatty acids by nitrogen dioxide and ozone. *Lipid peroxides in Biology and Medicine*, 1-22. New York: Academic Press.
- Pushpa, H., & Purushothoma, K. B. (2010). Nutritional analysis of wild and cultivated edible medicinal mushrooms. *World Journal of Dairy and Food Sciences*, 5(2), 140-144.
- Quereshi, S., Pandey, A. K., & Sandhu, S. S. (2010). Evaluation of antibacterial activity of different *Ganoderma lucidum* extracts. *Journal of Scientific Research*, 3(1), 9-13.

- Rai, M., Biswas, G., Mandal, S. C., & Acharya, K. (2011). Free radicals and human diseases. *Herbal Drugs: A modern approach to understand them better*. Kolkata, India: New Central Book Agency (P) Ltd, 479-96.
- Rajakumar, K., Greenspan, S. L., Thomas, S. B., & Holick, M. F. (2007). Solar ultraviolet radiation and vitamin D: a historical perspective. *American Journal of Public Health*, 97(10), 1746-1754. <https://doi.org/10.2105/AJPH.2006.091736>
- Rajarathnam, S., Shashirekha, M. N. J., & Bano, Z. (1998). Biodegradative and biosynthetic capacities of mushrooms: Present and future strategies. *Critical Reviews in Biotechnology*, 18(2-3), 91-236. <https://doi.org/10.1080/0738-859891224220>
- Rathee, S., Rathee, D., Rathee, D., Kumar, V., & Rathee, P. (2012). Mushrooms as therapeutic agents. *Revista Brasileira de Farmacognosia*, 22, 459-474.
- Rathore, H., Prasad, S., & Sharma, S. (2017). Mushroom nutraceuticals for improved nutrition and better human health: A review. *PharmaNutrition*, 5(2), 35-46. <https://doi.org/10.1016/j.phanu.2017.02.001>
- Ray, G., & Husain, S. A. (2002). Oxidants, antioxidants and carcinogenesis. *Indian Journal of Experimental Biology*, 40(11), 1213-1232.
- Ray, S. K. (2020). Modulation of autophagy for neuroprotection and functional recovery in traumatic spinal cord injury. *Neural Regeneration Research*, 15(9), 1601-1612. [doi.10.4103/1673-5374.276322](https://doi.org/10.4103/1673-5374.276322).
- Reddy, P. H., & Beal, M. F. (2005). Are mitochondria critical in the pathogenesis of Alzheimer's disease? *Brain Research Reviews*, 49(3), 618-632. <https://doi.org/10.1016/j.brainresrev.2005.03.004>
- Reis, F. S., Barros, L., Martins, A., & Ferreira, I. C. (2012). Chemical composition and nutritional value of the most widely appreciated cultivated mushrooms: An inter-species comparative study. *Food and Chemical Toxicology*, 50(2), 191-197. <https://doi.org/10.1016/j.fct.2011.10.056>
- Reis, F. S., Martins, A., Vasconcelos, M. H., Morales, P., & Ferreira, I. C. (2017). Functional foods based on extracts or compounds derived from mushrooms. *Trends in Food Science & Technology*, 66, 48-62. <https://doi.org/10.1016/j.tifs.2017.05.010>
- Reiter, R. J. (1995). Oxidative processes and antioxidative defence mechanisms in the aging brain. *FASEB J*, 9: 526-536. Proceedings of the 8th International Conference on Mushroom Biology and Mushroom Products (ICMBMP8). 9(7). 526-533. <https://doi.org/10.1096/fasebj.9.7.7737461>
- Reshetnikov, S. V., & Tan, K. K. (2001). Higher Basidiomycota as a source of antitumor and immunostimulating polysaccharides. *International Journal of Medicinal Mushrooms*, 3(4), 361-394. <https://doi.org/10.1615/IntJMedMushr.v3.i4.80>
- Ribble, D., Goldstein, N. B., Norris, D. A., & Shellman, Y. G. (2005). A simple technique for quantifying apoptosis in 96-well plates. *BMC biotechnology*, 5(1), 5-12. <https://doi.org/10.1186/1472-6750-5-12>

- Ribeiro, B., Valentao, P., Baptista, P., Seabra, R. M., & Andrade, P. B. (2007). Phenolic compounds, organic acids profiles and anti-oxidative properties of beefsteak fungus (*Fistulina hepatica*). *Food and Chemical Toxicology*, 45, 1805-1813.
- Rosen, K. M., Moussa, C. E. H., Lee, H. K., Kumar, P., Kitada, T., Qin, G., Fu, G., & Querfurth, H. W. (2010). Parkin reverses intracellular β -amyloid accumulation and its negative effects on proteasome function. *Journal of Neuroscience Research*, 88(1), 167-178. <https://doi.org/10.1002/jnr.22178>
- Rowe, J. W., Fulmer, T., & Fried, L. (2016). Preparing for better health and health care for an aging population. *Jama*, 316(16), 1643-1644. <https://doi.org/10.1001/jama.2016.12335>
- Roy, A., Ahuja, S., & Garg, S. (2021). Fungal secondary metabolites: Biological activity and potential applications. In *Recent Trends in Mycological Research: Volume 1: Agricultural and Medical Perspective* (pp. 159-188). Cham: Springer International Publishing. doi: 10.1007/978-3-030-60659-6_7.
- Rózsa, S., Măniuțiu, D. N., Poșta, G., Gocan, T. M., Andreica, I., Bogdan, I., Rózsa, M., & Lazăr, V. (2019). Influence of the culture substrate on the *Agaricus blazei* Murrill mushrooms vitamins content. *Plants*, 8(9), 1-19. <https://doi.org/10.3390/plants8090316>
- Ruan, W., & Popovich, D. G. (2012). *Ganoderma lucidum* triterpenoid extract induces apoptosis in human colon carcinoma cells (Caco2). *Biomedicine & Preventive Nutrition*, 2(3), 203-209.
- Ryvarden, L. (1993). Tropical polypores. In *British Mycological Society Symposium Series* (Vol. 19, pp. 149-149). Cambridge University Press.
- Rzeski, W., Stepulak, A., Szymański, M., Juszczak, M., Grabarska, A., Sifringer, M., Kaczor, J., & Kandefer-Szerszeń, M. (2009). Betulin elicits anti-cancer effects in tumour primary cultures and cell lines *in vitro*. *Basic & Clinical Pharmacology & Toxicology*, 105(6), 425-432. <http://dx.doi.org/10.1111/j.1742-7843.2009.00471.x>
- Saeidnia, S., & Abdollahi, M. (2013). Antioxidants: Friends or foe in prevention or treatment of cancer: the debate of the century. *Toxicology and Applied Pharmacology*, 271(1), 49-63. <https://doi.org/10.1016/j.taap.2013.05.004>
- Saha, S., & Roy, M. N. (2017). Encapsulation of Vitamin C into β -Cyclodextrin for Advanced and Regulatory Release. *Vitamin C*, 129.
- Sakamoto, S., Putalun, W., Vimolmangkang, S., Phoolcharoen, W., Shoyama, Y., Tanaka, H., & Morimoto, S. (2018). Enzyme-linked immunosorbent assay for the quantitative/qualitative analysis of plant secondary metabolites. *Journal of Natural Medicines*, 72(1), 32-42. doi.10.1007/s11418-017-1144
- Salinaro, A. T., Pennisi, M., Paola, R. D., Scuto, M., Crupi, R., Cambria, M. T., Ontario, M. L., Tomasello, M., Uva, M., Maiolino, L., Calabrese, E. J., Cuzzocrea, S., & Calabrese, V. (2018). Neuroinflammation and neurohormesis in the pathogenesis of Alzheimer's disease and Alzheimer-linked pathologies: Modulation by nutritional mushrooms. *Immunity & Ageing*, 15(1), 1-8. <https://doi.org/10.1186/s12979-017-0108-1>

- Salvesen, G. S., & Dixit, V. M. (1997). Caspases: Intracellular signaling by proteolysis. *Cell*, 91(4), 443-446.
- Sánchez, C. (2009). Lignocellulosic residues: Biodegradation and bioconversion by fungi. *Biotechnology Advances*, 27(2), 185-194. <https://doi.org/10.1016/j.biotechadv.2008.11.001>
- Sánchez, C. (2010). Cultivation of *Pleurotus ostreatus* and other edible mushrooms. *Applied Microbiology and Biotechnology*, 85(5), 1321-1337. <https://doi.org/10.1007/s00253-009-2343-7>
- Sánchez, C. (2017a). Bioactives from mushroom and their application. In *Food bioactives: Extraction and biotechnology applications* (pp. 23-57). Cham: Springer International Publishing.
- Sánchez, C. (2017b). Reactive oxygen species and antioxidant properties from mushrooms. *Synthetic and Systems Biotechnology*, 2(1), 13-22. <https://doi.org/10.1016/j.synbio.2016.12.001>
- Sato, M., Iga, H., Yoshioka, N., Fukui, K., Kawamata, H., Yoshida, H., Hirota, S., & Kitamura, Y. (1997). Emergence of osteoblast-like cells in a neoplastic human salivary cancer cell line after treatment with 22-oxa-1 α , 25-dihydroxyvitamin D3. *Cancer Letters*, 115(2), 149-160. [https://doi.org/10.1016/S0304-3835\(97\)04722-8](https://doi.org/10.1016/S0304-3835(97)04722-8)
- Schmid, F., Stone, B. A., McDougall, B. M., Bacic, A., Martin, K. L., Brownlee, R. T., Chai, E., & Seviour, R. J. (2001). Structure of epiglucan, a highly side-chain/branched (1 $\rightarrow$ 3; 1 $\rightarrow$ 6)- β -glucan from the micro fungus *Epicoccum nigrum* Ehrenb. ex Schlecht. *Carbohydrate Research*, 331(2), 163-171. [https://doi.org/10.1016/S0008-6215\(01\)00023-4](https://doi.org/10.1016/S0008-6215(01)00023-4)
- Schweitzer, C., & Schmidt, R. (2003). Physical mechanisms of generation and deactivation of singlet oxygen. *Chemical Reviews*, 103(5), 1685-1758.
- Seirafi, M., Kozlov, G., & Gehring, K. (2015). Parkin structure and function. *The FEBS Journal*, 282(11), 2076-2088. <https://doi.org/10.1111/febs.13249>
- Selkoe, D. J., & Hardy, J. (2016). The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Molecular Medicine*, 8(6), 595-608. <https://doi.org/10.15252/emmm.201606210>
- Selvendiran, K., Koga, H., Ueno, T., Yoshida, T., Maeyama, M., Torimura, T., Yano, H., Kojiro, M., & Sata, M. (2006). Luteolin promotes degradation in signal transducer and activator of transcription 3 in human hepatoma cells: An implication for the antitumor potential of flavonoids. *Cancer Research*, 66(9), 4826-4834. <https://doi.org/10.1158/0008-5472.CAN-05-4062>
- SH, K. (2000). Induction of apoptosis by cancer chemotherapy. *Experimetal Cell Research*, 256, 42-49. <https://doi.org/10.1006/excr.2000.4838>
- Sharma, A. K., & Sharma, A. (1990). *Chromosome techniques: Theory and practice*. Adithya Books, New Delhi.

References

- Shi, Y., Ji, M., Dong, J., Shi, D., Wang, Y., Liu, L., Feng, S., & Liu, L. (2024). New bioactive secondary metabolites from fungi: 2023. *Mycology*, 15(3), 283-321. <https://doi.org/10.1080/21501203.2024.2354302>
- Shieh, Y. H., Liu, C. F., Huang, Y. K., Yang, J. Y., Wu, I. L., Lin, C. H., & Lin, S. C. (2001). Evaluation of the hepatic and renal-protective effects of *Ganoderma lucidum* in mice. *The American Journal of Chinese Medicine*, 29(3-4), 501-507.
- Siddiq, M., Ravi, R., & Sami, A. (2018) Edible mushrooms: Production, processing, and quality. In: Handbook of vegetables and vegetable processing, pp 701–72. Wiley Blackwell.
- Siddique, H. R., Mishra, S. K., Karnes, R. J., & Saleem, M. (2011). Lupeol, a novel androgen receptor inhibitor: Implications in prostate cancer therapy. *Clinical Cancer Research*, 17(16), 5379-5391. <https://doi.org/10.1158/1078-0432.CCR-11-0916>
- Sies, H., & Jones, D. P. (2020). Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nature Reviews Molecular Cell Biology*, 21(7), 363-383. <https://doi.org/10.1038/s41580-020-0230-3>
- Simic, M. G. (1998). Mechanisms of inhibition of free-radical processes in mutagenesis and carcinogenesis. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 202(2), 377-386. [https://doi.org/10.1016/0027-5107\(88\)90199-6](https://doi.org/10.1016/0027-5107(88)90199-6)
- Singdevsachan, S. K., Auroshree, P., Mishra, J., Baliyarsingh, B., Tayung, K., & Thatoi, H. (2016). Mushroom polysaccharides as potential prebiotics with their antitumor and immunomodulating properties: A review. *Bioactive Carbohydrates and Dietary Fibre*, 7(1), 1-14. <https://doi.org/10.1016/j.bcdf.2015.11.001>
- Singh, K. P., Miaskowski, C., Dhruva, A. A., Flowers, E., & Kober, K. M. (2018). Mechanisms and measurement of changes in gene expression. *Biological Research for Nursing*, 20(4), 369-382. <https://doi.org/10.1177/1099800418772161>
- Sliva, D. (2006). *Ganoderma lucidum* in cancer research. *Leukemia Research*, 30(7), 767-768. <http://dx.doi.org/10.1016/j.leukres.2005.12.015>
- Smânia, E. D. F. A., Smânia Júnior, A., & Loguercio-Leite, C. (1998). Cinnabarin synthesis by *Pycnoporus sanguineus* strains and antimicrobial activity against bacteria from food products. *Revista de Microbiologia*, 29(4), 317-320.
- Smith, J. E., Sullivan, R., & Rowan, N. J. (2003). The role of polysaccharides derived from medicinal mushrooms in cancer treatment programs: Current perspectives. *International Journal of Medicinal Mushrooms*, 5(3), 217-234.
- Soares, R., Meireles, M., Rocha, A., Pirraco, A., Obiol, D., Alonso, E., Joos, G., & Balogh, G. (2011). Maitake (D fraction) mushroom extract induces apoptosis in breast cancer cells by BAK-1 gene activation. *Journal of Medicinal Food*, 14(6), 563-572. <https://doi.org/10.1089=jmf.2010.0095>
- Sofowora, A. (1993). Recent trends in research into African medicinal plants. *Journal of ethnopharmacology*, 38(2-3), 197-208.

- Sohal, R. S. (2002). Role of oxidative stress and protein oxidation in the aging process. *Free Radical Biology and Medicine*, 33(1), 37-44. [https://doi.org/10.1016/S0891-5849\(02\)00856-0](https://doi.org/10.1016/S0891-5849(02)00856-0)
- Song, K. S., Li, G., Kim, J. S., Jing, K., Kim, T. D., Kim, J. P., Seo, S. B., Yoo, J. K., Park, H. D., Hwang, B. D., Lim, K., & Yoon, W. H. (2011). Protein-bound polysaccharide from *Phellinus linteus* inhibits tumor growth, invasion, and angiogenesis and alters Wnt/ β -catenin in SW480 human colon cancer cells. *BMC Cancer*, 11(1), 1-11. <https://doi.org/10.1186/1471-2407-11-307>
- Song, W. S., & Griensven, L. V. (2008). Pro-and antioxidative properties of medicinal mushroom extracts. *International Journal of Medicinal Mushrooms*, 10(4), 315-324. <https://doi.org/10.1615/IntJMedMushr.v10.i4.30>
- Sontag, B., Arnold, N., Steglich, W., & Anke, T. (1999). Montadial A, a cytotoxic metabolite from *Bondarzewia montana*. *Journal of Natural Products*, 62(10), 1425-1426.
- Sorg, O., Schilter, B., Honegger, P., & Monnet-Tschudi, F. (1998). Increased vulnerability of neurones and glial cells to low concentrations of methylmercury in a prooxidant situation. *Acta Neuropathologica*, 96(6), 621-627.
- Sowndhararajan, K., & Kang, S. C. (2013). Free radical scavenging activity from different extracts of leaves of *Bauhinia vahlii* Wight & Arn. *Saudi Journal of Biological Sciences*, 20(4), 319-325. <https://doi.org/10.1016/j.sjbs.2012.12.005>
- Sreevidya, N., & Mehrotra, S. (2003). Spectrophotometric method for estimation of alkaloids precipitable with Dragendorff's reagent in plant materials. *Journal of AOAC international*, 86(6), 1124-1127.
- Stamets, P. (2002). Novel antimicrobials from mushrooms. *HerbalGram*, (54), 28-33
- Stampfer, M. J. (2006). Cardiovascular disease and Alzheimer's disease: Common links. *Journal of Internal Medicine*, 260(3), 211-223. <https://doi.org/10.1111/j.1365-2796.2006.01687.x>
- Stanley, G., Harvey, K., Slivova, V., Jiang, J., & Sliva, D. (2005). *Ganoderma lucidum* suppresses angiogenesis through the inhibition of secretion of VEGF and TGF- β 1 from prostate cancer cells. *Biochemical and Biophysical Research Communications*, 330(1), 46-52. <https://doi.org/10.1016/j.bbrc.2005.02.116>
- Stassen, H. H., Bachmann, S., Bridler, R., Cattapan, K., Herzig, D., Schneeberger, A., & Seifritz, E. (2022). Detailing the effects of polypharmacy in psychiatry: Longitudinal study of 320 patients hospitalized for depression or schizophrenia. *European Archives of Psychiatry and Clinical Neuroscience*, 272(4), 603-619. <https://doi.org/10.1007/s00406-021-01358-5>
- Staveren, V. W. C. G., Solís, D. W., Hebrant, A., Detours, V., Dumont, J. E., & Maenhaut, C. (2009). Human cancer cell lines: Experimental models for cancer cells in situ? For cancer stem cells? *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*, 1795(2), 92-103.
- Steffan, B. N., Venkatesh, N., & Keller, N. P. (2020). Let's get physical: Bacterial-fungal interactions and their consequences in agriculture and health. *Journal of Fungi*, 6(4), 1-18. <https://doi.org/10.3390/jof6040243>

- Stockert, J. C., Horobin, R. W., Colombo, L. L., & Blázquez-Castro, A. (2018). Tetrazolium salts and formazan products in Cell Biology: Viability assessment, fluorescence imaging, and labeling perspectives. *Acta Histochemica*, 120(3), 159-167. <https://doi.org/10.1016/j.acthis.2018.02.005>
- Sudirman, L. I., & Mujiyati, S. (1997). Preliminary detection of antimicrobial activity of fruiting bodies' extracts of tropical *Ganoderma* sp. In *Proceedings of the 1st International Symposium on Ganoderma lucidum in Japan*. (pp. 154-159). Toyo-Igaku-sha Co. Ltd., Tokyo.
- Suh, J. H., Shenvi, S. V., Dixon, B. M., Liu, H., Jaiswal, A. K., Liu, R. M., & Hagen, T. M. (2004). Decline in transcriptional activity of Nrf2 causes age-related loss of glutathione synthesis, which is reversible with lipoic acid. *Proceedings of the National Academy of Sciences*, 101(10), 3381-3386. <https://doi.org/10.1073/pnas.0400282101>
- Sui, X., Chen, R., Wang, Z., Huang, Z., Kong, N., Zhang, M., Han, W., Lou, F., Yang, J., Zhang, Q., Wang, X., He, C., & Pan, H. (2013). Autophagy and chemotherapy resistance: A promising therapeutic target for cancer treatment. *Cell Death & Disease*, 4(10), 1-12. <https://doi.org/10.1038/cddis.2013.350>
- Sun, K., Deng, W., Zhang, S., Cai, N., Jiao, S., Song, J., & Wei, L. (2013). Paradoxical roles of autophagy in different stages of tumorigenesis: Protector for normal or cancer cells. *Cell & Bioscience*, 3(1), 35. <https://doi.org/10.1186/2045-3701-3-35>
- Swapna, S., Abrar, S., & Krishnappa, M. (2008). Diversity of macrofungi in semi-evergreen and moist deciduous forest of Shimoga District, Karnataka, India. *Journal of Mycology and Plant Pathology*, 38(1), 21-26.
- Takahashi, S., & Mashima, K. (2022). Neuroprotection and disease modification by Astrocytes and Microglia in Parkinson disease. *Antioxidants*, 11(1), 1-18. <https://doi.org/10.3390/antiox11010170>
- Takaku, T., Kimura, Y., & Okuda, H. (2001). Isolation of an antitumor compound from *Agaricus blazei* Murill and its mechanism of action. *The Journal of Nutrition*, 131(5), 1409-1413. <https://doi.org/10.1093/jn/131.5.1409>
- Talarico, L. B., Zibetti, R. G., Faria, P. C., Scolaro, L. A., Duarte, M. E., Nosedá, M. D., Damonte, E. B. (2004). Anti-herpes simplex virus activity of sulfated galactans from the red sea weeds *Gymnogongrus griffithsia* and *Cryptonemia crenulata*. *International Journal of Biological Macromolecules*, 34(1-2), 63-71.
- Tarapore, R. S., Siddiqui, I. A., Adhami, V. M., Spiegelman, V. S., & Mukhtar, H. (2013). The dietary terpene lupeol targets colorectal cancer cells with constitutively active Wnt/ β -catenin signaling. *Molecular Nutrition & Food Research*, 57(11), 1950-1958. <https://doi.org/10.1002/mnfr.201300155>
- Tel-Cayan, G., Ozturk, M., Duru, M. E., & Turkoglu, A. (2017). Fatty acid profiles in wild mushroom species from Anatolia. *Chemistry of Natural Compounds*, 53(2), 351-353. <https://doi.org/DOI 10.1007/s10600-017-1986-3>
- Thébaud, A., Debray, D., & Gonzales, E. (2018). An update on the physiopathology and therapeutic management of cholestatic pruritus in children. *Clinics and Research in*

- Hepatology and Gastroenterology*, 42(2), 103-109.
<https://doi.org/10.1016/j.clinre.2017.08.007>
- Thongbai, B., Rapior, S., Hyde, K. D., Wittstein, K., & Stadler, M. (2015). *Hericium erinaceus*, an amazing medicinal mushroom. *Mycological Progress*, 14(10), 1–23.
<https://doi.org/10.1007/s11557-015-1105-4>
- Thornberry, N. A. (1997). The caspase family of cysteine proteases. *British Medical Bulletin*, 53(3), 478-490.
- Tomasi, S., Lohezic-Le Devehat, F., Sauleau, P., Bezivin, C., & Boustie, J. (2004). Cytotoxic activity of methanol extracts from Basidiomycete mushrooms on murine cancer cell lines. *Die Pharmazie-An International Journal of Pharmaceutical Sciences*, 59(4), 290-293.
- Tordera, R. M., Totterdell, S., Wojcik, S. M., Brose, N., Elizalde, N., Lasheras, B., & Del Rio, J. (2007). Enhanced anxiety, depressive-like behaviour and impaired recognition memory in mice with reduced expression of the vesicular glutamate transporter 1 (VGLUT1). *European Journal of Neuroscience*, 25(1), 281-290.
<https://doi.org/10.1111/j.1460-9568.2006.05259>
- Torel, J., Cillard, J., & Cillard, P. (1986). Antioxidant activity of flavonoids and reactivity with peroxy radical. *Phytochemistry*, 25(2), 383-385. [https://doi.org/10.1016/S0031-9422\(00\)85485-0](https://doi.org/10.1016/S0031-9422(00)85485-0)
- Torggler, R., Papinski, D., & Kraft, C. (2017). Assays to monitor autophagy in *Saccharomyces cerevisiae*. *Cells*, 6(3), 23.
- Torres, M. L., Tadosa, E. R., & Reyes, R. G. (2020). Species listing of macrofungi on the bugkalot tribal community in Alfonso Castaneda, Nueva Vizcaya, Philippines. *Current Research in Environmental & Applied Mycology (Journal of Fungal Biology)*, 10(1), 475-493. <https://doi.org/10.5943/cream/10/1/37>
- Trecek, O., Kindzorra, I., Pauser, K., Trecek, L., & Ortmann, O. (2005). Expression of icb-1 gene is interferon-gamma inducible in breast and ovarian cancer cell lines and affects the IFN γ -response of SK-OV-3 ovarian cancer cells. *Cytokine*, 32(3-4), 137-142.
<https://doi.org/10.1016/j.cyto.2005.08.008>
- Tripathy, S. S., Rajoriya, A., & Gupta, N. (2014). Wild mushrooms of Odisha: Prospective candidates of antioxidant sources. *Advances in Plants Agriculture Research*, 1(4), 2-7.
- Troshin, V. V. (2009). Pathogenesis and classification of chronic encephalopathy due neurotoxic chemicals (Review of literature). *Meditina Truda i Promyshlennaia Ekologiya*, (7), 21-26.
- Tu, H., Jacobs, S. C., Borkowski, A., & Kyprianou, N. (1996). Incidence of apoptosis and cell proliferation in prostate cancer: Relationship with TGF- β 1 and bcl-2 expression. *International Journal of Cancer*, 69(5), 357-363.
[https://doi.org/10.1002/\(SICI\)1097-0215\(19961021\)69:5%3C357::AID-IJC1%3E3.0.CO;2-4](https://doi.org/10.1002/(SICI)1097-0215(19961021)69:5%3C357::AID-IJC1%3E3.0.CO;2-4)

- Turkoglu, A., Duru, M. E., Mercan, N., Kivrak, I., & Gezer, K. (2007). Antioxidant and antimicrobial activities of *Laetiporus sulphureus* (Bull.) Murrill. *Food Chemistry*, 101(1), 267-273. <https://doi.org/10.1016/j.foodchem.2006.01.025>
- Valisolalao, J., Luu, B., & Ourisson, G. (1983). Steroides cytotoxiques de *polyporus versicolor*. *Tetrahedron*, 39(17), 2779-2785. [https://doi.org/10.1016/S0040-4020\(01\)82446-7](https://doi.org/10.1016/S0040-4020(01)82446-7)
- Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M., & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The international Journal of Biochemistry & Cell Biology*, 39(1), 44-84. <https://doi.org/10.1016/j.biocel.2006.07.001>
- Valverde, M. E., Hernández-Pérez, T., & Paredes-López, O. (2015). Edible mushrooms: Improving human health and promoting quality life. *International Journal of Microbiology*, 2015(1), 1-14. <https://doi.org/10.1155/2015/376387>
- Vauzour, D., Rodriguez-Mateos, A., Corona, G., Oruna-Concha, M. J., & Spencer, J. P. (2010). Polyphenols and human health: Prevention of disease and mechanisms of action. *Nutrients*, 2(11), 1106-1131.
- Viallet, J., Tsao, M. S., & Gallant, G. (1996). Etoposide and doxorubicin antagonize the in vitro activity of paclitaxel in human non-small cell lung cancer cell lines. *Lung Cancer*, 15(1), 93-101. [https://doi.org/10.1016/0169-5002\(96\)00574-0](https://doi.org/10.1016/0169-5002(96)00574-0)
- Wachtel-Galor, S., Tomlinson, B., & Benzie, I. F. (2004). *Ganoderma lucidum* ('Lingzhi'), a Chinese medicinal mushroom: Biomarker responses in a controlled human supplementation study. *British Journal of Nutrition*, 91(2), 263-269. <https://doi.org/10.1079/BJN20041039>
- Wang, H., & Ng, T. B. (2006). Ganodermin, an antifungal protein from fruiting bodies of the medicinal mushroom *Ganoderma lucidum*. *Peptides*, 27(1), 27-30. <https://doi.org/10.1016/j.peptides.2005.06.009>
- Wang, H., Gao, J., & Ng, T. B. (2000). A new lectin with highly potent antihepatoma and antisarcoma activities from the oyster mushroom *Pleurotus ostreatus*. *Biochemical and Biophysical Research Communications*, 275(3), 810-816. <https://doi.org/10.1006/bbrc.2000.3373>
- Wang, L., Yang, Y. J., Chen, S. H., Ge, X. R., Xu, C. J., & Gui, S. Q. (2006). Effects of beta-sitosterol on microtubular systems in cervical cancer cells. *Zhonghua Yi Xu eZa Zhi*, 86(39), 2771-2775.
- Wang, P., Guan, Y. F., Du, H., Zhai, Q. W., Su, D. F., & Miao, C. Y. (2012). Induction of autophagy contributes to the neuroprotection of nicotinamide phosphoribosyltransferase in cerebral ischemia. *Autophagy*, 8(1), 77-87.
- Wang, W., Chen, K., Liu, Q., Johnston, N., Ma, Z., Zhang, F., & Zheng, X. (2014). Suppression of tumor growth by *Pleurotus ferulae* ethanol extract through induction of cell apoptosis, and inhibition of cell proliferation and migration. *PLoS One*, 9(7), 1-14. <https://doi.org/10.1371/journal.pone.0102673>

- Wang, Y., & Chang, Q. (2020). MicroRNA miR-212 regulates PDCD4 to attenuate A β 25–35-induced neurotoxicity via PI3K/AKT signalling pathway in Alzheimer's disease. *Biotechnology Letters*, 42(9), 1789-1797.
- Wang, Y., Cheng, X., Wang, P., Wang, L., Fan, J., Wang, X., & Liu, Q. (2014). Investigating migration inhibition and apoptotic effects of *Fomitopsis pinicola* chloroform extract on human colorectal cancer SW-480 cells. *PloS one*, 9(7), 1-13. <https://doi.org/10.1371/journal.pone.0101303>
- Wani, B. A., Bodha, R. H., & Wani, A. H. (2010). Nutritional and medicinal importance of mushrooms. *Journal of Medicinal Plants Research*, 4(24), 2598-2604. <http://dx.doi.org/10.5897/JMPR09.565>
- Wasser, S. P. (2002). Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Applied Microbiology and Biotechnology*, 60(3), 258-274. DOI <https://doi.org/10.1007/s00253-002-1076-7>
- Wasser, S. P. (2010). Medicinal mushroom science: History, current status, future trends, and unsolved problems. *International Journal of Medicinal Mushrooms*, 12(1). 1-16. <https://doi.org/10.1615/IntJMedMushr.v12.i1.10>
- Wasser, S. P., & Weis, A. L. (1999a). Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: Current perspectives. *International Journal of Medicinal Mushrooms*, 1(1), 31-62. <https://doi.org/10.1615/IntJMedMushrooms.v1.i1.30>
- Wasser, S. P., & Weis, A. L. (1999b). Therapeutic effects of substances occurring in higher Basidiomycetes mushrooms: A modern perspective. *Critical Reviews in Immunology*, 19(1), 65– 96. <https://doi.org/10.1615/CritRevImmunol.v19.i1.30>
- Wasser, S. P., & Weis, A. L. (1999c). General description of the most important medicinal higher Basidiomycetes mushrooms. *International Journal of medicinal mushrooms*, 1(4), 351-370.
- Wasser, S. P., Sokolov, D., Reshetnikov, S. V., & Timor-Tismenetsky, M. (2000). Dietary supplements from medicinal mushrooms: diversity of types and variety of regulations. *International Journal of Medicinal Mushrooms*, 2(1), 1-9.
- Wasser, S. P., Weis, A. L., & Nevo, E. (1997). *Medicinal Mushrooms: Ganoderma Lucidum* (Curtis: Fr.) P. Karst.: Reishi Mushroom. International Centre for Cryptogamic Plants and Fungi Institute of Evolution-University of Haifa Israel.
- Wätjen, W., Weber, N., Lou, Y. J., Wang, Z. Q., Chovolou, Y., Kampkötter, A., Kahl, R., & Proksch, P. (2007). Prenylation enhances cytotoxicity of apigenin and liquiritigenin in rat H4IIE hepatoma and C6 glioma cells. *Food and Chemical Toxicology*, 45(1), 119-124. <https://doi.org/10.1016/j.fct.2006.08.008>
- Watling, R. (1997). Poisoning by fungi: Interesting cases. *Mycologist*, 11(3), 101.
- WHO (2015). World report on ageing and health. World Health Organization, Geneva.
- Wijeyaratne, W. D. N., & Wadasinghe, L. G. Y. J. G. (2019). *Allium cepa* bio assay to assess the water and sediment cytogenotoxicity in a tropical stream subjected to multiple

- point and nonpoint source pollutants. *Journal of Toxicology*, 2019(1), 1-10. <https://doi.org/10.1155/2019/5420124>
- Wolff, J., Reißner, P., Hefner, G., Normann, C., Kaier, K., Binder, H., Hiemke, C., Toto, S., Domschke, K., Marschollek, M., & Klimke, A. (2021). Pharmacotherapy, drug-drug interactions and potentially inappropriate medication in depressive disorders. *PLOS One*, 16(7), 1-16. <https://doi.org/10.1371/journal.pone.0255192>
- Won, D. P., Lee, J. S., Kwon, D. S., Lee, K. E., Shin, W. C., & Hong, E. K. (2011). Immunostimulating activity by polysaccharides isolated from fruiting body of *Inonotus obliquus*. *Molecules and Cells*, 31(2), 165-173. <https://doi.org/10.1007/s10059-011-0022-x>
- Wong, K. H., Ng, C. C., Kanagasabapathy, G., Yow, Y. Y., & Sabaratnam, V. (2017). An overview of culinary and medicinal mushrooms in neurodegeneration and neurotrauma research. *International Journal of Medicinal Mushrooms*, 19(3), 191-202. <https://doi.org/10.1615/IntJMedMushrooms.v19.i3.10>
- Wong, R. S. (2011). Apoptosis in cancer: From pathogenesis to treatment. *Journal of Experimental & Clinical Cancer Research*, 30(1), 1-14. <https://doi.org/10.1186/1756-9966-30-87>
- Wu, B., Zhang, Q., Shen, W., & Zhu, J. (2008). Anti-proliferative and chemosensitizing effects of luteolin on human gastric cancer AGS cell line. *Molecular and Cellular Biochemistry*, 313(1), 125-132. <https://doi.org/10.1007/s11010-008-9749-x>
- Wu, G. S., Lu, J. J., Guo, J. J., Li, Y. B., Tan, W., Dang, Y. Y., Zhong, Z. F., Xu, Z. T., Chen, X. P., & Wang, Y. T. (2012). Ganoderic acid DM, a natural triterpenoid, induces DNA damage, G1 cell cycle arrest and apoptosis in human breast cancer cells. *Fitoterapia*, 83(2), 408-414. <https://doi.org/10.1016/j.fitote.2011.12.004>
- Wu, H. T., Lu, F. H., Su, Y. C., Ou, H. Y., Hung, H. C., Wu, J. S., Yang, Y. C., & Chang, C. J. (2014). *In vivo* and *in vitro* anti-tumor effects of fungal extracts. *Molecules*, 19(2), 2546-2556. <https://doi.org/10.3390/molecules19022546>
- Wu, J., Xiao, Y. Z., & Yu, H. Q. (2005). Degradation of lignin in pulp mill wastewaters by white-rot fungi on biofilm. *Bioresource Technology*, 96(12), 1357-1363.
- Wu, X., Yang, J. S., & Yan, M. (2009). Four new triterpenes from fungus of *Fomes officinalis*. *Chemical and Pharmaceutical Bulletin*, 57(2), 195-197. <https://doi.org/10.1248/cpb.57.195>
- Wu, Y., Ye, L., Yuan, Y., Jiang, T., Guo, X., Wang, Z., ... & Xiao, J. (2019). Autophagy activation is associated with neuroprotection in diabetes-associated cognitive decline. *Aging and Disease*, 10(6), 1233. doi: 10.14336/AD.2018.1024.
- Xu, T., B. Beelman, R., & D. Lambert, J. (2012a). The cancer preventive effects of edible mushrooms. *Anti-Cancer Agents in Medicinal Chemistry-Anti-Cancer Agents*, 12(10), 1255-1263. <https://doi.org/10.2174/187152012803833017>
- Xu, W., Huang, J. J. H., & Cheung, P. C. K. (2012b). Extract of *Pleurotus pulmonarius* suppresses liver cancer development and progression through inhibition of VEGF-induced PI3K/AKT signaling pathway. *PLoS One*, 7(3), 1-13. <https://doi.org/10.1371/journal.pone.0034406>

- Yaltirak, R. (2009). Presence of antioxidants and compounds in mushroom. *International Journal of Agriculture and Biology*, 13, 2052-2056.
- Yang, C. Z., Wang, S. H., Zhang, R. H., Lin, J. H., Tian, Y. H., Yang, Y. Q., Liu, J., & Ma, Y. X. (2023). Neuroprotective effect of astragaloside via activating PI3K/Akt-mTOR-mediated autophagy on APP/PS1 mice. *Cell Death Discovery*, 9(1), 15. doi: 10.1038/s41420-023-01324-1.
- Yang, S. F., Yang, W. E., Chang, H. R., Chu, S. C., & Hsieh, Y. S. (2008). Luteolin induces apoptosis in oral squamous cancer cells. *Journal of Dental Research*, 87(4), 401-406. <https://doi.org/10.1177/154405910808700413>
- Yang, Z. J., Chee, C. E., Huang, S., & Sinicrope, F. A. (2011). The role of autophagy in cancer: Therapeutic implications. *Molecular Cancer Therapeutics*, 10(9), 1533-1541. <https://doi.org/10.1158/1535-7163.MCT-11-0047>
- Yang, Z., Slavin, M. J., & Sachdev, P. S. (2013). Dementia in the oldest old. *Nature Reviews Neurology*, 9(7), 382-393. <http://dx.doi.org/10.3390/ijms20205143>
- Yankner, B. A. (1996). Mechanisms of neuronal degeneration in Alzheimer's disease. *Neuron*, 16(5), 921-932.
- Yavorsky, V. A., & Lukyanetz, E. A. (1997). Blocking of the leakage potassium current mediated by activation of muscarinic receptors. *Neurophysiology*, 29, 205-211.
- Yazawa, Y., Yokota, M., & Sugiyama, K. (2000). Antitumor promoting effect of an active component of *Polyporus*, ergosterol and related compounds on rat urinary bladder carcinogenesis in a short-term test with concanavalin A. *Biological and Pharmaceutical Bulletin*, 23(11), 1298-1302. <https://doi.org/10.1248/bpb.23.1298>
- Yim, H. S., Chye, F. Y., Tan, C. T., Ng, Y. C., & Ho, C. W. (2010). Antioxidant activities and total phenolic content of aqueous extract of *Pleurotus ostreatus* (cultivated oyster mushroom). *Malaysian Journal of Nutrition*, 16(2), 281-291.
- Yu, X., Liu, Y., Wang, Y., Feng, X., Tu, M., & Chen, J. (2020). Role of bioengineering and laborers in integration of farmland resources toward to improve dimension of sustainable agriculture in China. *Bioengineered*, 11(1), 559-571. <https://doi.org/10.1080/21655979.2020.1765523>
- Yuen, J. W., & Gohel, M. D. I. (2005). Anticancer effects of *Ganoderma lucidum*: A review of scientific evidence. *Nutrition and Cancer*, 53(1), 11-17.
- Yurchenko, E. A., Menchinskaya, E. S., Pislyagin, E. A., Trinh, P. T. H., Ivanets, E. V., Smetanina, O. F., & Yurchenko, A. N. (2018). Neuroprotective activity of some marine fungal metabolites in the 6-hydroxydopamine- and paraquat-induced Parkinson's disease models. *Marine Drugs*, 16(11), 1-15. <https://doi.org/10.3390/md16110457>
- Zahn, R., Perry, N., Perry, E., & Mukaetova-Ladinska, E. B. (2019). Use of herbal medicines: Pilot survey of UK users' views. *Complementary Therapies in Medicine*, 44, 83-90.
- Zaidman, B. Z., Yassin, M., Mahajna, J., & Wasser, S. P. (2005). Medicinal mushroom modulators of molecular targets as cancer therapeutics. *Applied Microbiology and Biotechnology*, 67(4), 453-468. <https://doi.org/10.1007/s00253-004-1787-z>

- Zeevalk, G. D., Bernard, L. P., Song, C., Gluck, M., & Ehrhart, J. (2005). Mitochondrial inhibition and oxidative stress: Reciprocating players in neurodegeneration. *Antioxidants & Redox Signalling*, 7(9-10), 1117-1139. <https://doi.org/10.1089/ars.2005.7.1117>
- Zhang, B., Buya, M., Qin, W., Sun, C., Cai, H., Xie, Q., Xu, B., & Wu, Y. (2013). Anthocyanins from Chinese bayberry extract activate transcription factor Nrf2 in β cells and negatively regulate oxidative stress-induced autophagy. *Journal of Agricultural and Food Chemistry*, 61(37), 8765-8772. <http://dx.doi.org/10.1021/jf4012399>
- Zhang, H. W., Hu, J. J., Fu, R. Q., Liu, X., Zhang, Y. H., Li, J., Liu, L., Li, Y. N., Deng, Q., Luo, Q. S., & Gao, N. (2018). Flavonoids inhibit cell proliferation and induce apoptosis and autophagy through downregulation of PI3K γ mediated PI3K/AKT/mTOR/p70S6K/ULK signaling pathway in human breast cancer cells. *Scientific Reports*, 8(1), 1-13. <http://dx.doi.org/10.1038/s41598-018-29308-7>
- Zhang, M., Cui, S. W., Cheung, P. C., & Wang, Q. (2007). Antitumor polysaccharides from mushrooms: A review on their isolation process, structural characteristics and antitumor activity. *Trends in Food Science & Technology*, 18(1), 4-19. <https://doi.org/10.1016/j.tifs.2006.07.013>
- Zhang, Q., Wan, L., Guo, Y., Cheng, N., Cheng, W., Sun, Q., & Zhu, J. (2009). Radiosensitization effect of luteolin on human gastric cancer SGC-7901 cells. *Journal of Biological Regulators and Homeostatic Agents*, 23(2), 71-78.
- Zhang, Y., Mills, G. L., & Nair, M. G. (2003). Cyclooxygenase inhibitory and antioxidant compounds from the fruiting body of an edible mushroom, *Agrocybe aegerita*. *Phytomedicine*, 10(5), 386-390. <https://doi.org/10.1078/0944-7113-00272>
- Zhao, C. L., Cui, B. K., Song, J., & Dai, Y. C. (2015). Fragiliporiaceae, a new family of Polyporales (Basidiomycota). *Fungal Diversity*, 70(1), 115-126.
- Zhao, Y., Chang, S. K., Qu, G., Li, T., & Cui, H. (2009). β -Sitosterol inhibits cell growth and induces apoptosis in SGC-7901 human stomach cancer cells. *Journal of Agricultural and Food Chemistry*, 57(12), 5211-5218. <https://doi.org/10.1021/jf803878n>
- Zhao, Y., Cui, J., Liu, M., & Zhao, L. (2020). Progress on terpenoids with biological activities produced by plant endophytic fungi in China between 2017 and 2019. *Natural Product Communications*, 15(7), 1934578X20937204.
- Zhao, Z. Z., Liu, J. K., & Chen, H. P. (2020). Microporotriol, a new cadinane-type sesquiterpenoid from the cultures of the wood-decay fungus *Microporus affinis* HFG829. *Natural Product Research*, 34(15), 2194-2201. <https://doi.org/10.1080/14786419.2019.1582038>
- Zhou, J., Zhang, Z. X., & Li, D. C. (2010). Effects of lorglumide on growth and invasion of human pancreatic cancer cell line Mia PaCa-2 *in vitro* through the cholecystokinin-cholecystokinin-I receptor pathway. *Current Therapeutic Research*, 71(4), 239-251. <https://doi.org/10.1016/j.curtheres.2010.08.005>
- Zhou, X., Lin, J., Yin, Y., Zhao, J., Sun, X., & Tang, K. (2007). Ganodermataceae: Natural products and their related pharmacological functions. *The American Journal of Chinese Medicine*, 35(04), 559-574.

References

- Zhou, Y., Fang, S. H., Ye, Y. L., Chu, L. S., Zhang, W. P., Wang, M. L., & Wei, E. Q. (2006). Caffeic acid ameliorates early and delayed brain injuries after focal cerebral ischemia in rats. *Acta Pharmacologica Sinica*, 27(9), 1103-1110.
- Zjawiony, J. K. (2004). Biologically active compounds from Aphyllophorales (polypore) fungi. *Journal of natural products*, 67(2), 300-310.
- Zugazagoitia, J., Guedes, C., Ponce, S., Ferrer, I., Molina-Pinelo, S., & Paz-Ares, L. (2016). Current challenges in cancer treatment. *Clinical therapeutics*, 38(7), 1551-1566. <https://doi.org/10.1016/j.clinthera.2016.03.026>



Appendices

APPENDICES

Wagner's reagent

Appendix 1

Iodine : 1.27 g

KI : 2 g

Dissolve the above chemicals in 5 mL H_2SO_4 and make upto 100 mL.

Dragendorff's reagent

Appendix 2

Part A: Bismuth subnitrate (0.8 g), water and glacial acetic acid

Part B: Potassium iodide (8 g) and water

To prepare the reagent, both solutions are mixed, forming a soluble orange complex of potassium tetraiodobismuthate

Vanilin - glacial acetic acid solution

Appendix 3

Vanillin: 1.0 g

Take vanillin in a 100 mL volumetric flask

Add glacial acetic acid into the flask

Perchloric acid solution

Appendix 4

Perchloric acid: 1.72 mL

Dissolve this to about 800 mL of distilled water

Alkaline Copper solution

Appendix 5

Stock A (Copper and Chelator)

0.4 g of copper sulfate and 0.2 g of Rochelle salt in 20 mL of water are mixed, along with a sodium carbonate solution, to form the copper reagent.

Stock B (Alkaline solution)

Dissolve 4 g of NaOH in 100 mL of water to get 1 M NaOH solution

Alkaline Copper solution = Stock A + Stock B

Folin-Ciocalteu reagent

Appendix 6

Dissolve 100 g of sodium tungstate and 25 g of sodium molybdate in 700 mL of distilled water.

Add 50 mL of 85% phosphoric acid and 100 mL of concentrated hydrochloric acid to the solution.

Heat the mixture to reflux (boiling with condensation) for 10 hours.

After cooling, add 150 g of lithium sulfate and 5 mL of water.

Heat the mixture again to reflux for another 15 minutes to prevent precipitate formation.

Allow the solution to cool to room temperature and then dilute it to a final volume of 100 mL with water.

Phosphate buffer saline (PBS)

Appendix 7

NaCl : 8 g

KCl : 0.2 g

Na₂HPO₄ : 1.44 g

KH₂PO₄ : 0.24 g

Dissolve in 1 L double distilled water and adjust pH to 7.4.

Griess reagent

Appendix 8

Naphthyl ethylenediamine HCl: 0.1% in distilled water

Sulfanilamide: 1% in 5% H₃PO₄

Both solutions are mixed in 1:1 ratio

Modified Carnoy's fluid

Appendix 9

Acetic acid: 10 mL

Ethanol: 30 mL

Acetocarmine

Appendix 10

Carmine : 2 g
Acetic acid : 100 mL of 45% acetic acid

The solution is heated to dissolve carmine and is filtered to remove the undissolved carmine powder.

DMEM (Dulbecco's Modified Eagle's medium)

Appendix 11

Sodium bicarbonate : 1.85 g
HEPES : 2.95 g
DMEM powder : 1 packet
Distilled water : 1 L

Vacuum sterilized and stored at 4°C

Blocking buffer

Appendix 12

Dissolve 0.2% gelatin in 0.05% Tween 20

O-dianisidine hydrochloride

Appendix 13

It consists of the organic compound O-dianisidine (also known as 3,3-dimethoxybenzidine) combined with hydrochloric acid.

Green stain solution

Appendix 14

Prepared by mixing the cyto ID green dye with 1X assay buffer

TRIzol reagent

Appendix 15

A monophasic mixture of phenol and guanidine thiocyanate

TE (Tris-EDTA) buffer

Appendix 16

Tris HCl: 10 mM, pH 8
EDTA: 0.1 mM, pH 8