Bioprospecting of Lichens of the Genus *Parmotrema* A. Massal. (Parmeliaceae) in Kerala

Thesis submitted to
the University of Calicut in partial fulfilment of
the requirement for the degree of

DOCTOR OF PHILOSOPHY IN BOTANY

By

BIBIN JOSEPH

Research Supervisor

Dr. N.S. Pradeep

KSCSTE-Malabar Botanical Garden & Institute for Plant Sciences
Kozhikode, Kerala, India

2024

KSCSTE-MBGIPS

KSCSTE - MALABAR BOTANICAL GARDEN AND INSTITUTE FOR PLANT SCIENCES

(An Institution under Kerala State Council for Science, Technology & Environment)

Post Box No. 1, Kozhikode - 673 014, Kerala, India. Phone: +91 495 2430939

Email: malabarbot.garden@gmail.com, www.mbgs.in

KSCSTE

CERTIFICATE

This is to certify that the thesis entitled "**Bioprospecting of lichens of the genus *Parmotrema* A. Massal. (Parmeliaceae) in Kerala**"submitted to the University of Calicut by Mr. Bibin Joseph., in partial fulfilment for the award of the degree of Doctor of Philosophy in Botany is a bonafide record of the research work carried out by him under my supervision and guidance. No part of the present work has previously formed the basis for the award of any other degree or diploma.

Kozhikode
31.05.2025

Dr. N. S. Pradeep
Research Supervisor

Dr. N.S. Pradeep
Principal Scientist
KSCSTE - Malabar Botanical Garden
and Institute for Plant Sciences
Kozhikode - 673 014, Kerala

DECLARATION

I hereby declare that the work presented in the thesis entitled "**Bioprospecting of lichens of the genus *Parmotrema* A. Massal. (Parmeliaceae) in Kerala**" is based on the original work done by me under the guidance of **Dr. N. S. Pradeep** and has not been included in any other thesis submitted previously for the award of any degree. The contents of the thesis are undergone plagiarism check using iThenticate software at C.H.M.K. Library, University of Calicut, and the similarity index found within the permissible limit. I also declare that the thesis is free from AI generated contents.

Kozhikode
31.05.2025

Bibin Joseph

Dr. N. S. Pradeep
Principal Scientist
KCCSTE-MBGIPS, Kozhikode

Dr. N.S. Pradeep
Principal Scientist
KSCSTE - Malabar Botanical Garden
and Institute for Plant Sciences
Kozhikode - 673 014, Kerala

ACKNOWLEDGEMENTS

First and foremost, I express my heartfelt gratitude and indebtedness to my research guide, Dr N. S. Pradeep, Principal Scientist (Scientist in charge), KSCSTE-Malabar Botanical Garden and Institute for Plant Sciences, Kozhikode, for his full support, understanding, and consistent encouragement throughout my work. His academic enthusiasm and energy have always been a source of inspiration.

I would like to thank Dr S. Pradeep Kumar, former Member secretary of KSCSTE (former Director in charge, KSCSTE-MBGIPS), and Dr R. Prakashkumar, former Director, KSCSTE- MBGIPS, for providing the necessary facilities to carry out my work and for their valuable suggestions and support throughout it.

I sincerely acknowledge the help and encouragement of Prof. P.V. Madhusoodanan, Former Emeritus Scientist at KSCSTE-MBGIPS, and Prof. M. Sabu, Emeritus Scientist at KSCSTE-MBGIPS.

I am incredibly thankful to Dr. Stephen Sequira, Ass. Professor, P.G. Department of Botany, Govt. Maharajas College, Eranakulam, and his team for the help and support rendered during the study.

I thank Dr. V.B. Sreekumar, Principal Scientist at KSCSTE-KFRI, Peechi, and Dr.Siljo Joseph, Junior Scientist at KSCSTE-KFRI, Peechi, for their help and support during the study.

I wish to thank Dr Manjula K.M., Dr Hareesh V.S., Dr Raghupathi**,** Junior Scientists, KSCTSE-MBGIPS, for their help and support during the study.

I want to express my gratitude to Mr Mithun Venugopal, Mrs Jaseela V.T., Mrs Sinisha E.K., Ms Pooja Pushakaran, and Mr Harshid P., Research scholars at KSCSTE-MBGIPS, Kozhikode, for their wholehearted support, timely help, and encouragement throughout the study period.

The sincere assistance extended by my colleagues in MBGIPS, Kozhikode, Mrs Liji, M. Mr Hareesh K.T., Dr Sanal C. Viswam, Mrs Rajisha T. K., Mrs Priya Andrews, Mr Prashob, Mr Reeja, and Mr Bidhusha at various stages of this work is gratefully acknowledged.

I sincerely thank Dr Arun K. Das, Dr Anoop K. P., Dr Rajilesh, V. K., and Dr Anoop. P. Balan, Mr Ajesh P. P., Mr Jithu M., and Mr Vimal Raveendran, former staff of MBGIPS, for their timely help and encouragement throughout the study period.

I wish to thank all the administrative and non-administrative staff of MBGIPS, Kozhikode, for their valuable help on various occasions during this study.

I am deeply indebted to my beloved parents, my loving daughter, my wife, and all other family members and relatives for their support, encouragement, and blessings to accomplish this study.

Above all, I thank the Almighty for showering me with unseen blessings, which enabled me to complete my work successfully.

Bibin Joseph

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	:	Percentage
µg	:	Microgram
µM	:	Micro molar
^{0}	:	Degree
C	:	Centigrade
A	:	Absorbance
ABTS	:	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid
CALI	:	Herbarium of the Calicut University
DPPH	:	2,2-Diphenyl-1-picrylhydrazyl
G	:	Gram
IC_{50}	:	Inhibitory concentration 50
L	:	Litre
l.c.	:	loco cited
M	:	Molarity
MBGH	:	Malabar Botanical Garden Herbarium
MBGIPS	:	Malabar Botanical Garden & Institute for Plant Sciences
m	:	Meter
mg	:	Milligram
mg/ml	:	Milligram per millilitre
min	:	Minute
mL	:	Millilitre
mM	:	Millimolar
mm	:	Millimetre
NaCl	:	Sodium chloride
NaOH	:	Sodium Hydroxide
OD	:	Optical Density
S.lat.	:	Sensu lato
S.str.	:	Sensu Stricto

ABSTRACT

Parmotrema A. Massal.(Parmeliaceae family) is the dominant lichen genera of Kerala part of the Western Ghats. The present study has made two additions to the lichen biota of Kerala: *Parmotrema claavuliferum* (Räsänen) Streimann and *Parmotrema abnuens* (Nyl.) Hale, rediscovered one untraceable species *Parmotrema margaritatum* (Hue) Hale and also reported the extensive occurrence of three species *Parmotrema tsavoense* (Krog &Swinscow) Krog &Swinscow and *Parmotrema chinense* (Osbeck) Hale & Ahti. The ecological and bio-geographic evaluations of the 29 species were conducted regarding alpha diversity and IVI value. The biogeography of these species was recorded using GIS, and distribution maps were prepared. The chemical prospecting of these lichens was carried out to identify the active principles and to find out the novel phytochemicals with pharmaceutical and industrial applications. The GC-MS analysis elucidated several active principles in this genus. Benzoic acid, Benzaldehydes and Ethyl 2,4-dihydroxy-6-methylbenzoate Orcinol and Squalene are compounds with anti-cancer, antibacterial, anti-fungal, anti-oxidant, Anti-diabetic, and Anti-inflammatory properties. Phthalic acid is reported to have insect-repellant and larvicidal properties. The total phenol and flavonoid content was determined, and *P. clavuliferum* is the species with high phenol and flavonoid content. The quantity of phenols and flavonoids has correlation with several medicinal properties. The anti-microbial properties of the genus *Parmotrema* were evaluated against various pathogenic bacteria and fungi, and their antimicrobial properties were evaluated.

In summary, the rich lichen biodiversity of the genus *Parmotrema* of Kerala and the broad spectrum of chemical substances within this genus have immense biopharmaceutical potential and industrial applications. Hence, the conservation and sustainable utilization of these resources require immediate attention and action.

Keywords: *Parmotrema,* bio-diversity, phytochemicals, anti-microbial properties, antioxident properties

സംഗ്രഹം

പാർമോടിമ എ. മസൽ. (പാർമീലിയേസി കുടുംബം) പശ്ചിമഘട്ടത്തിന്റെ ഭാഗമായ കേരളത്തിലെ പ്രധാന ലൈക്കൺ ജനസ്സാണ്. ഇപ്പോഴത്തെ പഠനം, ലൈക്കിൻ വൈവിധ്യത്തിലേക്ക പാർമൊടീമാ ക്ലാവ്ലിഫറും എന്ന ഇനം കൂട്ടി ചേർക്കുകയും പർമോടിമ മാർഗരിറ്റിറ്റം വീണ്ടും കണ്ടെത്തുകയും കൂടാതെ നിലനിൽപ്പ് അപകടകരമായി കുറഞ്ഞു വരുന്ന മൂന്ന് ഇനം ലൈക്കനുകളുടെ വ്യാപകമായ ആവാസസ്ഥലങ്ങൾ റിപ്പോർട്ട്ചെയ്യുകയും ചെയ്തു. ആൽഫവൈവിധ്യവും IVI മൂല്യവുംസംബന്ധിച്ച് 29 ഇനങ്ങളുടെ പാരിസ്ഥിതികവും ജൈവ-ഭ്രമിശാസ്ത്രപരവുമായ വിലയിരുത്തലുകൾ നടത്തി. ഈ സ്പീഷിസുകളുടെ ബയോജിയോഗ്രഫി ജി.ഐ.എസ്. ഉപയോഗിച്ച രേഖപ്പെടുത്തുകയും വിതരണ ഭ്രപടങ്ങൾ തയ്യാറാക്കുകയും ചെയ്തു. സജീവതത്വം നിർണയിക്കുന്നതിനും ഫാർമസ്യൂട്ടിക്കൽ, വ്യാവസായികപ്രയോഗങ്ങളുള്ള നോവൽ ഫൈറ്റോകെമിക്കലുകൾ കണ്ടെത്തുന്നതിനുമാണ് ഈ ലൈക്കണുകളുടെ രാസപരിശോധന നടത്തിയത്. ജി.സി.-എം.സ്. വിശകലനം ഈ ജനസ്സിലെ നിരവധി സജീവതത്വങ്ങൾ വ്യക്തമാക്കി. ബെൻസോയിക് ആസിഡ്, ബെൻസാൽഡിഹൈഡുകൾ, എഥൈൽ 2,4-ഡൈഹൈഡ്രോക്സി-6-മീഥൈൽ ബെൻസോയേറ്റ്, ഓർസിനോൾ, സ്കാലീൻ എന്നിവ കാൻസർ, ആന്റിബാക്ടീരിയൽ, ആൻറിഫംഗൽ, ആന്റിഓക്സിഡന്റ് ആൻറി-ഡയബറ്റിക്, ആൻറി-ഇൻഫ്ലമേറ്ററി ഗുണങ്ങളുള്ളസംയുക്തങ്ങളാണ്. ഫ്താലിക് ആസിഡിന് പ്രാണികളെ അകറ്റാനും ലാർവിസൈഡൽ ഗുണങ്ങളുമുണ്ട്. മൊത്തം ഫിനോൾ, ഫ്ലേവനോയ്ഡ് ഉള്ളടക്കം നിർണ്ണയിക്കപ്പെട്ടു, ഉയർന്ന ഫിനോൾ, ഫ്ലേവനോയ്ഡ് ഉള്ളടക്കം എന്നിവയുള്ള പാർമൊടീമാ ക്ലാവ്ലിഫറും ഇനത്തെ കണ്ടെത്തി. ഫിനോളുകളുടെയും ഫ്ലേവനോയ്ഡുകളുടെയും അളവിൽ നേരിട്ട്നിരവധി ഔഷധഗുണങ്ങളുണ്ട്. വിവിധ രോഗകാരികളായ ബാക്ടീരിയകൾക്കും ഫംഗസുകൾക്കുമെതിരെ പാർമോടിമ ജനസ്സിലെ ആന്റിഓക്സിഡന്റ് ഗുണങ്ങൾ വിലയിരുത്തുകയും അവയുടെ ആന്റി മൈക്രോബയൽ ഗുണങ്ങൾ നിർണ്ണയിക്കുകയും ചെയ്തു.

ചുരുക്കത്തിൽ, കേരളത്തിലെ പാർമോടേമ ജനസ്സിലെ സമ്പന്നമായ ലൈക്കൺ ജൈവവൈവിധ്യവും ഈ ജനസ്സിലെ രാസപദാർത്ഥങ്ങളുടെ വിശാലമായ സ്പെക്ട്രവും ബയോഫാർമസ്യൂട്ടിക്കൽ സാധ്യതകളും വ്യാവസായിക പ്രയോഗങ്ങളും ഉൾക്കൊള്ളുന്നു. അതിനാൽ, ഈ വിഭവങ്ങളുടെ സംരക്ഷണത്തിനും സുസ്ഥിരമായ ഉപയോഗത്തിനും അടിയന്തിര ശ്രദ്ധയും പ്രവർത്തനങ്ങളും ആവശ്യമാണ്.

സൂചകപദങ്ങൾ: പാർമോടിമ, ജൈവവൈവിധ്യം, ഫൈറ്റോകെമിക്കലുകൾ, സൂക്ഷ്മജീവ വിരുദ്ധ ഗുണങ്ങൾ, ആന്റിഓക്സിഡന്റ് ഗുണങ്ങൾ

CHAPTER 1
INTRODUCTION

1. Introduction

Lichens are fascinating members of the living world with an intimate mutualistic relationship between fungi and photosynthesizing algae or cyanobacteria. This mutualistic association has created more than 20,000 species worldwide. The Indian subcontinent is home to about 3,028 lichen species (Awasthi, 2000; Sinha, 2021), with Kerala accounting for 27% of this total diversity (Purushothaman *et al.,* 2021; Anilkumar *et al*., 2022; Sequeira *et al*., 2022) of which India alone has about 2040 endemic species (Awasthi, 2000).A lichen is a self-supporting and self-regulating symbiotic organism where fungal (mycobiont) and algal or cyanobacterial (photobiont) partners exist and function together (Honegger, 1991; Kirk *et al.,* 2008). The dual components of the lichens are well established by studying the anatomy of lichen thalli as well as by gathering molecular evidences. The lichens are used for food, medicine, perfumery, cosmetics, dyes, preservatives and biocontrol agents as the list extends. The biopharmaceutical and industrial applications of lichens have not yet been fully disclosed. The wide spectrum of phytochemical substances unique in the living world due to their capacity to adapt to diverse climatic conditions, their presence in almost diverse regions of the globe and their versatility in reproduction and occurrence in diverse habitats make the lichens unique (Hale,1979).

The classical definition of lichens describes them as a group of plants holding a unique and harmonious association between the algae (phycobiont) and fungi (mycobiont), and the relationship is termed mutualism. Nowadays, critical studies and developments in lichenology have revealed mycobiont's dominance over the phycobiont. Hence, the term heliotropism is more appropriate to describe such an association. Lichens are one of the pioneer inhabitants of barren rock or a dry substratum in xeric ecological succession. They are very sensitive to environmental pollution and specific to the growing substratum. Either Ascomycota (ascocarp bearing) or Basidiomycota (typical mushrooms) constitute the fungal thallus and algal component predominantly a Chlorophyceae or Cyanobacterium. They are economically important, being the source of food, medicine, preservatives, dyes, energy sources, *etc.* They can also be used as potential systems in pollution

monitoring, being very sensitive to atmospheric changes and other experimental purposes (Scheidegger, 2002).

1.1. Origin, Evolution, and History of *Parmotrema*

The Genus *Parmotrema* A. Massal.; belongs to the family Parmeliaceae and is one of the most common and well-known ascomycete families. It is the largest family of lichen in the world and is currently estimated to comprise more than 60 genera. Acharius (1803), the disciple of Linnaeus and the father of lichenology, proposed a genus *Parmelia* as an umbrella term to include all the then-known foliose lichen with leporine apothecia, rhizine and simple spores (Fries, 1861). At that time, systematic problems prevailed in the proposed genus since the members included were not only dissimilar in morphological-reproductive structures but also in growth forms. Hence, the 19th and 20th centuries witnessed tremendous changes and voluminous works in the original genus proposed by Acharius. In the early sixties, with the aid of scanning electron microscopy and the vast knowledge gained through the phytochemical studies of lichen substances, the monographic revision study of most of the taxa coming under the family Parmeliaceae was carried out by Hale (Hale, 1974). The studies originated in the early sixties and were continued for three decades, and as a result of these studies, the heterogeneous members of the genus were separated into several homogeneous genera. Thus, the taxonomic structure and hierarchy of the family Parmeliaceae have a solid structure.

The early sixties of the 20th century witnessed momentum in entire fields of lichenology, which is obtained through the application of SEM in analyzing detailed morphology; increased knowledge of lichen substances and secondary metabolites has contributed much to lichen species delimitation. The developments are well visible in the series of monographic revisions of the majority of taxa of Parmelioid lichen by Hale, and it was continued for almost three decades. As a result, the species of the Genus *Parmelia* were successfully segregated into several genera, which are more or less homogeneous in nature.

The number of Genera in this family has increased over the last three decades partially due to the narrow species concept and the momentum attained in the fields

of taxonomy by the combined application of phytochemistry and molecular aspects in species delimitation. All the lichens in this genus show foliose growth form (Ohmura, 2009). The family Parmeliacee is mostly found in tropical regions of the world. It comprises more than 2400 species (Blanco *et al*., 2004). Within this large family, parmelioid lichens, formerly in the huge genus *Parmelia*, are a monophyletic group based on mitochondrial SSU sequence analysis (Crespo *et al*., 2010). They also are defined morphologically in typically havingrhizinate thalli with laminallecanorine apothecia, a Lecanora-type ascus and simple hyaline ascospores. Parmelioid lichens comprise more than 1500 species and exhibit remarkable diversity, especially in oceanic-temperate, tropical and subtropical ecosystems (Elix, 1993).

1.1.1. Morpho-Taxonomy

Morpho-taxonomic characterization of the species is the pivotal step in all scientific processes related to an organism. Nowadays, information about internal chemistry and genetic information through DNA analysis is also significant with the advent of molecular biology. Even though morpho-taxonomy is the keystone of species determination, this is also valid for lichens. The growth form of the thallus is the primary character of lichen. There are three major growth forms, and these are crustose lichens, which form a crust over the surface they grow; foliose lichens, which are leafy, and have two easily distinguishable sides; and fruticose lichens, which have pendants or hair-like thallus. Just like any other vascular plants, lichens also have their own asexual and sexual reproductive structures, and these structures also have to play the systematic of lichen.

1.1.2. General Characters of the Genus *Parmotrema*

Globally, approximately 350 *Parmotrema* A. Massal. species have been reported so far. Itis a common lichen genus scattered throughout the Indian Peninsula, and it is widely distributed in the high elevations of the Western Ghats ranging from 500 ft. above the mean sea level. The genus *Parmotrema* belongs to the family Parmeliaceae, one of the largest genera of parmelioid core in the family Parmeliaceae. (Blanco *et al*., 2006) All the members of the genus *Parmotrema* have

foliose thallus, and with very few exemptions, all possess nude or rhizine-free lower margins. This character can be used to distinguish *Parmotrema* from other similar foliose lichens. The broad thallus is loosely attached to the substratum, and the substratum either is corticolous or saxicolous. The lobe margins are often rotund. In the genus *Parmotrema*, ciliate and non-ciliate species are there. The ciliate lichens show variations in the morphology of cilia, such as simple, bifurcate or branched. The thallus is pale grey to grey-green in colour, and some members have maculate thallus. Sometimes, the maculation in the thallus creates a reticulate pattern on the upper surface. The thallus is heteromerous in nature it is due to the differential arrangement of the algal layer and fungal layer in a thallus. The thallus is corticated on both the upper and lower sides. The medulla is formed by the fungal hyphae. The medulla is often white or sometimes pigmented to various colourations. The lower surface of the thallus is black or tan brown in the genus *Parmotrema*. While the lower marginal area shows peculiar shades in various *Parmotrema* species, the lower margin appears white in some species. The rhizinesare mostly found in the centre and are often simple but rarely branched (Crespo *et al*., 1999).

The occurrence of vegetative reproductive structures like isidea, soredia and pycnidia create demarcation arrangements in the surface of the thallus. Isidea is often in the form of simple, coralloid branched columnar, columnar branched or even absent whereas the soralia may be marginal submarginal or labriform in arrangement. These characters are specific to species to species, and hence, these can be used as key characters in species delimitation in the genus *Parmotrema*. Pycnidia are located laminal in position, immersed in the thallus and an urn in shape, filled with conidial spores. The conidia in the pycnidia are sub-lageniform, filiform or bacilliform in origin and structure. In this genus, spores are 8 in number in a single ascocarp, and the number of asci is around 20, and its size ranges from 1–1.5 µm. like the common dung fungi peziza, apothecia is a common reproductive structure. As ascomycete fungi, the apothecia present in *Parmotrema* are located in the laminal position. The apothecia are leporine type and generally pedicellate. The disc of the apothecia shows brown, entire, perforate or imperforate. Epithelium is brownish, but the hypothelium is colourless, and hymenium is also colourless. The

Iodine test gives a positive result by the generation of blue colour.TheAscospore bears 8 asci ortheascocarp is 8-spored; ascospores are colourless, simple, ellipsoid, sub-lageniform in shape, thick-walled, measuring 8–37µm X 5–8 µm (Lawrey, 1980).

1.1.3 Economic Importance of the Genus *Parmotrema*

Lichens are useful to mankind in different aspects of life. Primitive humans classified plants according to their daily needs, starting with food, medicine, fodder, aesthetics, and dyes, but modern man gives adequate importance to plants with economic interests and environmental aspects. Lichens are a group of plants that fascinated aborigines as well as modern man for their wide spectrum of applications.

Pioneer Colonizers in Xerarch Succession

The establishment of biotic communities over uninhabited patches of land is one of the miracles of nature, and it is an essential phenomenon of life on earth. The formation of biotic communities over dry or xeric areas is known as xerarch succession. The pedogenesis or the process of formation of soil is also invariably related to xerarch succession. Xerarch succession cannot be proceeding without the action of crustose lichen. The igneous rock or the mother rock, which is formed directly from the action of volcanic eruption, will initially lead to the lava flow, which is effluent with the minerals and will finally deposit on cooling and solidify to form igneous rocks. All other forms of rocks, including granites, are derived from igneous rocks (mother rock). Here arises the importance of crustose lichen. Many of the crustose lichens, especially *Caloplaca, Lecanora, Rhizocarpon*, etc., which often appear as greyish or greenish patches, are actively involved in the rock weathering process.(Adamo and Violante, 2000) The organic acids, especially the oxalic acid produced by the mycobionts, will actively participate in the soil formation and xerach succession. In addition to oxalic acid, phenol compounds often referred to as lichen substances or lichen acids, can react with these minerals and form metal complexes. From this, it is clear that the thalus or the hyphae don't penetrate into the substratum. Still, the substances in lichens, which act as solvents, break down the toughest rocks by targeting the less resistant particles. This causes the rock to

crumble, allowing the lichen's hyphae to grow between the loosened fragments. Consequently, the lichen can become almost completely integrated with the disintegrated rock, with the gelatinous hyphae binding the entire mass together and attaching it to the intact rock. (Adamo and Violante, 2000) As the season progressed, especially during the dry summer, the lichen died, and the decayed thallus, along with the disintegrated rock particles, rightly termed as soil primordial, formed the suitable substratum for the higher lichen such as foliose and fruticose lichens. The biomass and lichen substance left behind by these higher lichens are always larger when it is comparing with biomass left behind by the crustose lichens. This will lead to suitable climatic and ecosystem formation by nature. Subsequently, higher forms of organisms will be established here as serial communities (Jones, 2019). Therefore, it is fitting to consider lichens as one of the most selfless forms of life in nature, as they exist entirely for the benefit of others. Unlike lichens, all other eukaryotes depend on a substrate formed from the death and decay of other organisms. The only notable exceptions to the lichens' altruistic nature are certain chemoautotrophic bacteria, which are comparatively less significant in this context.

Food, Fodder and Spices

Lichens are very slow-growing cryptogams, so it is not included in the food basket of man since we cannot depend on them in large quantities over a long time for all the seasons. Their bitter or unpleasant taste is another reason. At the same time, there are exemptions to this, especially in the polar regions of the globe. In the Biblical story of the divine ‘Manna’, There has long been a range of opinions within the scientific community regarding the true origin and nature of the 'Manna' that is said to have fallen from heaven to feed the Israelites during their Exodus from Egypt to the Promised Land. One candidate for this 'heavenly Manna' is *Lecanora esculenta*, lichen found in the highlands of North Africa and the deserts of West Central Asia (Peveling, 1973). It is also notable those in times of drought, *Lecanora esculenta* forms very light balls easily taken by air, which are carried away by the strong winds blowing along the desert. The formation of these lichens on rocks (crustose lichens) gives a thick wrinkled appearance on rocks and gradually by the effect of heat and radiation from the sun they eventually dried and were carried by

the wind and deposited in large quantities by rain showers in the depressions of deserts and these were used by the people and merchants in their way through the deserts, especially in times they are facing a shortage of food. Their massive quantity often gives the feel that these were gathered and showered by the heavens for the exhausted travelers, and hence it got the name 'Manna' of the heavens. *Cetraria islandica* belongs to the *Parmaliaceae family* and is commonly known as Iceland moss. It is used by Scandinavian countries as a food supplement since it contains active principles such as lichenin or lichen starch (Svanberg and Eggisson, 2012). It has the same chemical composition as starch but differs in that it usually does not give the blue reaction with iodine. It is to this substance that lichens owe their nutritive properties. The peasantry of Norway, Iceland, Sweden and Scandinavian countries powdered *Cetraria islandica* and mixed it with flour from various cereals and mashed potatoes, from which (the lichen) an "uncommonly palatable and healthful and easily digestive bread is prepared." It has been used as a natural remedy against scurvy, known as Iceland scurvy, once prevalent in Iceland and other Scandinavian countries. *Umbilicaria mühlenbergii* is lichen that is also used as food in North Africa and West Central Asian deserts. *Umbilicaria esculenta* is lichen, and it is utilised by the people and explores the Artic (Wang *et al.,* 2014). Notable Artic explorationled by Captain Sir John Franklin, reported the usage of Rock-tripe, a species of Umbilicaria, *Umbilicaria esculenta* as a food supplement, also of trappers and hunters in Canada and Alaska (Thajuddin *et al*., 2019). Some of Franklin's companions discovered, to their regret that consuming this lichen led to severe intestinal inflammation, likely due to the bitter substance it contains. This bitter principle is found in most lichens and is believed to be removed through repeated washing or soaking in a solution of potash or another alkali. In Northern Europe, a common method to prepare lichens for consumption involved boiling them in milk after extensive washing in water. However, completely eliminating the bitter taste is challenging, which is why lichens were not widely used as food except during famines. In Norway and Sweden, people gather large quantities of various lichens to feed their livestock, and reindeer moss (*Cladonia rangiferina*) is well-known as a food source for the reindeer in Lapland of Finland (Pulliainen,

1971).*Cladina rangiferina*, *Cetraria islandica*, *Evernia prunastri* were processed into the ingredients of special bread, which was used in winter and famine in the Czech Republic, Iceland, Norway, and Estonia. (Airaksinen, *et al*., 1986)

Tropical countries like India with Monson climate have witnessed the luxuriant growth of leafy lichens and all other forms of lichen. This region will not undergo a severe winter and snowfall as the temperate regions of the globe witness. Hence, the lichens here are not considered as food, but it is traditionally an ethnobotanical considered as a spice. In the traditional cuisines of lichen-growing regions in India, *Parmelia*, *Heterodermia*, *Pyxine*, and *Physcia* are widely used to enhance the flavor of the Indian spicy dish 'Biryani'.

Parmotrema tinctorum, Parmotrema austrosinensis and *Parmotrema reticulatum* are the three species that are mainly collected from the Western Ghats of Kerala side and have high market demand and are extensively used as spices.A wild collector in the urge to full fill his monetary needs will unknowingly destroy more than 45 x 103 cm^2 well-grown lichen cover in one shot to collect 1 kg of lichen biomass. At the same time, the major ecological setback is that it will take approximately one year to grow 2-3 mm in length.

Lichens as Dyes

Probably the first reported industrial application of lichens is its colouring property over the fabrics. Theophrastus, the father of Botany and PedaniusDioscorides, the foremost pharmacologist of Europe, described *Rocella tinctoria* as a "marine fungus growing upon rocks of the shallow region, possessing colouring properties," from which it is concluded that dye made from the lichen was known before that time (Melo *et al*., 2016). It was probably in use before the time of Pliny the Elder. Later, it was found that the species *Lecanora, Pertusaria, Umbilicaria, Gyrophora,* etc., yielded excellent dyes (Bolton, 1991). It was reported by many travellers of Europe that two dyes, purple and red dyes, orchil and cudbear, were obtained from the lichens *Roccella* and *Ochrolechia*. The detailed industrial application for fabric processing using these dyes is also interesting. The fabrics were first immersed in a mixture of human urine and the dyes. The human urine will act as a mordent to the

dyes. Ammonia salts in the urine functioned as mordents to make the dyes permanent. The advantage of lichen dyes has also been noted in that the same lichen can induce colour variants as the reagent that is treated with the lichen. Generally, lichens are crushed and soaked in an alkaline solution or ammonia for a period, resulting in a vibrant purple color. As it is treated with an acidic reagent, the resultant color is pink and allied shades (Monaghan, 2001).

Lichen as an Indicator

Another grand old application of lichen is its application as an acid-base indicator or litmus. *Roccella montagnei* is one of the widely using litmus lichens. The wide spectrum of chemical compounds present in the lichens, which are derived as secondary metabolites, are unique to this group, and some of these secondary metabolites also have a chromomeric effect, from which the colouring matter is derived. Under the combined influence of ammonia and oxygen, lecanoric acid and erythrin in *Roccella montagnei* give orcin and subsequently orcein, which is the colouring matters of orchil and which, in the presence of sodium or potassium carbonates, form azolitmin and erythrolitmin (colouring matters of litmus) (Shah, 2024).

Lichen as Medicine

Before the advent of modern medicine and scientific practices, crude medicines were used, and the practices were often not standardized. According to London Pharmacopoea (1721 to 1788), *Nux vomica* and not powdered glass, was mixed with the lichen, which seems more probable the edible dog-lichen (*Peltigera canina*) formed the basis of the noted "anti-hydrophobia powder." It is mentioned in the records as pulvisantilyssns or pulvis contra rabiem) of the London Pharmacopoea (1721 to 1788) (Crawford, 2019). *Usnea barbata*, the fruticose lichen commonly called "beard moss," was initially recommended for specific female ailments. It later gained great value as a treatment for whooping cough and epilepsy, and as a pain reliever. Additionally, it is a key component in powders suggested to encourage hair growth. The yellow thallus coloured *Physcia parietina* was considered specific in jaundice (Gandhi *et al*., 2020) and used as a quinine substitute. During the

Napoleonic wars (1809-1815) fevers raged in the military hospitals. However, most of these medicines are now not recommended or the least preferred because of their drawbacks in curative properties for these particular diseases. Modern medicine is based mainly on an empirical and experimental basis, mainly on compounds rather than crude plants. The chemical constituents in lichens are very effective in many ailments. 'Sailaia' is the vernacular name for *Parmotrema perlatum*, is an essential component in the famous Navaratna oil, which is the formulation of nine herbs. *Parmotrema perlatum* plays the role of headache reliever, and it gives a cool and smoothening effect to effect to the head while using the Navarathna oil. Himalaya Pharmacy has three major products based on *Parmotrema perlatum ie.*V Gel, Confido and Speeman. The first formulation is based on the antifungal, antibacterial and anti-inflammatory properties of *Parmotrema perlatum,* and the later two medicines Promote spermatogenesis by improving the testicular, seminal vesicle and epididymal & Seminiferous tubules, bringing about improvement in semen quality (Balaji *et al* ., 2016). Usnea is a common ingredient in many pharmaceutical products. It is reported to have a special dermatoprotective function against solar radiation and profound antioxidant and antimicrobial properties due to the presence of Usnic acid in its thallus (Sharma and Mohammad, 2020).

1.2. Bioprospection

Bioprospecting can be defined as "the discovery and development of recent merchandise of chemical compounds, genes, micro-organisms, macro-organisms supported by biological resources". In contrast, Biodiversity refers to the variety of species within an ecosystem. The imperative to safeguard biodiversity and ensure equitable use of genetic resources and traditional knowledge has sparked one of the most contentious debates of the 21st century between developed and developing nations.This debate has fundamental implications for how basic and applied research on genetic resources and biodiversity is conducted and its results are made available between and within people and societies (Juan, 2017).

1.2.1. Lichen Compounds and their Biomedical Applications

Lichens are regarded as the reservoir of phytochemicals, and among these, certain compounds are the resultants of the symbiotic association between the mycobiont and the phycobiont. Hence, these are unique to lichens. These compounds are primary and secondary metabolites, and these compounds are essential for the survival of these symbionts in harsh environments, and these compounds have considerable biological activities (Vartia, 1973). Lichen substances can be broadly categorized into three primary groups. The first group comprises aliphatic compounds, including acids, Zeorin, and Polyhydric alcohols. The second group comprises aromatic compounds, such as pulvic acid derivatives, depsides, depsidones, quinones, xanthone derivatives, diphenylene oxide derivatives, nitrogen-containing compounds, triterpenes, and tetronic acids. The third group includes carbohydrates, specifically polysaccharides. Research has identified around 800 lichen substances globally (Zambare and Christopher, 2012) with the structures of approximately 300 of these substances having been determined. Typically, lichen secondary metabolites are insoluble in water, so organic solvents are employed for their extraction.

1.2.2 Secondary Metabolites of Lichens

Wherever lichens grow abundantly in nature that is whether in a high-elevation mountain peak, a branch of a roadside tree, in the left and right arms of the holy cross or even in an old monument which is kept undisturbed for years, impart fabulous colour to the substrate and also the thallus often develops colour and aroma for their self-protective mechanism against herbivorous animals and solar protection as well as metabolic functions. This is the basic reason behind the production of various phytochemicals, often referred to as lichen substances (Huneck, and Yoshimura, 1996), to protect from solar radiation and herbivorous animals. In addition to this, some compounds are produced as a result of symbiotic association more clearly to be in a symbiotic lifestyle or not to be a heliotropic association. As the degree of symbiotic relationship changes to a heliotropic relationship or the master-slave association, there come these metabolites to protect the symbiotic

association. The common lichen substances are usnic acid, lecanoric acid, salazinic acid, atranorin, etc. These entire compounds have remarkable medicinal and ecological functions (Condò *et al*., 1976; Cole *et al*., 2005; Kostyuk *et al*., 2012; Ginwala *et al*., 2019). The traditional and ethnobotanical practices were based on the formulations with the crude application as well as the decoction method, but the modern practices are much more refined; hence the active principle was isolated and utilized. From this, it is clear that irrespective of the mode application, lichen diversity plays a crucial role in traditional and modern therapeutic practices.

Strictic acid has been identified as an effective superoxide anion scavenger, exhibiting potency comparable to ascorbic acid while demonstrating minimal toxicity to human cell lines. This suggests its potential use in preventing proliferative diseases in humans (Ismed *et al*., 2017). Additionally, Leconoric acid shows moderate antibacterial activity against *Pseudomonas aeruginosa* and limited activity against *Staphylococcus aureus*. In vitro cytotoxicity assessments have indicated that Leconoric acid is cytotoxic to HELA cell lines and significantly affects A549 cell lines (Zhang *et al*., 2024). These findings underscore the potential of parmelioid lichens as valuable sources of medicinal compounds.

Usnic acid is a solid yellow substance that is said to protect the lichens against sunlight, also, being bitter, it protects from herbivorous animals. Usnic acid is a natural antibiotic and hepatoprotective agent found exclusively in lichens, and its presence can be easily detected with the pale green or yellow thallus colour. Atranorin is one of the common chemical substances in paemelioid lichens produced as a secondary metabolite in cortical and medullary substances and has profound antiviral properties, especially against HIV (Neamati *et al.*, 1997). All these show the potential application of parmelioid lichens as a true source of resourceful medicine against various ailments.

This thesis explores the diverse applications of these lichens in addressing various human health issues as well as the scope of lichens in which the foliose lichens of the genus *Parmotrema* can explored such as functional food and spice, medicines and enzymes, commercially useful merchandise and ecological indicator. Lichens,

known for their unique symbiotic relationship between fungi and algae, have garnered significant interest due to their diverse bioactive properties. This thesis aims to delve into the potential of *Parmotrema* lichens from Kerala, with a focus on several key objectives. The study will involve the systematic exploration, collection, and preservation of *Parmotrema* specimens from various regions within Kerala. This foundational step is crucial for ensuring the availability of high-quality samples for subsequent analyses. Following collection, the research will employ a multifaceted approach to identify these lichens. This will involve morphological assessments, molecular techniques, and phytochemical evaluations to accurately classify and characterize the specimens.The antimicrobial properties of the *Parmotrema* lichens will be rigorously tested to evaluate their effectiveness against a range of microbial pathogens. This includes examining their potential to inhibit bacterial growth, which could reveal novel antimicrobial agents. In addition to antimicrobial testing, the antioxidant potential of these lichens will be assessed. Antioxidants play a vital role in neutralizing harmful free radicals, and evaluating this property could highlight their potential therapeutic benefits. The study will conduct phytochemical screenings and bioprospecting to identify and characterize the chemical compounds present in the lichens. This step aims to uncover valuable bioactive molecules that could contribute to new medicinal applications.Overall, this research seeks to uncover the medicinal potential of *Parmotrema* lichens through comprehensive exploration, identification, and bioactivity assessments, potentially leading to new insights and applications in the field of natural products.

1.3 Objectives of the Study

- Exploration collection and preservation of the lichen (*Parmotrema)* from Kerala.
- Identification of the specimens based on morphological, molecular and phytochemical evidence.
- Studies on the antimicrobial potential of the specimens.
- Studies on the anti-oxidant potential of the specimens.
- Preliminary phytochemical screening and bioprospecting.

CHAPTER 2
STUDY AREA

2. The Study Area

Kerala, a region of unparalleled natural beauty, is a treasure trove of biodiversity and cultural heritage. Its diverse ecosystems, species, and genetic resources make it a fascinating area of study. With an average of 10000 km^2 patches of original tropical forests, Kerala is home to about 10000 species of plants, spanning vascular, non-vascular, and lower groups in its pristine habitats. The state boasts about 5100 taxa under 1537 genera and 221 families of flowering plants (Sasidharan, 2012). A total of 1709 taxa, unique to Peninsular India, find their home in Kerala, with 237 species distributed in 47 families exclusively endemic to the state's current political boundary (Nayar *et al*., 2008). Kerala, nestled in the 'hottest' of hotspots of endemism in India, proudly hosts one Biosphere Reserve, two National Parks, and twelve Wildlife Sanctuaries.

2.1 Kerala Part of the Western Ghats

Kerala, located between $8^o18'$ and $12^o4'$ North latitudes and between $74^o52'$ and $77^o22'$ East longitudes, is a region of stable lichen diversity. The diversity of lichens, influenced by climate, elevation, pollution, and land use pattern, is particularly stable based on elevation. The state's major lichen habitats have been identified and these regions have been grouped into three based on elevation, providing a reliable framework for our research.

The study area experiences tropical monsoons with seasonally excessive rainfall and hot summer as per Koppen's classification. Because of the uniformity, the entire state is classified as one meteorological sub-division for climatological purposes. The state has four types of climate such as winter, summer, South west monsoon and North east monsoon. As the state extends from north to south with the Arabian Sea on its west border, it also has high relative humidity. The total annual rainfall in the State varies from 360 cm to 180 cm.

2.1.1 Zone 1- Regions with Elevation below 800m

The topology and elevation of Kerala play a significant role in shaping the faunal and floral diversity of the region. The coastal zone orlowland area contributes the

major portion ofzone 1. While the coastal zone is generally devoid of macro lichens, the higher elevations of zone one boast a significant diversity of lichens. The lichens, belonging to the genus Graphidaceae, are luxuriantly found in the plantation crops in this region. The lowest point in Kerala, the Kuttanad region, is also part of Zone 1 and is located below sea level. The highest point where samples were collected is Meppadi and adjoining regions in the Wayanad district of Kerala. Puthurvayal, Karappuzha, and Kolagappara are other regions in the Wayanad district listed in Zone 1. Zone 1 also includes the rain shadow region of Kerala, Chinnar of Idukki district. Thus, zone 1 encompasses various geographic and climatic regions, each contributing to the unique and diverse nature of Kerala's biodiversity.

Sl. No	**Location**	**Zone**	**Elevation**	**Lattitude (N)**	**Longitude (E)**
1	Vaduvanchal	Zone 1 Altitude below 800 m	400	11.5210364	76.2393654
2	Chennalode		690	11.6631996	75.98487763
3	Arijermala		719	11.7146403	76.06218148
4	Neelimala		725	11.5327544	76.23645906
5	Kolagapara		753	11.61955001	76.17823095
6	Karappuzha		766	11.58407531	76.15903076
7	Puthoorvayal		766	11.58748825	76.09855323
8	Periya		769	11.81961661	75.83803087
9	Meppadi		785	11.57728355	76.14728895
10	ChinnarWildlifeSanctuary		600	10.307230	77.206821
11	Rosemala		400	8.914981	77.170086
	MBGIPS		22	11.237659	75.82796

Table 1 – list of sample sites in zone 1

2.1.2. Zone 2- Regions with Elevation between 800m- 1200 m

Zone 2 of the study area contains ten sample sites, and all the sites are hilly regions with plantations and forests prevailing land use patterns. Most of the sites were protected areas, so that, human encroachments are limited. Four sample sites belong to the districts Wayanad and Idukki. Palakkad and Pathanamthitta districts have one

sample site. Plantation crops and cash crops are luxuriantly grown, and the coconut and areca nut trees provide nesting places for lichens. Here, the climate of the same contours is uniform in the sample sites irrespective of the district where they are situated. All the sample sites and their geo coordinates and elevation of zone 2 are listed in Table 2.

Sl. No	Location	Zone	Elevation	Lattitude (N)	Longitude (E)
1	ChooralMalai	Zone 2 Altitude between 800m-1200m	818	11.51799489	76.14309851
2	Thirunelli		851	11.88324358	76.02617202
3	Wayanad Wildlife Sanctuary		856	11.67626877	76.36811484
4	Irulam		890	11.75776861	76.19951054
5	Idukki WLS		800	9.781676	76.957735
6	Bison valley.		900	10.005237	77.112427
7	Pettimudi		950	10.017922	76.980379
8	Cardamom hills		1000	9.866387	77.148758
9	Kakki		1000	9.316901	77.142507
10	Nelliampathy		1000	10.537857	76.693735

Table 2 – list of sample sites in zone 2

2.1.3. Zone 3- Regions with Elevation above 1200m

Nine sample sites were selected, all of which are hill stations and protected areas. Anamudi is the highest point of the Western Ghats and South India. Chembra Peak is the highest peak in the Wayanad district. The study area includes two national parks, Eravikulam National Park and Anamudi Shola National Park, and Idukki Wildlife sanctuary. Tea plantations are the common land use pattern in the sample sites of the Idukki district. The sample sites in zone three of Wayanad district have various plantation crops, such as coffee and coconut, as the major crops, and agriculture is the major land use pattern. All the sample sites and their geo coordinates and elevation of zone 3 are listed in Table 3.

The elevations in Tables 1, 2, and 3 are the regions where maximum lichen diversity is observed. It is also noted that the highest peaks of zone three have significantly less lichen biota compared with the ecotones of the peak. The foliose lichens prefer open forests, and they appear in the canopy when the forest is dark and scanty sunlight is present. In such forest types, samples were collected from fallen twigs.

Sl. No	Location	Zone	Elevation	Lattitude (N)	Longitude (E)
1	Chembra	Zone 3 Altitudeabove 1200m	1252	11.51220149	76.07799674
2	Vellarimala		1389	11.47523043	76.13555719
3	Banasuramalai		1911	11.69295873	75.90831713
6	Periyar WLS		1200	9.4622	77.2368
7	Gundumalai		1400	10.177959	77.105693
8	Eravikkulam		1600	10.1354	77.0366
9	Anamudi Shola NP		2200	10.2083	77.0833

Table 3 – list of sample sites in zone 3

Among the 31 sample sites listed in Table 1, 2 and 3, most of them belong to the Wayanad and Idukki districts of Kerala. Wayanad district of Kerala stands on the southern top part of the Deccan Plateau, and its major attraction is the grand Western Ghats with the lofty ridges interspersed with dense forests and green valleys. Most of the area in this district is covered by dense forests and protected areas. Brahmagiri (1608 meters), Banasura Peak (2073 meters), and Chembara Peak (2100 meters) are the main mountains in Wayanad. These mountains are surrounded by rainforests, where rare species of animals and plants are found. Kabani River and its three tributaries, the Panamaram, Mananthavady, and Kalindy, flow through the Wayanad. Kabini is an important tributary of the Kaveri River. Kabini is one of the only three rivers in Kerala that flows to the east. Wayanad has a salubrious climate. Generally, the year is classified into four seasons: cold weather, hot weather, southwest monsoon and northeast monsoon. This place experiences a high relative humidity, which goes even up to 95 per cent during the southwest monsoon period.

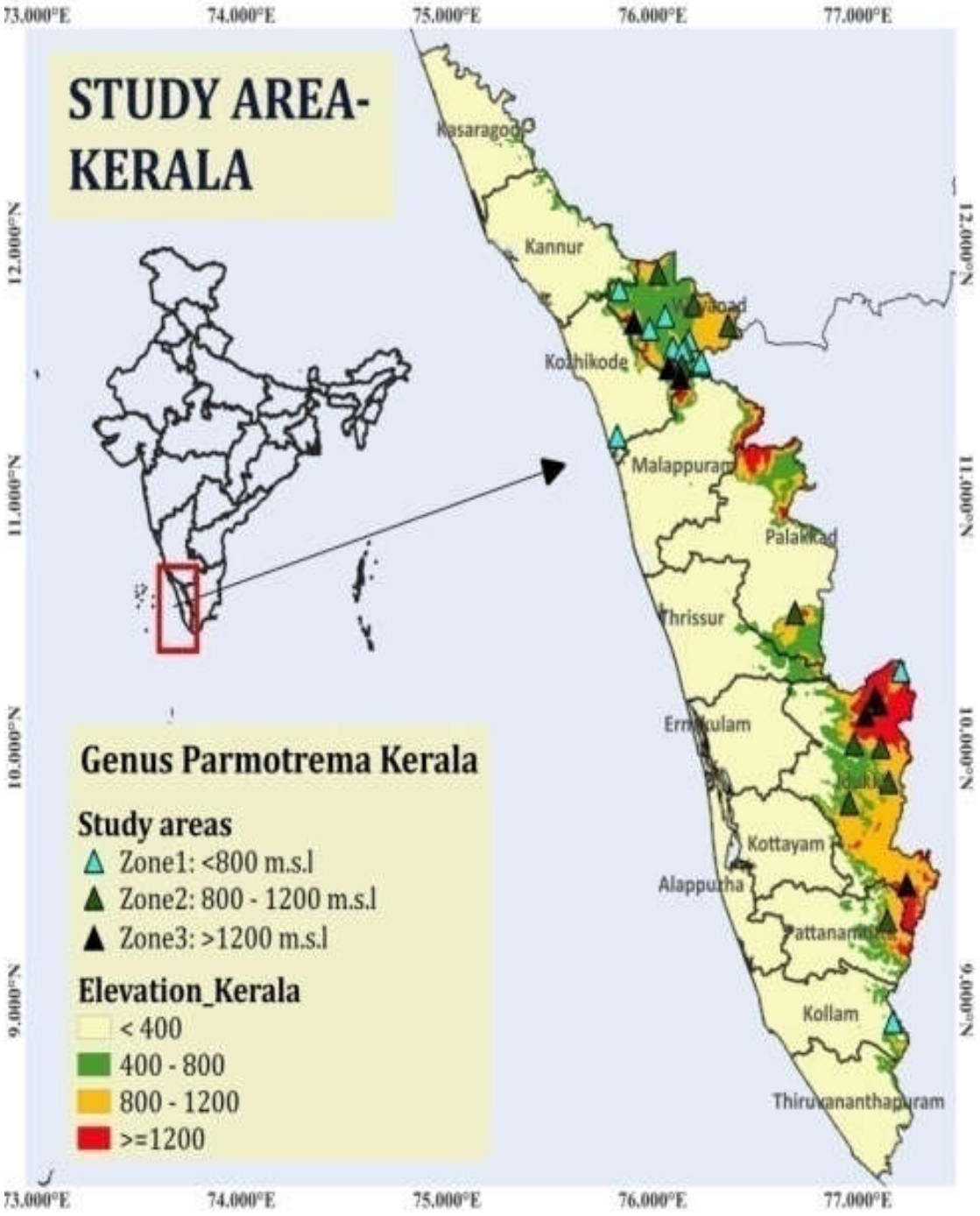


Figure 1- Study Area

CHAPTER 3

REVIEW OF LITERATURE

3. Review of Literature

Lichens are composite organisms consisting of a fungus (the mycobiont) and a photosynthetic partner (the phycobiont or photobiont), which can either be a green alga or a cyanobacterium (Schwendener, 1868, 1869). This symbiotic relationship allows lichens to thrive in various environments, from arctic tundra to rocky shores and even on the bark of tree trunks. This dual nature of lichens, where the fungus provides structure and protection while the photobiont provides energy through photosynthesis, is a remarkable example of mutualism in nature (Ahmadjian, and Hale, 1973).

3.1 Taxonomic Study

A taxonomic study in lichens involves classifying, identifying, and naming lichen species based on their morphological, chemical, and molecular characteristics. Taxonomic studies are crucial for understanding lichen diversity, ecology, and evolution, and they provide essential information for conservation efforts and ecological monitoring.

3.1.1. History of the Genus *Parmotrema* A. Massal

The first documentation of lichen in a systematic thesis can be traced back from the accounts of the father of botany, Theophrastus (371-286 B. C). He was curious about the beard or foliose-like outgrowths in the tree (oak) trunks. From his descriptions, we can assume he was familiar with *Usnea barhata* and *Rocella tinctoria*. After that, it took several centuries to consider lichens as a significant group as important as mosses or vascular plants. He has also coined the term Lichen for this group of plants (Nash, 1996 & 2008). Hawksworth and Hill (1984) reported that 'Tournefort was the first scientist who considered lichens as a distinct group", and up to his time, the lichens were placed with algae, fungi or even bryophytes' (Plitt, 1919). The history of lichenology in India can be traced back to the phenomenal book of Carl Linnaeus, 'Species Plantarum' in 1753 AD. Linnaeus mentioned the occurrence of *Lichen fuciformis* (L.) DC. from India (Nayaka and Upreti, 2005). From his descriptions, it aligns with *Rocella Montagnei* Bél. Even

though approximately 176 species of lichens were known then, the Species Plantarum mentioned only 86 species. Linnaeus believed the apothecia to be male organs and the soredia female organs. Heller, one of the foremost lichenologists and a contemporary of Linnaeus, believed in Micheli who proposed the idea, the soredia were seeds, and the apothecium was a calyx cup. Eric Acharius, the father of lichenology (Nayaka and Upreeti, 2021), and his book "Lichenographia Universalis" is considered the most important text of the nineteenth century (Thel *et al*., 2013). It is the most voluminous and complete work on lichens yet has been issued. It is simply a manual of lichens with rather imperfect descriptions of species. He has described more than 3000 lichen species. Acharius's classification system was based on the characteristics of lichen thallus morphology (the vegetative part of the organism), the colour of the upper cortex, and reproductive structures. His descriptions became the foundation for subsequent lichenological studies. His significant contribution was the introduction of standardized nomenclature for lichens. "Acharian system" established consistent naming conventions and facilitated communication and collaboration among lichenologists worldwide. Furthermore, Acharius's writings and works also encompassed the comprehensive study of lichen ecology, distribution, and the functional role of lichens as environmental indicators (Niche). His research helped to establish the ecological significance of lichens and their sensitivity to environmental conditions, earning him recognition as a pioneer in ecological studies and environmental and pollution monitoring (Plitt, 1919).

Eschweiler (1824) proposed the family Parmeliaceae with the shield-like apothecia, surrounded by a thalline margin. The structure of the apothecia is considering as the most important character for delimiting species in the family. *Parmelia sensu lato* is one of the largest genera in the family. Parmeliaceae comprises several heterogeneous groups of species. *Parmelia* Ach. *sensu lato* proposed by Acharius in 1803 belongs to the major ‘parmelioid’ clade within the large and widely distributed family Parmeliaceae is one of the largest genera which was proposed by Acharius comprised of several heterogeneous groups of species in a very broad sense, encompassing a large number of foliose lichens with lecanorine apothecia, including

such diverse genera as Cetraria, Lobaria, *Parmelia s. lat*., Physcia, and Xanthoria. It includes the lichens with foliode thallus and rhizinate margins along with laminal apothecia and simple spores. Parmelioid lichens can be distinguished by their mainly foliose thalli, rarely subcrustose, subfruticose, peltate and umbilicate, the rhizinate lower surface, laminal cupulate apothecia, Lecanora-type asci, and simple ellipsoid hyaline ascospores (Blanco *et al,*. 2006; Crespo *et al.*, 2007, 2010). By the second half of 19th century, the genera *Parmelia* Ach. *sensu lato* has begun to assume its modern circumscription, being described as a genera with foliose thallus, rhizinate margins with laminal apothecia and simple spores (Fries, 1861).

Massalongo (1860) proposed the genus *Parmotrema* which was based on the species, namely *Parmelia perforata*. The key taxonomic feature or generic character used by Massalongo was the presence of perforated apothecia. However, the generic description proposed by Massalongo was not widely accepted by contemporary lichenologists. Among these Vainio (1890) used the name *Parmelia*, never mentioning the genus *Parmotrema* in his detailed paper on Brazilian Parmeliaceae. On the other hand, Vainio's section *Amphigymnia* within *Parmelia* very well aligns with Massalongo's concept of *Parmotrema*. Zahlbruckner (1926) prepared the catalogue of the genera *Parmelia sensu lato*, comprised of 700 species arranged under four subgenera. In 1959, Dodge (1959) raised *Amphigymnia* to the status of subgenus, the level used by Hale (1965) in his classic world monograph on the group of parmelioid lichens.

Hale (1974) reestablished the genus *Parmotrema* as a generic segregation of the large genus Parmelia, including all of the species previously classified in the *Parmelia* subgenus *Amphigymnia* (Hale, 1965). The genus is distinguished by its broad, rounded lobes at the tips, often featuring a clearly defined bare rim along the margins (erhizinate zone). Rhizines are simple and often sparse. Apothecia are usually substipitate to stalked and rather frequently perforate. All species have palisade plectenchyma in the upper cortex and a pored epicortex (Hale, 1973). The genera *Parmelia s. str.* species are characterized by adnate, sublinear to subirregular lobes without cilia; an upper surface with effigurate pseudocyphellae, lower surface black, rhizinate (rhizines simple, furcate or squarrosely branched); 8 simple spores

per ascus; conidia cylindrical or weakly bifusiform, less than 80 mm long; with atranorin and chloratranorin in the cortex (Hale, 1987). Hale also included *Parmelia reticulate* (Mackay, 1836) in the genus Parmotrema by pointing out the variations in the nature of rhizine in the thalline margin which is now corroborated by the modern phylogenetic analysis.

3.1.2. Diversity of the Genus *Parmotrema* A. Massal

Hale (1974) in the monumental record of the monograph of the genus *Parmotrema*, listed 124 species that originally belonged to *Parmelia s. lat.,* thus forming the foundation of the new genera. Lücking *et al.,* (2017) reported the present status of species diversity as 350 lichens are known to the genus *Parmotrema* A. Massal.

Elix and Streimann (1989) reported nine taxons belonging to the genus *Parmotrema* A. Massal. from the Pacific Island complex called Norfolk Island. The sixteen species belong to the family Parmeliaceae and comprise seven genera. The genus *Parmotrema* includes *;Parmotrema austro-cetratum* Elix& Johnson, *Parmotrema chinense* (Osbeck) Hale & Ahti., *Parmotrema crinitum* (Ach.) Chois, *Parmotrema cristiferum* (Taylor) Hale, *Parmotrema gardnen* (Dodge) Serusiaux, *Parmotrema rampoddense* (Nyl.) Hale, *Parmotrema reticulalum* (Taylor) Choisy, *Parmotrema sancti-angelii* (Lynge) Hale and *Parmotrema tinctorum* (Despr .exNyl.) Hale. The detailed description of the species makes the study as a resourceful contribution to the species assessments in the genus *Parmotrema* A. Massal.

Spielmann and Marcelli (2009) have done the taxonomic revision of the genus *Parmotrema* A. Massal. at Serra Geral slopes of central Rio Grande do Sul State in Brazil revealed 31 species of *Parmotrema* A. Massal. These species formerly belong to genera *Canomaculina* Elix & Hale, *Parmotrema s. str.* and *Rimelia* Hale & Fletcher. The cortical and medullary substances of lichens were taken as a prime species delimiting character and the correspondence between morphological structures and the presence of medullar substances of the lichens were also illustrated in this study.

Kukwa *et al.*, (2012) reported thirty-six new taxa belonging to the genus *Parmotrema* A. Massal from Bolivia. Among the reported species *Parmotrema brasiliense* Hale and *P. nylanderi* (Lynge) Hale were discovered for the first time outside of Brazil. *Parmotrema sorediiferum* Hale, P. *soredioaliphaticum* Estrabou & Adler and *P. wrightii* L. I. Ferraro & Elix are reported in this study. With this addition, the diversity of the genus *Parmotrema* in Bolivia became forty-nine species.

Jayalal *et al.,* (2013) conducted the taxonomic or revisionary study of the genus *Parmotrema* A. Massal in South Korea and revised the taxonomy of this genus *Parmotrema* based on specimens deposited in the lichen herbarium at the Korean Lichen Research Institute, and samples were identified using recent literature. A total of eighteen species were recorded, and eight were new to South Korea. The newly reported species are *Parmotrema cetratum* (Ach.) Hale, *Parmotrema cristiferum* (Taylor) Hale, *Parmotrema grayanum* (Hue) Hale, *Parmotrema defectum* (Hale) Hale, *Parmotrema dilatatum* (Vain.) Hale, *Parmotrema margaritatum* (Hue) Hale, *Parmotrema pseudocrinitum* (Abbayes) Hale, and *Parmotrema subsumptum* (Nyl.) Hale.

Bawingan *et al*., (2017) presented the taxonomic treatment of *Parmotrema* lichens (Ascomycota, Parmeliaceae) in the Philippines at high altitudes where they are found abound, particularly in the mountainous regions of northern Luzon and Mindanao. A total of 30 lichen species from the genus *Parmotrema* were identified, including twelve new records. The findings indicate that the Philippines likely harbors a substantial and yet undiscovered lichen diversity that warrants further investigation.

Bungartz and Spielmann (2019) as part of a comprehensive inventory of all Galapagos lichens, the genus *Parmotrema* has been revised with several additions. In the Galapagos genus, *Parmotrema* is represented by thirty-five species of lichens belongs to *Parmotrema*. Among these, seven are described as new to science. They are *Parmotrema cactacearum*, *P. erectociliatum*, *P. lawreyi*, *P. marcellianum*, *P. pustulotinctum*, *P. saxoisidiatum*and *P. weberi.* The species *P. weberi.* was

described by M E Hale. However, the description was vague and inadequate. Thus, the species is formally described by Bungartz and Spielmann (2019); hence, the name is validated.

Spielmann and Marcelli (2020) conducted Type studies on *Parmotrema* (Parmeliaceae, Ascomycota) with salazinic acid and provided the account of 66 species with the synonymy, chemistry, distribution and taxonomic affinities. They have established three new species and are: *Parmotrema austromaculatum* sp. nov., *P. bifidum* sp. nov. and *P. clercianum* sp. nov. They have also proposed 13 new combinations in *Parmotrema*: *P. acanthifolium* comb. nov., *P. concors* comb. nov., *P. foliolosum* comb. nov., *P. granulare* comb. nov., *P. lividotessellatum* comb. nov., *P. magnum* comb. nov., *P. maximum* comb. nov., *P. nudum* comb. nov., *P. petropoliense* comb. nov., *P. radiatum* comb. nov., *P. reterimulosum* comb. nov., *P. sieberi*comb. nov. *P. warmingii* comb. nov. They have renamed *Parmotrema elixii* nom. nov. is proposed for *Rimelia pustulata*.

3.1.3. Diversity of the Genus *Parmotrema* A. Massal. in India

In India, the presence of 2368 species of lichens belonging to 305 genera and 67 families showcases a significant diversity of lichens reported by Nayaka and Asthana (2014) contributes to our understanding of lichen taxonomy and distribution in the country and reminds the opportunities of this biological wealth.

Kumar (2000), a pioneer in lichenology, conducted research on the macro lichen flora of Kerala in the Western Ghats, revealing the rich lichen diversity of the region. His study and extensive fieldwork identified 254 macro lichen species across 43 genera and 18 families in Kerala. The family *Parmeliaceae* is the most diverse, with 80 species in 14 genera, followed by *Physciaceae* (43 species in 6 genera), *Usneaceae* (40 species in 1 genus), and *Collemataceae* (29 species in 2 genera). Within the genera, *Usnea* is the most prevalent with 40 species, followed by *Parmotrema* (26 species), *Heterodermia* (24 species), and *Leptogium* (23 species). Among the 24 species of *Parmotrema* studied, 12 were new records for Kerala

In 2003, Divakar and Upreti revised the genus *Parmotrema* A. Massal, identifying and describing 46 species within this genus. Their extensive study provided a list of 108 lichen species from 35 genera, found on twelve major tree species and various other substrates across thirteen forest sites within the Jim Corbett Tiger Reserve, which spans 1318.54 sq. km in Uttaranchal. The research revealed that crustose lichens were the most prevalent, with 88 species identified across all sites. In contrast, only 20 species of foliose lichens from nine genera—namely *Bulbothrix, Cladonia, Coccocarpia, Collema, Heterodermia, Leptogium, Parmotrema, Phaeophyscia, and Physcia*—were observed on different substrates.

Balaji and Hariharan (2004) investigated lichen diversity and distribution patterns in the tropical dry evergreen forest of Guindy National Park (G.N.P) in Chennai. Their quantitative ecological data revealed the presence of 31 lichen species distributed across fewer than 26 genera, 19 families, and 9 fungal orders. Balaji and Hariharan (2013) assessed the Diversity of Macrolichens in the Bolampatti II Forest Range of Siruvani Hills in the Western Ghats and reported 13 species of lichens belonging to the genera *Parmotrema.*

Divakar and Upreti (2005) published a revisionary study of the Parmelioid lichens of India and reported that 192 species represent the Parmeloid taxa in India. It comprises about 11% of the total Parmeloid diversity of the world. The study has also reported that 20 species are endemic to India. He also illustrated about 46 species of *Parmotrema* that dominate other genera in terms of diversity. Out of the 192 species of Parmeloid lichens, 3 species may be extinct or untraceable, one is endangered, 17 are vulnerable, and 81 species are rare in India. 90 species were catagorised as least concerned or commonly occurring taxa.

Biju *et al.*, 2010 reported the occurrence of *Parmotrema pseudocrinitum* and *Parmotrema robustum* from peninsular India and *Parmotrema andinum* and *Parmotrema melanothrix* are new reports of lichen to Kerala. This was the first report of these species from peninsular India and Kerala's geographical boundaries, respectively.

Baral (2015) conducted research on lichens in the high-altitude regions of Nepal, focusing on the Manaslu Conservation Area and Sagarmatha National Park. He documented 13 lichen species across 4 families in the Manaslu Conservation Area and 69 species across 15 families in Sagarmatha National Park. This finding underscores the rich diversity of lichens and suggests that these organisms exhibit specialized adaptations to varying altitudes and ecological conditions within the Himalayan region.

In the same year, Goni *et al.,* (2015) documented 356 species of lichens in Jammu and Kashmir, belonging to 35 families and 91 genera. The Parmeliaceae family stood out with 65 species across 24 genera, followed by the Physciaceae family with 57 species from 11 genera. This detailed checklist provides valuable insights into the region's distribution patterns and ecological preferences of lichens.

Mishra and upreti (2017) revised the genus *Parmotrema* in India and reported 53 species. It is the largest compailation of taxons of the genus *Parmotrema*, as Awasthi (2007) reported 49 species, and Singh and Sinha (2010) reported 51 species. Tamil Nadu has the richest *Parmotrema* diversity, with 34 species, followed by Kerala, with 24 species. Also, the study determines the parmotremoid lichens such as *P. praesorediosum* (Nyl.) Hale, *P. reticulatum* (Taylor) M. Choisy, *P. Sancti-angelii* (Lynge) Hale and *P. tinctorum* (Despr. ex Nyl.) Hale exhibits their common occurrence in a different part of the country. And these four lichens were extensively collected by people for various purposes.

Sequeira *et al.*, (2022) add one more species to the Lichen genera, *Parmotrema sahyadrica* from Wayanad district of Kerala. The species has a characteristic yellow medulla and densely isidiate thallus with 6-10 mm wide lobes. The species contains entothein and atranorin as major compounds. The taxon is distributed at an elevation of 750 m.

Christy *et al.,* (2022) reported 97 species of macro lichens from the Wayanad district of Kerala. Of these, 19 species belong to the genus *Parmotrema*. *P. cetratum* was described as a new species for Kerala. Habitat destruction, encroachment, habitat

loss, and pollution were identified as the major threats to the lichen biota of the Wayanad district.

Anilkumar *et al.,* (2022) has reported nine species of *Parmotrema* lichens from the Mathikettan Shola national park of kerala and in this study *P. chinense* is reported as a new species to Kerala. Nayaka and Biju (2024) conducted analyses of lichen-forming and lichenicolous fungi in the Western Ghats, based on current publications, revealed a total of 1,617 taxa of lichen-forming fungi, including 1,597 species, 19 varieties, 2 subspecies, and 1 forma. Additionally, there are 28 species of lichenicolous fungi. Of these, 251 lichen taxa are endemic, with 129 confined solely to the Western Ghats. The lichen biota in this region is predominantly composed of crustose lichens, totalling 1,117 taxa, while foliose and fruticose lichens account for 393 and 107 taxa, respectively. Among the states, Tamil Nadu, Kerala, and Karnataka have the highest lichen taxa, with 963, 783, and 658, respectively.

3.2. Bioprospection

Beattie *et al*., (2011) define bioprospection as the exploration of biodiversity to discover new biological resources with social and economic value. This process is undertaken by a range of industries, most notably the pharmaceutical sector, but also by various fields such as agriculture, manufacturing, engineering, construction, and more. UNEP defines bioprospection as "the exploration of biodiversity for commercially valuable genetic and biochemical resources" (Source: UNEP/CBD/COP/5/INF/7).

Juan (2017) defines bioprospection as the discovery and development of novel merchandise of chemical compounds, genes, micro-organisms, and macro-organisms supported by biological resources. Biodiversity is the essential 'lifeblood' of bioprospection. Biodiversity refers to the variety of species within an ecosystem. Without this diversity, the biological resources essential for bioprospection would not exist. The imperative to conserve biodiversity and ensure equitable access to genetic resources and associated traditional knowledge has sparked one of the most contentious debates of the 21st century between developed and developing nations.

A successful bioprospection should call for resource and benefit-sharing between haves and haven't. This debate has fundamental implications for how basic and applied research on genetic resources and biodiversity is conducted and its results are made available between and within people and societies (Juan, 2017).

3.2.1. Lichen as a Bio-Monitoring Agent

Lichens are highly sensitive to pollution. Generally, the greater the diversity of lichens, the lower the levels of pollution in the atmosphere and water (Larsen et al., 2007).

Aslan *et al.,* (2011) studied the extent of heavy metal pollution in roadside soils by utilizing eight different lichen species as bio-indicators. The results indicated that the presence of Pb and Cd in roadside soils was predominantly attributed to traffic-related activities, as evidenced by their distribution patterns and enrichment factors. Among the lichen species studied, *Xanthoria candelaria, Lecanora muralis,* and *Xanthoria elegans* were highlighted for their ability to indicate pollution from traffic sources. Specifically, *Xanthoria candelaria* was identified as a particularly effective indicator species for traffic-related pollution. Using lichens in environmental monitoring offers several advantages, including ease of collection, cost-effectiveness, and sensitivity to air pollution. Lichens accumulate heavy metals and other pollutants from the atmosphere over time, providing valuable insights into the environmental quality of their surroundings. This study underscores the potential of lichens, especially *Xanthoria candelaria*, as reliable bio-indicators for monitoring air pollution from traffic and assessing its impact on roadside soils and adjacent ecosystems.

Kuldeep and Prodyut (2015), in the review "Lichen as a bio-indicator tool for assessment of climate and air pollution vulnerability," summarize the use of lichens in studying climate change, air pollution, and heavy metal contamination. Lichens accumulate pollutants from the air, providing valuable insights into local and regional environmental quality. Studies have utilized lichens to monitor changes in biodiversity, growth patterns, and physiological responses influenced by climate variability and pollutant concentrations.

Koch *et al.*, (2018) has showed the method to monitor air pollution and the value of using biological organisms for this purpose, the primary aim of this study was to evaluate air quality in urban areas through the use of lichen transplants. Additionally, the study sought to identify differences among various cities to highlight the main pollution characteristics of each area. Monitoring was conducted in seven cities in Rio Grande do Sul, southern Brazil, ranging from predominantly rural to mostly industrial settings. The foliose lichen *Parmotrema tinctorum* was selected as the biomonitor species. The study measured physiological parameters and concentrations of sulfur and heavy metals in the lichen thalli. Air quality data, including pollutant levels from air samplers and modeled concentrations of fine particulate matter and nitrogen oxides, were available for some cities. Results showed that air pollution negatively affected the physiological parameters of the lichen, and the concentration of heavy metals increased after exposure, particularly in industrial areas. The impact of pollutants on *Parmotrema tinctorum* was evident in most of the thalli, with an overall increase in physiological parameters, except for the percentage of live algal cells. Previous research has indicated that more dead algal cells are associated with increased air pollution.

Air quality assessments using lichen transplants were also carried out in India. The major experiments were done by Bhat *et al.*, (2014). In the Indian Himalayan state of Jammu and Kashmir (J & K), air quality parameters were monitored using transplanted lichen samples. These samples showed elevated levels of lead, iron, copper, chromium, zinc, cadmium, nickel, and mercury compared to control samples, with a minimum metal concentration of 4391.5 μg g^{-1}. Lichens transplanted near the city center exhibited the highest concentrations of these heavy metals, reaching up to 6912.7 μg g^{-1} of dry weight. In contrast, metal concentrations decreased progressively as the distance from the city center increased.

3.2.2. Lichen Compounds and their Traditional Biomedical Applications

Lichens are considered reservoirs of phytochemicals. Certain compounds are the resultants of the symbiotic association between the mycobiont and the phycobiont; hence, they are unique to lichens. These compounds are primary and secondary

metabolites and are essential for these symbionts' survival in harsh environments. These compounds have considerable biological activities.

Vartia (1973) was the first to document that lichens have been used medicinally in various folklore traditions and are listed in different global pharmacopoeias. Historically, lichens have been used to treat coughs and pulmonary tuberculosis in various cultures worldwide. The chlorine-containing lichen compound ‘diploicin’, which was first isolated from *Buellia canescens*, has shown inhibitory effects against human tuberculosis and diphtheria bacilli at concentrations of 1:100,000 and *Mycobacterium smegmatis* at 1:70,000 in vitro. Additionally, lichens have been used in cattle breeding to treat udder inflammation, a serious condition in dairy farms. The study also highlighted the antiseptic properties of phenols and their derivatives, which are components of lichen depsides and depsidones. Kumar and Upreti (2001) compiled the world's folklore knowledge on lichen and summarized the world's traditional wisdom on the utilization of lichens. According to Shri Pad Damodar Satvalekar (1958 & 1985), references in the Atharvaveda and Rigveda indicate that the term "Shipal" was used for algae in the Rigveda (6000-4000 B.C.), which is the earliest known record of Oshadhi (medicine). The medicinal properties of Shipal as a lichen are also noted in the Avkolva of the Atharvaveda (1500 B.C.) (Singh, 1972; Sharma, 1984, 1998). Ancient texts such as the Sushruta Samhita (1000 B.C.), Charak Samhita (300-200 B.C.), and several Nighantu (A.D. 1100-1800) document various Sanskrit synonyms for lichens, including Shailaya and Shilapushp. These names were later linked to species of the genus *Parmelia* (Upreti et al., 2005), such as *P. cirrhata* and *P. perforata*. In Ayurveda, an ancient Indian medicinal system, the vernacular name "Chharlia" is commonly used for treating a range of ailments including headaches, skin diseases, urinary issues, boils, vomiting, diarrhea, dysentery, heart problems, coughs, fevers, leprosy, and as a blood purifier. Additionally, *Parmotrema sulcata*, another species from the genus *Parmotrema*, is also recognized in Indian folklore for its medicinal properties and is reported by Hale (1983) to be effective in treating cranial ailments Lichens have played a significant role in traditional medicine practices, particularly in the Middle East, where they were prominently featured in herbals used by medieval medical

practitioners. Llano (1948) highlighted that the use of lichens in medicine dates back to ancient times, citing *Evernia furfuracea*, which was found in an Egyptian vase from the 18th Dynasty (1700-1600 B.C.) and is still imported into Egypt from Europe alongside *Cetraria islandica* as a foreign drug. Additionally, Gonzalez-Tejero *et al.,* (1995) emphasized that lichens have been utilized in traditional medicine since the early Chinese and Egyptian civilizations.

3.2.3. Important Secondary Metabolites in *Parmotrema* A. Massal.

Phytochemical substances produced as a result of metabolism or biochemical reactions in plants are generally termed metabolites, and the metabolites are broadly classified into two major groups: primary and secondary. Primary metabolites are primary in origin and formed due to primary metabolism. They include sugars, proteins, amino acids, fatty acids, lipids, nucleic acids, etc.; secondary metabolites are secondary in origin. They are derived from primary metabolites as a result of secondary-level metabolism. Examples are phenols, terpenoids, alkaloids etc. The primary metabolites act as the basic molecules and structural, functional, energetic, and hereditary components of organisms. The secondary metabolites perform some specific functions, but they are restricted to certain specific cells or appear at a specific stage of growth and development. They have no direct role in growth and development, but they are important in regulating many physiological processes and some ecological roles. Most of these secondary metabolites have profound commercial significance as phytomedicines, esters, fungicides, insecticides etc. The vital substance and primary metabolite glucose, which is produced via photosynthesis is often acts as the precursor for synthesizing the secondary metabolites. Moreover, glucose is the most favoured substance for respiration, and all other carbohydrates are usually converted to glucose for respiration. Respiration is a process capable of breaking down respiratory substrate for the release of chemical energy, and it can also synthesize materials. For example, fatty acids are broken down into acetyl CoA before entering the respiratory pathway when used as a substrate. However, acetyl CoA would be withdrawn from the respiratory pathway when the organism needs to synthesise fatty acids. Similarly, respiratory intermediates form the link during the synthesis of secondary metabolites. Lichens

produce a variety of secondary substances through several biosynthetic pathways. The polyketide biosynthetic pathway is primarily responsible for most classes of lichen compounds. In contrast, pulvinic acids are derived from shikimate, while the abundance of di- and tri-terpenoids in lichens is generated via the mevalonate pathway. Numerous studies have reported the isolation and characterization of individual components from lichen extracts (Goga *et al.,* 2020). Among the several biosynthetic pathways in lichens, the poly malonate pathway is responsible for producing fingerprint lichen compounds. This pathway also starts from acetyl CoA. In addition to the poly malonate pathway (APP), the shikimic acid pathway (SAP) and the mevalonic acid pathway (MAP) are also important in secondary metabolite production in lichen.

The lichen substances can be widely classified into three major groups. The first group belongs to the members, including aliphatic compounds; the second group of compounds, including aromatic compounds; and the third group, including carbohydrates. Aliphatic compounds in lichens encompass acids, zeorin compounds, and polyhydric alcohols. Aromatic compounds include derivatives of pulvic acid, depsides, depsidones, quinones, xanthone derivatives, diphenyleneoxide derivatives, nitrogen-containing compounds, triterpenes, tetronic acids, and carbohydrates, such as polysaccharides. Past studies worldwide identified 350 lichen substances, and the structural elucidation of approximately 200 ones was carried out. Generally, the lichen secondary metabolites are insoluble in water, and organic solvents are used to extract these lichen substances. Most of the species of lichens of the genus *Parmotrema* lacks usnic acid but a few lichens in this genus contain usnic acid and *Parmotrema reticulatum* reported to have usnic acid (Torres-Benítez *et al*., 2017; .Saha *et al*., 2021). Usnic acid has Antioxidant, pro-oxidant activities, (Halici *et al.,* 2005; Mayer *et al.,* 2005; Odabasoglu *et al.*, 2006).

Din *et al.,* (2010) investigated the chemical composition of lichens from Bukit Larut, Peninsular Malaysia. They analyzed lichen samples, including *Parmotrema tinctorum, P. clavuliferum*, and *P. reticulatum*, identifying ten classes of compounds present: depsides (10 compounds), depsidones, quinones, xanthones,

naphthopyrones, pulvinic acid derivatives, diphenyl ethers, dibenzofurans, aliphatic acids, and terpenoids.

The traditional and ethnic medicinal practices directly use lichen thallus for their formulations against various diseases. All these practices indicate the presence of therapeutic substances in the genus *Parmotrema*. Atranorin is one of the β-orcinol derivatives often found in various families of lichen and all the members of the genus *Parmotrema.* In the comprehensive study of Variable responses of different human cancer cells such as; nine human cancer cell lines (A2780, HeLa, MCF-7, SK-BR-3, HT-29, HCT-116 p53(+/+), HCT-116 p53(-/-), HL-60 and Jurkat) to the lichen compounds parietin, atranorin, usnic acid and gyrophoric acid has analysed by Bačkorová *et al*., (2011). This study has confirmed a differential sensitivity of cancer cell lines to lichen secondary metabolites and compared with parietin and gyrophoric acid, usnic acid and atranorin effectively suppressed cell viability and proliferation at equitoxic doses. This effect correlated more strongly with increased floating cells or a higher apoptotic index.

Galanty *et al.,* (2021) studied the effect of Usnic acid and atranorin in viability, proliferation, apoptosis and motile activity and actin cytoskeleton organization of melanoma HTB-140, prostate cancers DU-145 and PC-3, normal human skin fibroblasts and prostate epithelial PNT2 cells. The study focuses on the selectivity and versatility of the compounds in inhibiting cancer cells and inducing programmed cell death (apoptosis). The findings demonstrate that both compounds are effective in inhibiting cancer cell proliferation, migration, and the organization of the actin cytoskeleton. However, their impact on the apoptosis process was less significant. Usnic acid was found to be more effective than atranorin in affecting the cancer cells. Both compounds exhibit selective effects on tumor cells.

Zhou *et al*., (2017) investigated the anticancer properties of atranorin extracted from *Everniastrum texanum*. This secondary metabolite effectively inhibits A549 cell motility and migration at a concentration of 10 μg/mL, which is not cytotoxic and thus safe for biological systems. Atranorin reduces β-catenin-mediated 'TOPFLASH' activity by inhibiting β-catenin nuclear import and downregulating β-

catenin/LEF and c-jun/AP-1 target genes. 'KITENIN', an enzyme that enhances the carcinogenic potential of epidermal growth factor (EGF), was found to be a carcinogenic marker due to its role in stimulating 'KITENIN'-mediated AP-1 activation. Atranorin reduced AP-1 activity by approximately 70% at 10 µg/mL and also diminished EGF-activated 'KITENIN'-mediated AP-1 activity. Additionally, atranorin's inhibitory effect on lung cancer cell lines was assessed through invasion assays in H460, H1650, H1975, and LLC cells, where it significantly reduced cell invasion by 50%, 24%, 30%, and 80%, respectively, compared to untreated cells.

Harikrishnan *et al.,* (2021) studied the anti-breast cancer properties of atranorin. Molecular docking studies conducted by these researchers on breast cancer oncoproteins, including Bcl-2, Bax, Akt, Bcl-w, and Bcl-xL, revealed that atranorin exhibited the strongest molecular interaction with the oncoprotein Akt, followed by Bax, Bcl-xL, and Bcl-2, with the weakest interaction observed with Bcl-w. Cytotoxicity studies further elucidated the mechanism by which atranorin selectively inhibits the MDA-MB-231 and MCF-7 breast cancer cell lines. All the assays atranorin significantly reduces the production of reactive oxygen species generation and hence by oxidative stress in cell lines.

Galanty *et al.*, (2021) has studied the Anti-Melanoma and Anti Inflammatory Potential of Usnic Acid Enantiomers using in vitro analysis. The viability of all three melanoma cell lines has decresed drastically in the presence of Usnic acid. (+)-Usnic acid at a sub-cytotoxic dose strongly inhibited melanoma cells migration. They have effectively decreased the release of pro-inflammatory mediators. The study was conducted on three melanoma cell lines HTB140, A375 and WM793. The analysis also considered the cyto-toxic properties of Usnic acid, and hence, the cytotoxic level of the usnic acid concentration was used for the study.

Shukla *et al*., (2022) have analyzed the wound-healing properties of usnic acid in the light that the compound has profound antimicrobial and antioxidant properties. The study has proved the wound healing properties of usnic acid by analyzing the enhancement in collagen deposition in the lower epithelium, angiogenesis, and re-epithelialization of the outer boundary. In addition to this, usnic acid increases the

activity of antioxidant enzymes. It stimulates the expression of growth factors that are involved in tissue repair, among other advantages that are beneficial for wound healing and prevent the formation of ROS. Furthermore, usnic acid can be incorporated into wound dressings to promote prolonged release and enhance therapeutic efficacy as a wound healer.

Tripathi *et al*., (2022), in the review of anti-cancer and related properties of lichen-extracts and metabolites, narrate lichen as "The symbiotic organisms are naturally equipped with distinct characteristics as compared to constituting organisms separately. Lichens, characterized by their unique anatomy and physiology, serve as reservoirs for over 600 distinct secondary metabolites, often referred to as 'lichen substances. Experimental studies have demonstrated that lichen substances exhibit remarkable antioxidant, antimicrobial, antiviral, anti-tumor, and anti-inflammatory properties. Usnic acid, a prominent metabolite in various lichen species, is particularly noted for its potent antioxidant and anti-inflammatory effects. Moreover, it has shown significant antiproliferative properties in various cancer cell lines during research investigations. Atranorin, gyrophoric acid, norstictic acid, lecanoric acid, lobaric acid, stictic acid, ramalin, salazinic acid, protolichesterinic acid, and fumarprotocetraric acid are among the purified lichen metabolites known for their potent anti-cancer activities.

Galla *et al*., (2023) have studied the anti-viral properties of usnic acid in the scenario of airborne viral diseases becoming a great threat to the survival of human beings. They have analyzed the possibilities of usnic acid as a nasal spray against viral pathogens by which the drug can create a physical barrier against viral uptake without cell damage and incorporate different substances with antiviral activity. The study summarizes the anti-viral properties of usnic acid as it can create a mechanical protective barrier, confirming its ramification property against virus pathogens to modify its structure by creating a branch. UA was effective in preventing the infection of Vero E6 and HNEpC cells by disrupting the interaction between the cells and the viruses. It can inhibit viral activity by creating a physical barrier, without affecting the normal physiological balance of the nasal environment.

Usnic acid has been extensively studied for its antibacterial effects against Gram-positive bacteria such as *Staphylococcus aureus* and *Streptococcus pneumoniae*. It inhibits bacterial growth by disrupting cell membrane integrity and interfering with essential metabolic processes. Maciag *et al*., (2014) suggested the molecular mechanism behind the antibacterial properties of Usnic acid against bacteria. They demonstrated that the lichen secondary metabolite Usnic acid can cause rapid and strong inhibition in DNA and RNA synthesis of Gram-positive bacteria. The study was conducted on two Gram Positive Bacteria, *Bacillus subtillis* and *Staphylococcus aureus.* The inhibition of RNA synthesis is the general mechanism of the anti-bacterial property of usnic acid. Still, in Gram-positive bacteria, the compound can halt the DNA synthesis by interfering with the chain elongation of the DNA synthesis. It also inhibits bacterial growth by disrupting cell membrane integrity and interfering with essential metabolic processes. Francolini *et al*., 2004 also reported the antibacterial properties of Usnic acid against three Gram-positive Bacteria such as S*taphylococcus aureus*, *Enterococcus faecalis*, and *Enterococcus faecium.*The study also postulated the suitability of lichens in biofilm production against pathogens using Usnic acid as the coating material.

Studies have demonstrated usnic acid's efficacy against various fungal pathogens, including *Candida albicans*, *Aspergillus spp*., and dermatophytes. It exhibits fungicidal activity by affecting fungal cell wall synthesis and mitochondrial function. Pires *et al*., (2012) have demonstrated the activity of usnic acid against Candida parapsilosis and Candida orthopsilosis under planktonic and biofilm conditions. They conducted the assay using broth microdilution and microplate methods. The metabolic activity of the test organism was reduced by 80%.

The UV protective properties of lichens are another area of research in bioprospection. The compound, enzymes, proteins or genes can contribute to innovations in the pharmaceutical and chemical industry. Huneck and Hoefle, (1978) first coined the properties of certain lichen substances as the U V protective substances “Aromatic lichen substances that are characterized by high conjugated bonds can strongly absorb UV light and thereby shielding the photosynthetic pigments against photo-oxidative radiation. MacKenzie (1952) was the pioneer in

the UV protective properties of Usnic acid by preparing the UV absorption spectrum of Usnic acid. The proper case study of usnic acid and UV light was revealed by the documentation of ozone depletion. Quilhot *et al*., (1996) found that the amount of usnic acid in Antarctic lichens is directly correlated with the extent of ozone depletion. This implies that usnic acid could be a protective measure against UV radiation.

Galanty *et al.,* (2021) have validated the traditional use of topical applications of various xantholichens for their dermato-protective properties, particularly against sunlight. Their study evaluated skin-penetrating properties using the 'skin-pampa' assay, assessed safety on normal human skin cells (keratinocytes, melanocytes, fibroblasts), and determined the photostability and photoprotective properties of usnic acid across different cell lines. The results revealed that both enantiomers of usnic acid exhibit comparable and effective skin-penetrating properties. The synergic effect of usnic acid was proven when it was combined with octocrylene, a standard drug and has revealed enhanced photoprotection and photostability. Thus usnic acid can act as a UV filter in cosmetic products.

The ecological implication of variation in the secondary metabolites in parmelioid lichens concerning altitude was studied by Shukla *et al.,* (2016) and confirmed the direct correlation between the quantity of lichen substance in the cortex and medulla with increasing altitudes. Atranorin and salazinic acid, two frequently found lichen substances, exhibit antioxidant and photoprotective properties that vary based on environmental stresses, whether from abiotic or biotic factors. As altitude increased, all three lichen species displayed greater amounts of chemical substances. Among them, *E. cirrhatum* exhibited the highest overall quantity of lichen compounds. The increased prevalence of *E. cirrhatum* at higher altitudes compared to *B. setschwanensis* and *P. reticulatum* may be linked to its higher levels of photoprotective and antioxidant chemicals, particularly salazinic acid.

The ethnic people use lichens for several purposes, such as dyes, food, fodder, medicine, insect repellents, etc. Several lichen substances have insect-repellent properties. Cetin *et al*., (2012) investigated the larvicidal activity of several lichen

metabolites, including (+)-usnic acid, atranorin, 3-hydroxyphysodic acid, and gyrophoric acid. They found that these compounds exhibited significant larvicidal effects against the second and third instar larvae of the mosquito *Culiseta longiareolata*. The toxicity was measured by LC (50) values, with gyrophoric acid showing the highest toxicity (0.41 ppm), followed by (+)-usnic acid (0.48 ppm), atranorin (0.52 ppm), and 3-hydroxyphysodic acid (0.97 ppm). When comparing LC(90) values, the order of toxicity shifted: (+)-usnic acid (1.54 ppm) was the most toxic, followed by gyrophoric acid (1.93 ppm), 3-hydroxyphysodic acid (4.33 ppm), and atranorin (5.63 ppm). The study concludes that lichen secondary metabolites hold potential as effective larvicides.

Cetin *et al*., (2008) investigated the insecticidal properties of two enantiomers of usnic acid, (-)-usnic acid and (+)-usnic acid, which are common lichen secondary metabolites. In laboratory tests, both compounds demonstrated significant larvicidal activity, achieving 100% mortality in the fourth larval stage of *Culex pipiens* L. (Diptera: Culicidae) within 24 hours at concentrations of 5 and 10 ppm. The bioassays revealed LC50 values of 0.8 ppm for (-)-usnic acid and 0.9 ppm for (+)-usnic acid. The study suggests that lichen compounds may be valuable in the development of new insecticides.

Gyrophoric acid is another depside molecule found in the genus parmotrema. Mohammadi *et al.,* (2022) have analysed the lichen-derived metabolite gyrophoric acid that modulates various cellular pathways pertinent to several biomedical conditions and disorders, such as cancer, diabetes, and cardiovascular disease. The study was conducted on human and rodent cell types. The therapeutic potential of gyrophoric acid and similar metabolites derived from lichens is linked to their chemical versatility as polyaromatic depsides. These compounds feature functional carboxyl and hydroxyl side groups, which enable them to interact with specific enzymatic active sites selectively. This property enhances their efficacy in potential therapeutic applications. The study proved the potentiality of Gyrophoric acid as an effective anticancer agent due to its ability to inhibit topoisomerase 1 activity, induce cell cycle arrest, inhibit cell survival pathways, and promote apoptosis. Its cytostatic properties suggest that beyond impacting cancer cells, hydrophobic acid's

biological roles and potential medicinal applications may extend to many other processes controlled by cell growth and differentiation more broadly.

Rajan *et al*., (2016) conducted a study to evaluate the antibacterial and antioxidant properties of acetone and methanol extracts from four lichen species of the genus *Parmotrema*: *P. praesorediosum*, *P. rampoddense*, *P. tinctorum*, and *P. reticulatum*. Several key phytochemical compounds were isolated from these lichens, including praesorediosic acid, protocetraric acid, usnic acid, α–collatolic acid, β-alectoronic acid, atranorin, and chloroatranorin. Antibacterial activity was assessed using the broth dilution method, revealing that acetone extracts (except for *P. reticulatum*) had significant inhibitory effects against *S. aureus* and *B. subtilis*, with MIC values ranging from 500 to 125 µg/mL. No antibacterial activity was found in the methanol extracts, and none of the extracts were effective against *E. coli*. Antioxidant activity was measured using the DPPH free radical scavenging assay, with only the methanol extract of *P. praesorediosum* showing more than 50% scavenging activity. Among the isolates, usnic acid exhibited the strongest antibacterial activity, with an MIC value of 7.81 µg/mL against *S. aureus* and *B. subtilis*. Praesorediosic acid and protocetraric acid specifically inhibited *E. coli* at a concentration of 125 µg/mL and showed significant scavenging activities of 57.57% and 63.97%, respectively.

Killari *et al*., (2023) studied the health benefits of Salazinic acid constituent, a secondary metabolite of Parmotrema lichens, against the acute health effects of Diabetes in animal models. This study demonstrates that salazinic acid (Sa) plays a beneficial role in mitigating the adverse effects of streptozotocin-induced diabetes on the male reproductive system. Salazinic acid effectively restored the weights of reproductive organs, improved sperm characteristics, and preserved testicular histology in a dose-dependent manner among diabetic rats. Additionally, Salazinic acid treatment led to significant increases in insulin, follicle-stimulating hormone, luteinizing hormone, and testosterone levels in the serum of diabetic rats. Furthermore, Salazinic acid supplementation resulted in decreased levels of malondialdehyde—a marker of oxidative stress—and increased activities of antioxidant enzymes (glutathione, superoxide dismutase, glutathione peroxidase, and catalase) both in the blood serum and testicular tissue. Salazinic acid also reduced

the protein expression levels of tumor necrosis factor-α in the serum. Notably, the higher dose of Salazinic acid showed marked improvements in glycemia and provided substantial protection to the testicular tissue. These findings underscore the potential of Salazinic acid as a therapeutic agent in ameliorating diabetic-induced damage to the male reproductive system through its antioxidative and hormonal regulatory properties.

In addition to the anti-diabetic properties Killari *et al.,* (2023) reported Salazinic acid also possesses free radical scavenging activities. The In vitro screening of salazinic acid (Sa) for antioxidant activity against free radicals and metal ions demonstrated its substantial efficacy, surpassing that of ascorbic acid. Salazinic acid exhibited significantly lower IC50 values of 121.47 ± 4.53 nM ($p < 0.001$) against ABTS+·, 110.79 ± 4.32 nM ($p < 0.0001$) against DPPH·, and 131.17 ± 7.60 nM ($p < 0.05$) against superoxide radicals. In comparison, the IC50 values for ascorbic acid were higher at 168.99 ± 12.86 nM, 163.10 ± 5.94 nM, and 178.63 ± 14.33 nM, respectively, indicating that salazinic acid possesses stronger antioxidant activity across these assays than ascorbic acid. These findings highlight salazinic acid potential as a potent antioxidant agent with efficacy against various free radicals.

Candan *et al.*, (2007) analyzed the anti-microbial properties of salazinic acid derived from *Parmelia sulcata* using the solvents such as acetone, diethyl ether, chloroform, methanol, and petroleum, against *Aeromonas hydrophila, Bacillus cereus, Bacillus subtilis, Listeria monocytogenes, Proteus vulgaris, Staphylococcus aureus, Yersinia enterocolitica, Streptococcus faecalis, Candida albicans, Candida glabrata, Aspergillus fumigatus, Penicillium notatum and Aspergillus niger*,. Salazinic acid exhibited antimicrobial activity against *Pseudomonas aeruginosa* and *Salmonella typhimurium,* but did not show activity against *Listeria monocytogenes, Proteus vulgaris, Yersinia enterocolitica*, and *Streptococcus faecalis.*

The neuro-protective properties of three lichen-derived compounds were studied by Reddy *et al.,* (2016) in the contest of Natural products derived from lichens have been extensively studied for their diverse biological properties; however, their potential as therapeutic agents for the central nervous system (CNS) remains

relatively underexplored. In the study, the neuroactive properties of atranorin, perlatolic acid, physodic acid, and usnic acid were investigated for their effects on neurotrophic factors, neurogenesis, and acetylcholine esterase (AChE) activity and found that Atranorin, perlatolic acid, physodic acid, and (+)-usnic acid were found to exhibit neurotrophic activity in a preliminary cell-based screening using Neuro2A cells to assess neurite outgrowth.

Among these compounds, atranorin and perlatolic acid did not show any cytotoxic effects, but usnic acid is cytotoxic, Perlatolic acid showed promising results as it not only exhibited AChE inhibition activity but also demonstrated potent pro-neurogenic effects. The neurotrophic lichen compounds atranorin, perlatolic acid, and physodic acid were observed to modulate the gene expression of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF). Additionally, perlatolic acid showed increased acetyl histone H3 and H4 protein levels in Neuro2A cells, indicating potential epigenetic regulatory effects by the lichen substances that could contribute to its neurotrophic properties.

Aparna *et al.,* (2012) assessed the Anti-Inflammatory Property of *n*-Hexadecanoic Acid which is present as a secondary metabolite in the genus *Parmotrema,* by analyzing the structural Evidence and Kinetic Assessment of the compound. The study proposes that inflammation begins with the hydrolysis of ester bonds in membrane phospholipids by Phospholipase A2, leading to the release of fatty acids. The inhibition Phospholipase A2 could be a strategy to control inflammation. The study revealed that n-hexadecanoic acid binds within the active site of phospholipase A2.The enzyme kinetics study demonstrated that n-hexadecanoic acid inhibits phospholipase A2 through competitive inhibition. Analysis using crystallography at a resolution of 2.5 revealed that n-hexadecanoic acid binds within the active site of phospholipase A2. From the structural and kinetics studies, it can be concluded that the fatty acid n-hexadecanoic acid acts as an inhibitor of phospholipase A2, thereby demonstrating anti-inflammatory properties. These findings support the traditional use of medicated oils containing n-hexadecanoic acid in Ayurvedic medicine in India for treating rheumatic symptoms. This validates

using such oils as therapeutic agents based on their ability to inhibit phospholipase A2, a key enzyme involved in initiating inflammation.

Ravi and Krishnan (2017) carried out an *in-silico* molecular docking study on N-hexadecanoic acid (palmitic acid) and found that it has a strong binding affinity with DNA topoisomerase-I, evidenced by a free binding energy of -6.71 kcal/mol. The compound showed a notable IC50 value of 0.8 µg/mL against HCT-116 cancer cells. The results from the docking analysis indicate that the cytotoxic effects of N-hexadecanoic acid are likely due to its interaction with DNA topoisomerase-I. This suggests that N-hexadecanoic acid has potential as an anticancer agent and merits further investigation to explore its effectiveness against additional cancer-related protein targets

Zorrilla *et al.,* (2022) investigated the antimicrobial properties of orcinol extracted from *Ramalina implexa* Nyl. and *Roccella phycopsis* Ach. Their study found that the crude organic extracts from both lichen species exhibited strong antibiotic effects against certain bacterial strains and demonstrated nematocidal activity. The compounds (+)-usnic acid, orcinol, and (+)-montagnetol showed significant nematocidal effects against the tobacco pest *Meloidogyne incognita*, comparable to the commercial nematocide "Velum," suggesting their potential use as biopesticides in agriculture. Additionally, orcinol exhibited antibacterial activity against all tested Gram-positive and Gram-negative bacterial strains.

Yanik and Ates (2023) studied the effects of orcinol on proliferation and apoptosis of sw480 human colorectal cancer cells and the cell viability was determined by MTT test. The MTT analysis results indicated that orcinol caused a significant decrease in cell viability at concentrations of 5 mM and above. In the control group, cell viability was measured as 100.00 ± 6.14%, whereas in cells treated with 25 mM orcinol, it decreased significantly to 12.50 ± 0.65%. Furthermore, Annexin V binding analysis revealed that the early apoptotic cell population was 12.06 ± 1.22% in the group treated with 25 mM orcinol, compared to 0.60 ± 0.11% in the control group. These findings collectively indicate that orcinol exhibits cytotoxic effects at high concentrations on SW480 colorectal cancer cells.

Tuan *et al*., (2020) investigated the antifungal properties of various chemical constituents from *Parmotrema tinctorum* and evaluated the activity of four compounds against different fungal pathogens: *Colletotrichum* sp. (which causes chili anthracnose), *Fusarium oxysporum* (which causes sesame wilt), and *Pyricularia oryzae* (which causes rice blast). The compounds studied were methyl β-orsellinate, methyl orsellinate, ethyl orsellinate, and n-butyl orsellinate. Methyl β-orsellinate exhibited complete inhibition of *Pyricularia oryzae*. The antifungal activity of the compounds was ranked in decreasing order as follows: methyl β-orsellinate, methyl orsellinate, ethyl orsellinate, and n-butyl orsellinate.

Benzoic acid ($C_7H_6O_2$, BA) is the colourless crystalline solid, which is the simplest aromatic carboxylic acid and is commonly found in the secondary metabolite profile of the genus Parmotrema. Del Olmo *et al.,* 2017 reported that Benzoic acid and its diverse derivatives and related benzenic compounds—including salts, alkyl esters, parabens, benzyl alcohol, benzaldehyde, and benzoyl peroxide—are widely employed as preservatives with antibacterial and antifungal properties, as well as flavour enhancers in food, cosmetics, personal hygiene items, and pharmaceuticals.

Kluge *et al.,* (2006) conducted a study to evaluate the effects of dietary benzoic acid in animal models. They investigated how adding potassium formate at 12 g/kg, benzoic acid at 10 g/kg, and benzoic acid at 5 g/kg to a basal diet affected growth performance, nutrient digestibility, nitrogen balance, and gastrointestinal microflora in piglets. The results showed that piglets receiving the diet supplemented with benzoic acid at 10 g/kg had a 9% increase in average feed intake, a 15% increase in body weight gain, and a 6% improvement in feed conversion ratio compared to the control group. Benzoic acid did not alter the pH or ammonia levels in the gastrointestinal tract but significantly reduced bacterial counts in the digesta. In the stomach, it decreased the levels of total aerobic, total anaerobic, lactic acid-producing, and gram-negative bacteria. In the duodenum, benzoic acid reduced gram-negative bacteria, and in the ileum, it lowered the number of total aerobic bacteria in a dose-dependent manner. Additionally, it significantly reduced acetic acid concentrations in the duodenum. These results indicate that benzoic acid has

strong antimicrobial properties in the gastrointestinal tract, which may enhance growth performance and nitrogen utilization in animals.

Torrallardona *et al.,* (2007), evaluated pig performance and the ecology of their gastrointestinal microbiota after administering 0.5% Benzoic acid. Digesta samples were analyzed through culturing to quantify specific bacteria such as Lactobacillus spp., Escherichia coli, Enterococcus spp., and Clostridium perfringens. Additionally, restriction fragment length polymorphism was employed to assess the biodiversity and similarity of microbiota profiles. The presence of benzoic acid was correlated with enhanced performance in piglets, which was linked to increased biodiversity of the microbiota in the ileum.

The pharmaceutical importance of lichens is manifold and these properties are fascinated by scientists all time. Some important lichen compounds with drug like properties are given in table 4

Sl. No.	**Name of the compound**	**Rt time**	**medicinal properties**	**Reference**
1	Orcinol	16.698	Anti cancer agent antibacterial, anti-fungal, anti-oxidant, anti-diabetic, and anti-inflammatory	Yanik and Bakar-Ates(2023) Lawrey (1986); Boustie and Grube (2005); Shukla *et al.'* (2010); Shrestha and Clair (2013).
2	Ethyl 2,4-dihydroxy-6-methylbenzoate	24.859	Flavouring agent Antibacterial	Gomes *et al.*, (2003)
3	n-Hexadecanoic acid	29.391	Antibacterial activity against Bacillus subtilus (NCIM 2718), Staphylococcus aureus (ATCC 25923), Pseudomonas aurginosa (ATCC 27853), Klebseilla pneumoniae (ATCC 70063), and Escherichia coli (ATCC 25922).	Omotoso *et al.,* (2024)

4	Benzoic acid,		antimicrobial food additive	Torrallardona *et al.,* (2007)
5	Squalene	43.438	Antibacterial activity against Escherichia coli, Klebsiellapneumoniae, Vibrio harveyi Micrococcus roseus, and Staphylococcus aureus. Chemopreventive	Brown and Herbert, (2007) Smith,(2000)
6	Phthalic acid	29.555	insect repellents, larvicidal properties in mosquito	Xu and He, (2010)
7	Octadecanoic acid	33.105	cytotoxic	Kerboua (2022)
8	Benzaldehyde	20.618	Anti microbial, fragrant, food preservative, cosmetic , food additive	Andersen (2006)

Table 4 - Pharmaceutical Importance of Various Lichen Substances

3.2.4. Biochemical and Species-LevelBioprospection

The study conducted by Goel *et al.,* (2011) focused on evaluating the antifungal activity of extracts from the lichen *Parmelia reticulatum* against several soil-borne pathogenic fungi using the poisoned food technique. Hexane, ethyl acetate, and methanol extracts of *Parmelia reticulata* were tested for their antifungal properties. Pathogenic Fungi Tested included *Sclerotium rolfsii*, *Rhizoctonia solani, Rhizoctonia bataticola, Fusarium udum, Pythium aphanidermatum* and *Pythium debaryanum*. Several secondary metabolites were isolated and identified from the extracts using analytical techniques such as 1H NMR, 13C NMR, and mass spectroscopy. The identified metabolites included: Isousnic acid, Protolichesterinic acid, Atranorin, Ethyl hematommate, Ethyl orsellinate, Methyl hematommate, Baeomycesic acid and Salazinic acid. The study found that protolichesterinic acid exhibited the highest antifungal activity against Rhizoctonia solani (ED50 = 23.09 µg/mL) and Pythium debaryanum (ED50 = 16.07 µg/mL). Atranorin also showed significant antifungal activity against Sclerotium rolfsii (ED50 = 39.70 µg/mL).These findings are significant as they highlight the potential of secondary

metabolites from *Parmotrema reticulatum* as natural antifungal agents against economically important soil-borne pathogens.

Tiwari *et al*., (2011) investigated the antifungal properties of the Himalayan foliose lichen *Parmotrema tinctorum* (Despr. ex Nyl.) Hale. Their study analyzed acetone, methanol, and chloroform extracts of *Parmotrema tinctorum* for antifungal activity against ten plant pathogenic fungi, including *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Alternaria alternata*, *Fusarium oxysporum*, *Fusarium solani*, *Fusarium roseum*, *Ustilago* spp., *Albugo candida*, and *Penicillium citrinum*. The effectiveness of these extracts was compared to the synthetic antifungal drug Ketoconazole using a disk diffusion assay. The findings revealed that the methanol extract was the most effective against all tested fungi, with acetone and chloroform extracts showing slightly less activity. Principal component analysis (PCA) indicated that while Ketoconazole was effective against five of the fungi, the extracts from *Parmotrema tinctorum* were more effective against the other five fungi, namely *Aspergillus fumigatus*, *Fusarium solani*, *Fusarium roseum*, *Penicillium citrinum*, and *Ustilago* spp.

Sati and Joshi (2011) investigated the antibacterial properties of methanol, ethanol, chloroform, and aqueous extracts obtained from the lichen *Parmotrema nilgherrense* collected in Nainital, Kumaun Himalaya. The extracts were tested against five pathogenic bacteria *Bacillus subtilis, Erwinia chrysanthemi, Escherichia coli, Agrobacterium tumefaciens, and Xanthomonas phaseoli* using the agar-well method. All extracts of *P. nilgherrense* showed significant antibacterial activity. The chloroform extract exhibited the highest antibacterial activity against the tested microorganisms, with inhibition zones ranging from 23 to 38 mm, followed by the ethanol and methanol extracts which showed zones of inhibition ranging from 12 to 24 mm. Negative controls consisted of wells treated with solvents, while positive controls included wells filled with standard antibiotics. These results indicate that extracts from *P. nilgherrense* possess broad-spectrum antibacterial properties effective against a range of bacteria responsible for common plant and animal diseases.

Kekuda *et al.,* (2015) investigated the inhibitory effects against *Colletotrichum capsici* using extracts from three macrolichens, namely *Parmotrema tinctorum, P. grayanum*, and *P. praesorediosum*, collected from the Western Ghats of Karnataka, India. The antifungal activity was assessed based on mycelial growth inhibition using the Poisoned Food Technique. *Parmotrema tinctorum* exhibited significant inhibitory activity, achieving more than 50% inhibition

Sharma and Kalikotay (2012) studied the antioxidant properties of two commonly found lichens, *Parmotrema reticulatum* and Usnea sp., collected from the Darjeeling hills of eastern Ghas. The antioxidant activity of ethanolic and methanolic extracts of these lichens was evaluated using DPPH radical scavenging activity, total antioxidant activity, reducing power ability, flavonoid content, phenolic content,etc. The results indicated that the methanol extracts of *Parmotrema reticulatum* and Usnea sp. exhibited DPPH radical scavenging activities ranging from 10% to 31.5%, respectively. The reducing power, measured by absorbance values, varied between 0.376 and 0.514. Furthermore, the total phenolic content of the extracts was found to be high, while the total flavonoid content was moderate. Overall, both lichen species demonstrated significant antioxidant activities based on the parameters tested in this study. The findings of the study suggest that *Parmotrema reticulatum* and Usnea sp. could serve as valuable natural sources of antioxidants.

Traditional medicine uses the lichens of the genus *Parmotrema* against various diseases. Some of these practices and formulations were scripted in various folklore, and some are still followed. Table 5 compiles such ethnobotanical practices of lichens.

No	Name of the Species	Traditional uses	Medicinal Property	Reference
1	*Parmotrema austrosinense* (Zahlbr.) Hale	No property reported	Beta - glucosidase inhibitor	Lee and Kim (2000), Balaji and Hariharan (2007)
2	*Parmotrema chinense* (Osbeck) Hale and Ahti	Chharila, derived from a medicinal plant, serves multiple purposes in traditional medicine. It is applied to treat wounds, cardio vascular diseases, gastric disease, dyspepsia, scabies, leprosy, sore throat, toothache, general pain, kidney stones, painful urination, hemorrhoids, lack of menstruation, menstrual pain, broken bones, rheumatism, and reducing swelling. Additionally, it functions as a carminative, aphrodisiac, diuretic, sedative, and astringent.	It is noted for its antioxidative, cardioprotective, anticancer, antifungal, cytotoxic, antiobesity (pancreatic lipase inhibitory), antimicrobial, anti-helminthic, insecticidal, antimycobacterial, antibacterial, and amylase inhibitory activities. Diuretic and amenorrhoea, dysentery antibacterial potential against *Staphylococcus aureus* and *Eschirechia coli*	Chandra and Singh (1971) Nadkarni (1976) Abdulla *et al.* (2007)
3	*Parmotrema hababianum* (Gyeln.) Hale	Using for spice and medicinal purposes. It is known to relieve kidney disorders and venereal diseases, and it is also used as a treatment for skin diseases. Mixture of ash and mustard or linseed oil for skin diseases.	Anti-hyperglycemic, antioxidant	Upreti *et al.* (2005), Ganesan (2017)

4	*Parmotrema nilgherrense (Nyl.) Hale*	Traditional practices did not distinguish between *P. nilgherrense* and *P. chinense*	Antioxidative, cardioprotective,, anticancer, antifungal, cytotoxic, antiobesity antimicrobial, anti-helmintic, insecticidal, antimyco-bacterial, antibacterial, amylase inhibitory	Kekuda *et al.* (2015), , Swathi *et al.* (2010), Gupta (2007), Nayaka (2010), Thadhani (2017)
5	*Parmotrema dilatatum* (Vain.) Hale	No medicinal activity reported.	immunological modulating activities	Santos *et al.* (2004)
6	*Parmotrema praesorediosum* (Nyl.) Hale	No property reported		Lee and Kim (2000) Balaji and Hariharan (2007)
7	*Parmotrema reticulata* (Taylor) M. Choisy	Used as spice, relieves urinary diseases and as medicine for skin diseases. The fresh plant is burnt to repel insects, used for ringworm like skin disease	kidney disorder or venereal disease Anti-hyperglycemic, antioxidant antibacterial activity against virulent strain of *Mycobacterium tuberculosis* H37Rv	Pennington (1969) Upreti *et al.,* (2005), Ganesan (2017), Gupta *et al.,* (2007)
8	*Parmotrema saccatilobum* (Taylor) Hale	No property reported	inhibition of Epstein-Barr virus activation induced teleocidin B-4 and superoxide dismutase like activity Antioxidant, and cytoprotective	Yamamoto *et al.* , (1998) Fernandez (2015)
9	*Parmotrema sancti-angelii* (Lynge) Hale	Spice, insect repellant	to treat ring-worm like skin disease of the neck	BrijLal and Upreti (1995)

10	*Parmotrema stuppeum* (Taylor) Hale	Spice, insect repellant	Moderate antioxidant activity	Jayaprakasa and Rao (2000)
11	*Parmotrema tinctorum* (Nyl.) Hale	Spice, insect repellant and flavoring agents. Used against bleeding and swelling Skin protective and chronic dermatitis and localized swelling	Anti proliferaive lipid peroxidation and tyrosinase enzyme activity free radical scavenging activity property	Kumar and Muller (1999a,b) Verma *et al.* (2008a) Verma *et al.* (2008b)
12	*Parmotrema praesorediosum* (Nyl.) Hale	No activity reported	Beta-glucosidase inhibitor, antimicrobial, antibacterial, antifungal	Lee and Kim (2000), Balaji and Hariharan (2007)

Table 5 - Ethno botanical significance and traditional uses of lichens of the genus *Parmotrema* A. Massal.

CHAPTER 4
MATERIALS AND METHODS

4. Materials and Methods

4.1 Systematic Treatment of the Genus *Parmotrema A. Massal.*

The systematic treatment of the genus *Parmotrema* is not just a fundamental aspect of our research, but a cornerstone of lichenology and taxonomy. It plays a crucial role in enhancing taxonomic accuracy, understanding ecological roles, leveraging medicinal properties, advancing conservation efforts, and contributing to scientific research and education. More than that, it ensures that species are correctly identified, understood, and preserved, thereby supporting both practical applications and theoretical knowledge in our field. The systematic treatment of the genus *Parmotrema* is essential for several reasons, each reflecting its significance in taxonomy, ecology, medicine, and conservation.

4.1.1. Collection, Identification and Preservation of Lichens as Herbarium

For the present work, more than 300 specimens were meticulously collected from various terrains and altitudes of Kerala. Each sample was systematically processed and analyzed. The study area, which comprises twenty-four sample sites across four districts of Kerala (Table 1), was a rich source of diverse lichen species. The specimens were carefully processed by the methods of Nayaka, (2014), deposited in the herbarium of Malabar Botanical Garden and Institute for Plant Sciences (MBGH). The lichen herbaria of Kerala Forest Research Institute were also utilized for species identification and comparison. The morphological characters were studied under a stereo zoom microscope, and the photographs were recorded with a camera mounted. The anatomy of the thallus, the main body of the lichen, and the anatomy of apothecia and perithecia, the reproductive structures, was studied under a compound microscope to study the organization of algal and fungal components and spore characters.

Identification Characterization and Documentation of Lichen

The identification, characterization, and documentation of lichens, particularly those of the genus *Parmotrema*, were a meticulous and thorough process. These lichens exhibit similarity in growth forms and growth patterns, so the initial segregation was

based on the presence or absence of the cilia, the character of rhizines, and reproductive structures. The specimens displayed variations in surface structures, such as vegetative reproductive structures, i.e. Isidia, medullary chemistry, and shape of the margin. From the various accessions of the specimens, those with fertile lobes were selected for identification. Vegetative reproductive structures like pycnidia, isidia, soredia, and bulbils, and sexual reproductive characters like perithecia and apothecia were observed and recorded. Some species are sterile. The lichens were identified by studying their morphology, anatomy, and phytochemistry. Spot tests, TLC, and microcrystallography were used to study the chemistry of the specimen, ensuring a comprehensive and reliable identification process.

Spot Test

When applied, certain chemicals impart a colour change to thallus parts. A colour change was denoted by a positive (+) symbol, followed by the colour produced and no change in colour was denoted by a negative (-) symbol.

K Test: In the K Test, a 10-25% aqueous solution of potassium hydroxide is applied to the cortex, medulla, and apothecia. This solution serves as a clearing agent for slices of fruiting bodies and thalli, as it frequently dissolves crystalline lichen substances and eliminates some mucilage, which can obscure details in the sections. The resultant colour forms in the thallus gives the result.

C Test: A freshly made aqueous solution of calcium hypochlorite, also known as bleaching powder, or a commercial bleaching fluid with active chlorine was utilized for the procedure. The resultant colour forms in the thallus gives the result.

KC test: At a specific location on the thallus, K was initially applied, and C was then applied immediately afterward.

P Test: A 1-5% solution of paraphenylenediamine was made in ethanol in a small batch for daily use, as it is unstable and cannot be stored for the following day. For longer-term use, a more stable alternative, known as Steiner's Pd, was prepared by dissolving 1.0 gram of paraphenylenediamine and 10 grams of sodium sulfite in 100

milliliters of distilled water, along with 1.0 milliliter of liquid detergent. This reagent remains effective for approximately a month.

The chemical components of the lichens were identified by the standardized TLC methods and crystallography (Orange *et al.*, 2001). Chromatograms were developed in solvent system A (toluene: dioxan: acetic acid, 180:60:8). The specimens were identified and authenticated following literature on lichens by (Hale 1983 & 1987) ; (Patwardhan,1983); (Awasthi 1991& 2007); (Singh and Sinha 2010); (Mishra and Upreti 2017), (Hekking and Sipman 2018); (Sinha 2021), and comparing with the specimens housed at KSCSTE- KFRI Peechi, Kerala (Sreekumar *et al.,* 2017).

The external morphology of the species is often the most valuable evidence in systematic studies. Morphological and habitat information were recorded from the field itself. A detailed photograph of the specimen and the substratum was captured with a Digital camera, EOS 77D, Canon and saved with the specimen's field number. Detailed morphological studies were carried out using the Leica stereo microscope and Leica binocular compound microscope.

4.1.2. Thin Layer Chromatography and Micro Crystallography

Thin-layer chromatography is used to identify the compounds in the thallus. The lichen substances were extracted using acetone, and the extract was plated on aluminium plates coated with silica gel. Merck TLC Silica gel 60 F_{254} was used for TLC. And the lichen extract made with acetone was applied over the TLC plate with the help of a capillary tube. The plate was immersed in a shallow layer of the solvent system. While moving the mobile phase, *ie.* The solvent ascends through the stationary phase and the loaded substance tends to move along with the mobile phase as fractions. The components with higher affinity or adsorption will lag, and as a result, the components will get separated. 10% sulphuric acid is sprayed over the TLC plate and kept in an oven preheated at 1100 c for a few minutes to make the plates clear enough to make the spots. The developed plate is divided into approximately 7 equal parts called Rf classes. The lichen substances of *P. reticulatum* i.e. Atranorin is the uppermost point at Rf class-7, while salazinic acid is at Rf class- 2

Lichen substances extracted with acetone can be crystallized on gentle vaporization near dryness along with a suitable crystallizing solution. The nature and shape of crystals vary based on secondary metabolites, and they are frequently used to detect the presence of gyrophoric acid, salazinic acid, skyline, etc. (Orange *et al*., 2001).

4.2. Biogeography of Lichens

The study was carried out in various sample sites of Kerala where lichens grew abundantly, and these sites were grouped into three zones based on elevation. The ecological studies were conducted using narrow frequency grid (sampling ladder) (Scheidegger *et al.,* 2002). The geographical parameters and zonal classification are given in Table 1. In the district, a dataset of biogeography of lichens was generated using a quadrant study, which was randomly laid to assess the diversity of lichens in each zone. Twenty quadrants were randomly laid in each zone. The diversity and species richness of lichens depends on several factors (John, 1992). Lichens are sensitive to pollution and environmental factors such as altitude, relative humidity, precipitation etc. (Misra, 1968). A diversity index is a numerical metric used to quantify the variety of different types, such as species, present within a particular dataset or community. This index serves as a statistical tool to represent various dimensions of biodiversity, including the richness of species, their evenness or uniform distribution, and the degree of dominance by certain species. Essentially, diversity indices provide a way to assess and compare the complexity and distribution of species within different ecological contexts.

4.2.1. Shannon Index

The Shannon Diversity Index, also known as the Shannon-Wiener Index, is a method used to quantify the diversity of species within a community (Shannon, 1949). It is represented by the symbol H. this index is calculated as:

$$H = -\Sigma p_i * ln(p_i)$$

where:

- **Σ:** A Greek symbol that means “sum”
- ***ln*:** Natural log
- p_i**:** The proportion of the entire community made up of species *i*

The higher the value of H, the higher the species. A value of H = 0 indicates a community with only one species.

4.2.2. Simpson Index

Simpson's Index (D) measures the probability that two individuals randomly selected from a sample will belong to the same species (or some category other than species) (Simpson, 1949).

Denoted as *D*, this index is calculated as:

$$D = \Sigma n_i(n_i-1) \ / \ N(N-1)$$

where:

- **n_i:** The number of organisms that belong to species *i*
- **N:** The total number of organisms

The value for Simpson's Diversity Index ranges between 0 and 1. The higher value of D indicates a lower diversity

4.2.3. Eveness`

Evennessof the region expresses the Shannon – Weiner function (H') relative to the maximum value that H can be obtained. Evenness reaches a maximum when all the species in the sample have the same number of individuals.

$$E = \left(\frac{H'}{H'max}\right)$$

Where,

H' = Shannon-Wiener Index

H' max = Species Richness

4.2.4. Importance Value Index (IVI)

The importance Value Index (IVI) is calculated by

IVI = Relative frequency + Relative Density (Curtis and McIntosh, 1950; Phillips, 1959; Pinokiyo *et al.,* 2008)

Frequency- refers to the degree of dispersion of individual species in an area. It can also be defined as the change or probability of an individual of a given species being present in a randomly placed quadrate.

Frequency is calculated by

$$\textbf{Frequency} = \left(\frac{\textbf{Number of quadrates in which a species occurs}}{\textbf{Total number of transect sampled}}\right)$$

Relative frequency- determined by the following formulae using the data obtained by the quadrate method.

$$\textbf{Relative Frequency} = \left(\frac{\textbf{Frequency of the species}}{\textbf{Total frequencies of all the species}}\right)\textbf{100}$$

Density- an expression of the numerical strength of a species where the total number of individuals of each species is divided

Density is calculated by

$$\textbf{Density} = \left(\frac{\textbf{total number of individual species}}{\textbf{total number of transect}}\right)$$

Relative density- the study of the numerical strength of a species in relation to the total number of individuals of all species

Relative density can be calculated as:

$$\textbf{Relative Density} = \left(\frac{\textbf{Total no. of individuals of the species in all the transect}}{\textbf{Total no. of individuals of the species in all the transect}}\right)\textbf{100}$$

Abundance – It is an appreciation of the relative number of individuals of each species entering into the constitution of the plant population of the territory under study, but thus obtained in quantitative terms gives little idea of the distribution of the species

Abundance is calculated by

$$\text{Abundance} = \left(\frac{\text{Total no. of the individuals of the species}}{\text{Total no. of quadrate in which the species occurred}}\right)$$

4.3. Qualitative and Quantitative Determination of Lichen Metabolites

Preparation of Lichen Extracts

Based on the abundance and quantitative availability, seven species of lichens were selected for further study. The selected lichens were washed in distilled water to remove the dust and other impurities and also make the samples free from contaminants. The washed lichens were dried in the shade and kept at room temperature and in air-tight containers. The lichen materials were made into fine powder and the metabolites were extracted by soxhlet apparatus using Ethyl acetate as the solvent. Five grams of dried samples were used to extract the metabolites, and the final extract was collected after two days of continuous soxhlet extraction. The extracts were filtered using Whatman No. 1 filter paper and concentrated at 40^{o}C under reduced pressure. The condensed solvent extracts were stored at 4^{o}C until use.

4.3.1. Identification and Quantification of Bioactive Compounds

Identifying bioactive compounds in lichens involves chemical, biological, and analytical techniques.

4.3.1.1. Gas Chromatography Mass-Spectroscopy (GC-MS) Analysis

1mg of purified sample was dissolved in 1ml of ethyl acetate from which 1 µl was subjected to GC-MS analysis. GC- MS equipment Agilent 5975C series selective detector interfaced to a quadrupole mass analyser employing the following conditions: column DB 5 MS (30m L x 0.25mm ID x 0.25um film thickness), operating in Electron Impact Ionization system with an impact mode of 70eV was used, helium was used as carrier gas at a constant flow rate of 1ml/min and injection volume of 1µl with an split made of 1:20 ratio. The oven temperature was programmed from 400 C, with an increase of 100C/min to 3000C/min isothermal; then holds for 5 minutes, scan mass range was from 30m/z – 600m/z with positive

polarity. 4.5 Identification of compound was based on the interpretation of the mass spectrum obtained from GC- MS was carried out using the database of National Institute Standard and Technology (NIST 2011), having more than 95,000 spectra. The name, molecular weight and structure of the components of the sample were ascertained using NIST Ver. 2.0 (2011) MS data library.

Instrumentation

GC SPECIFICATION	:	Shimadzu Nexis GC- 2030
AUTO SAMPLER	:	AOC-30/20i
COLOUMN SPECIFICATIONS		
Column Name	:	SH-I- 5Sil MS
Length	:	30.0 m
Inner Diameter	:	0.25 mm
Film thickness	:	0.25 μm
GCMS Software	:	GCMS Solutions
Libraries used	:	NIST 20

Quantification of Flavonoids and Phenols

All the extracts were subjected to systematic quantitative phytochemical investigations. A known aliquot of the crude extract (I mg) was diluted with 1 ml DMSO to attain a concentration of I mg/ml. The quantitative phytochemical analysis of all the extracts was performed to quantify the amount of Flavonoids and Phenolic compounds. Samples were analyzed in triplicates.

4.3.1.2. Determination of Total Flavonoids

To analyze the total flavonoid content, the Aluminum chloride method was employed following Chang *et al*., (2002). First, the nearly dried lichen extract (I mg) was dissolved in 1 ml of DMSO. To this solution, 0.1 ml of a 10% Aluminum chloride solution (prepared by dissolving 10 ml of $AlCl_3$ in 100 ml of distilled water), 0.1 ml of 1M Potassium acetate, and 2.8 ml of distilled water were added.

The resulting mixture was allowed to stand at room temperature (25°C) for 30 minutes. Afterward, the absorbance was measured at 415 nm using a UV-visible spectrophotometer. A calibration curve was constructed, and the total flavonoids were expressed as mg of quercetin equivalents (QE) per gram. Each sample was analyzed in triplicate (Kaur and Kapoor, 2002)

4.3.1.3. Determination of Phenols

To determine the total phenol content, the Folin-Ciocalteu method was utilized. Aliquots of 0.25 ml of 1 mg/ml extract were combined with 1.25 ml of Folin-Ciocalteu reagent (0.2 N, diluted in methanol). After mixing, the volume in each tube was adjusted to 3.0 ml with distilled water. Following this, 0.5 ml of Folin-Ciocalteu reagent was added, and the tubes were placed in a boiling water bath for precisely one minute. Once cooled, the absorbance was measured at 650 nm using a spectrophotometer, with a reagent blank as the reference. The total phenol content was calculated and expressed as milligrams of Gallic Acid Equivalents (GAE) per gram of extract (Baba and Malik, 2015).

4.4. Bioprospection

4.4.1. Determination of Antimicrobial Potential

The agar well diffusion method is widely used to evaluate the antimicrobial activity of plant extracts.

Media Preparation

The media were prepared by dissolving 38 grams of commercially available Mueller-Hinton Agar (for bacterial cultures) and 39 grams of Potato Dextrose Agar (for fungal cultures) in 1000 milliliters of distilled water. This mixture was then autoclaved at 15 pounds of pressure and 121°C for 15 minutes. After autoclaving, the medium was thoroughly mixed and poured into 100 mm petri dishes, with 20-25 milliliters per plate, and allowed to solidify.

Antibacterial Assay

A standardized inoculum of the test organism was evenly distributed on the surface of Petri dishes containing Mueller Hinton Agar using a sterile cotton swab. Four wells, each 8 mm in diameter and spaced 20 mm apart, were then created aseptically in each plate using a sterile cork borer. The plates were incubated under conditions appropriate for the test microorganism. Following incubation, the plates were examined for clear zones around the wells. The inhibition of bacterial growth was measured in millimeters (Valgus *et al.,* 2007).

Antifungal Assay

A standardized fungal culture inoculum was evenly spread over the surface of Petri plates containing Potato Dextrose Agar using a sterile cotton swab. Afterward, four wells, each 8 mm in diameter and spaced 20 mm apart, were aseptically created in each plate with a sterile cork borer. A pipette was used to introduce 20 and 40 µl of the sample into the wells, with one well reserved for a solvent-only negative control. The plates were then incubated at room temperature for 2 days. Following incubation, the plates were examined for zones of inhibition, which were measured using a caliper (Magaldi *et al*., 2004).

4.4.2. Anti-Oxidant Assay

DPPH Radical Scavenging Assay

The DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate) radical assay is a commonly employed method for assessing the free radical scavenging capability of natural compounds. This assay measures how effectively antioxidant substances can neutralize the stable DPPH radical. To evaluate the free radical scavenging activities of the extracts in vitro, the procedure outlined by Brand-Williams *et al.* (1995) was followed.

In this assay, DPPH interacts with an antioxidant compound that can donate hydrogen, leading to the reduction of DPPH. The resulting color change from deep violet to light yellow is quantified by measuring absorbance at 515 nm using a UV-

visible spectrophotometer. Ascorbic acid was used as a reference standard, dissolved in distilled water to prepare a stock solution at a concentration of 1 mg/100 ml. A fresh 60 µM solution of DPPH in methanol was prepared daily for the measurements.

For the assay, 3.9 ml of the DPPH reagent was mixed with 100 µl of extracts at various concentrations. The mixtures were incubated in the dark at room temperature for 15 minutes, after which the decrease in absorbance was recorded. The experiment was conducted in triplicate. A control sample, containing the same volume of DMSO without any extract and ascorbic acid, was prepared, and 95% methanol was used as the blank (Brand-Williams *et al.*, 1995). The radical scavenging activity was calculated using the appropriate formula.

$$\% \text{ of inhibition} = \left(\frac{\text{Absorbance of Control at 0 minute} - \text{Absorbance of Test \%}}{\text{Absorbance of Control at 15 minutes}}\right) 100$$

ABTS Radical Scavenging Assay

To prepare the ABTS stock solution, an aqueous solution of ABTS (Sigma Aldrich, India) at 7 mM was mixed with an equal volume of a 2.45 mM aqueous potassium persulphate solution (Merck, India). This mixture was allowed to stand in the dark at room temperature for 12 to 16 hours prior to use.

The working ABTS solution was then prepared by diluting the stock solution with methanol until the absorbance reached 0.70 ± 0.02 at 734 nm. For the assay, 2.0 mL of this ABTS solution was combined with 1 mL of the aqueous extracts at varying concentrations (0.5 to 5.0 mg/mL). The mixture was incubated in the dark at room temperature for precisely 10 minutes (Kaur, and Kapoor, 2002).

A control was set up by mixing 2.0 mL of ABTS solution with 1 mL of double-distilled water. The absorbance of the mixture was measured at 734 nm using a spectrophotometer (Systronics Visiscan 167), with a blank used for calibration. BHT (Merck, India) served as the standard reference. Each sample was prepared and analyzed in triplicate.

The percentage of scavenging activity of each extract on ABTS was calculated as,

% inhibition (I%) = I% = [(Ao-As)/ Ao] × 100

Where as I% = Percentage of inhibition, A_0 = absorbance of the control, As = absorbance of the sample

CHAPTER 5
RESULTS

5. Results

5.1. Systematic Treatment of the Genus *Parmotrema*A. Massal.

The assessment of the diversity of the genera was carried out by collecting samples from the various lichen-growing regions of the state of Kerala, systematically preserving these specimens as herbarium, and depositing them at KSCSTE-MBGIPS with the acronym MBGH. Field-based data include latitude, longitude, and altitude for geographical mapping, habitat or the substratum where the specimen is growing, and colour of the thallus and all other characters relevant to the taxon. 29 species of lichens belong to the genera *Parmotrema* A. Massal. in Kerala were collected during the study. The specimens were described, and the key for the species identification was indicated as follows.

5.1.2. Key to the Species

1. Margin of lobes ciliate 2

1a. Margin of lobes lacking cilia 19

2. Thallus sorediate or pustulate sorediate 3

2a. Thallus lacking soredia or pustulate soredia..... 15

3. Medulla K+ red, salazinic acid present..... 4

3a. Medulla K+ (yellow/orange) or K−..... 7

4. Sorediate lobes are forming lacinules..... 5

4a. Sorediate lobes are not forming lacinules..... 6

5. Medulla K+ yellow → red (salazinic acid) lower surface of the lcinulesarewhiteandgyrophoric acid present ..... ***P. clavuliferum***

5a. Medulla K+ yellow → red (salazinic acid) lower surface of the lcinulesarewhiteandgyrophoric acid absent..... ***P. margaritatum***

6. Thallus white maculate, reticulately cracked, margins rhizinate .. ***P. reticulatum***

6a. Thallus not white maculate, not reticulately cracked margins erhizinate. ..... ***P. stuppeum***

7. Medulla K- 8

7a. Medulla K+ (yellow/orange) 13

8. Medulla C+ rose.....9

8a. Medulla C- 11

9. Isidia coralloid, cylindrical and often ciliate and C+ faint rose.. .... ***P. planatilobatum***

9a. Isidia not prominent or absent and *C+ rose* *10*

10. Lobes reticulately cracked in older parts and soraliate lobes involute..... .....***P. sancti-angelii***

10a. Soraliate lobes ascending .....***P. indicum***

11. Medulla KC- ..... ***P. grayanum***

11a. Medulla KC+ 12

12. Lower margin wide, ivory to brownish molted and gyrophoric acid present…… ..... ***P. hababianum***

12 a. Lower margin not ivory colour and lecanoric acid present..... ***P. cooperi***

13. Thallus densely isidiate and isidia often coralloid branched..... ***P. crinitum***

13a. Thallus smooth and isidia absent or poorly developed *14*

14. Soralia marginal aswell as submarginal and the sorediate lobes *revolute* ....***P. chinense***

14a. Soralia on the margins of lacinules and the sorediatelacinules convolute ....***p. robustum***

15. Medulla P+ and rhizines restricted to the central part of the thallus .***P. cetratum***

15a. Medulla P-.....16

16. Medulla KC- .....***P. melanothrix***

16a. Medulla KC+ 17

17. Medulla C- ..... ***P. nilgherrense***

17a. Medulla C+ *18*

18. Lobes faintly maculate and KC+ deep orange red ..... ***P. abnuens***

18a. Lobescracked and densely white maculate and KC+ red.....***P. eunetum***

19. Thallus sorediate or pustulate sorediate 20

19a. Thallus lacking soredia or pustulate soredia.....27

20. Medulla K- 21

20a. Medulla K+ (yellow/orange) 25

21. Medulla K-, C-,KC- and P-.....***P. praesorediosum***

21a. Medulla either K+,C+,KC+,or P+ *22*

22. Thallus margin has wide margine zone with ivory or yellow brown color ....***P. austosinense***

22a. Thallus margin has lacking ivory or yellow brown color 23

23 Thallus closely adnate to the substratum and medulla P+ red ... ***P. saccatilobum***

23a. Thallus loosely adnate with the substratum and P- 24

24. Isidia thick, inflated, irregular and branched. ..... ***P. pseudotinctorum***

24a. Isidia cylindrical and filiform . ..... ***P. tinctorum***

25. Sorediate lobes are lacinulate and KC+.....***P. dialatum***

25a. Lobes rotund and KC 26

26 Thallus densely isidiate and lobes upto 6 mm wide .....***P. crinitoides***

26a. Thallus lacking isidia and lobes 5 mm to 10 mm wide.....***P. cristiferum***

27 Thallus isidiate lobe margins claviform and KC+ **.....*P. tsavoense***

27a. Thallus lacking isidia and KC- 28

28 Thallus loosely attached lobes8-20mm wide..... .***P. lattissimum***

28a. Thallus adnate and lobes 3 – 5 mm wide ..... ***P. mesotropum***

5.1.3. Nomenclature and Description

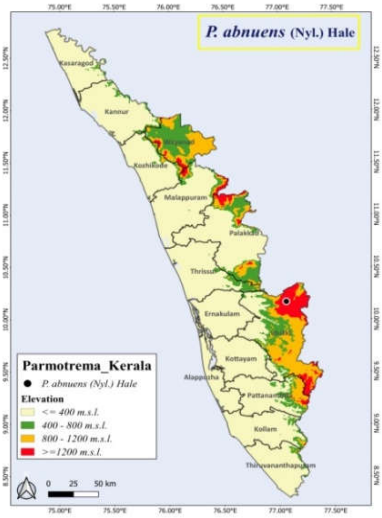
1. ***Parmotrema abnuens*** (Nyl.) Hale

Phytologia 28 (4): 334. 1974; — *Parmelia abnuens* Nyl, Flora 68: 610. 1885.

Index Fungorum Registration Identifier 343005

Thallus pale green in colour,corticolous, loosely attached to the substratum (Fig. 2), 3- 8 cm across; lobes rotund, 8–15 mm wide, becoming narrowly laciniate-canaliculate; marginal cilia present, ciliadense and long, up to 5 mm long; upper side pale grey, shiny, distinctly to faintly maculate, pitted, rugose and cracked with age,

lacking isidia, soredia and pustules ; lower side centrally black, marginal area dark brown, mottled, erhizinate; rhizines sparse in centre; medulla white. Apothecia common, stipitate, 5– 10 mm in diameter, disc pale brown in colour, disc imperforate; hymenium 80–120 µm high, ascospores 25–35 × 12–20 µm. Pycnidia present, conidia 6–10 µm long.



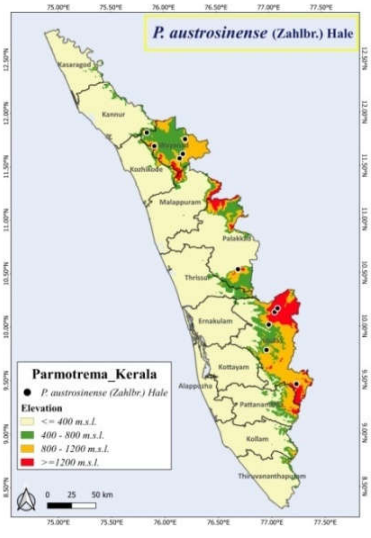
Chemistry: cortex K+ yellow; medulla K—, C+ orange-red, KC+ deep orange-red, P—.

Distribution: this taxon collected from Anamudi peak of Kerala above 1200 MSL

2. Parmotrema austrosinense (Zahlbr.) Hale

Phytologia 28: 335. 1974; — *Parmelia austrosinensis* Zahlbr., Symb. Sin. 3:192. 1930.Index FungorumRegistration Identifier: 343014

Thallus ash or faint green colour,foliose, corticolous, loosely attached to the substratum,5 to 10 cm across (Fig 3a); lobes rotund, each lobes 5 to 20 mm wide, margins ascending imbricate, sinuous; eciliate (Fig 3b); upper surface pale green or grey colour, smooth, white-maculate or emaculate, more or less rugose in the centre; soralia marginal, linear, soredia farinose to granular (Fig. 3c);, sorediate margins are wavy and assenting imbricate, wide marginal zone ivory, tan or brown mottled; erhizinate shiny marginal zone; lower side centrally black; rhizines sparse in the centre part, simple; short; up to 1 mm long, medulla white. Apothecia rare and isidia absent.



Chemistry: Cortex K+ yellow; medulla K—, C+ rose red, KC+ red, P—

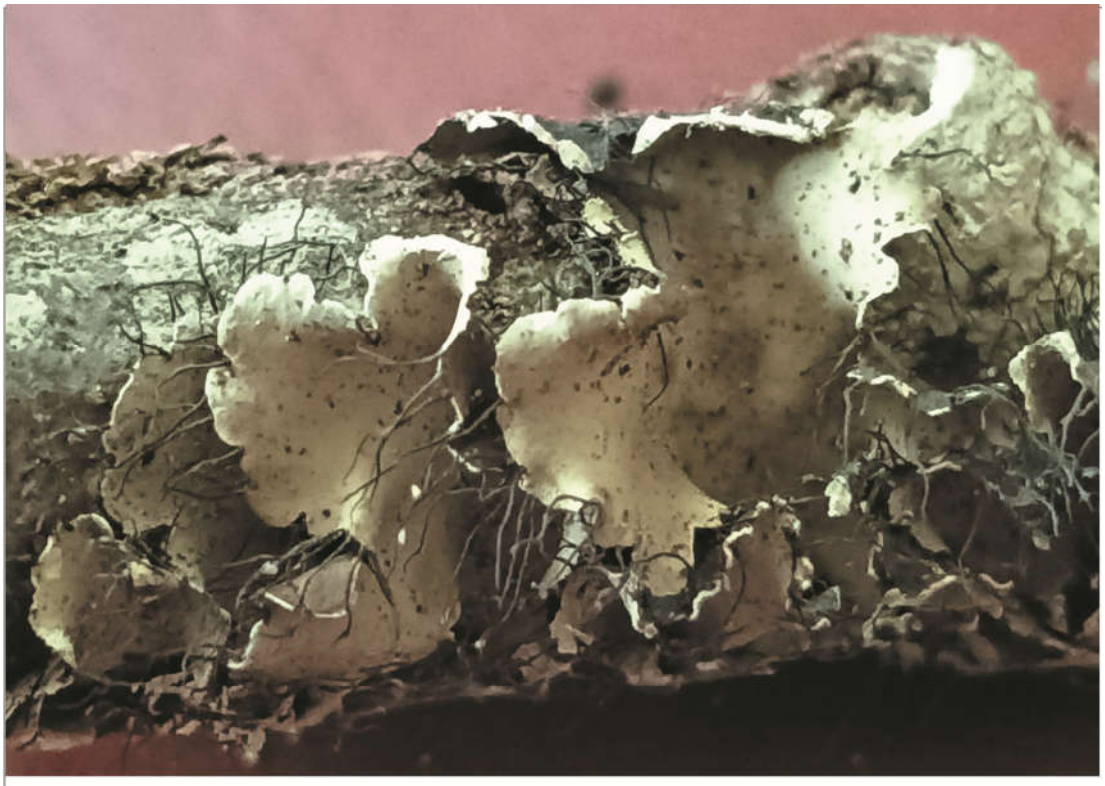


Fig.2. *P. abnuens (Nyl.)* Hale:a. Thalus with marginal cilia and pycnidia.

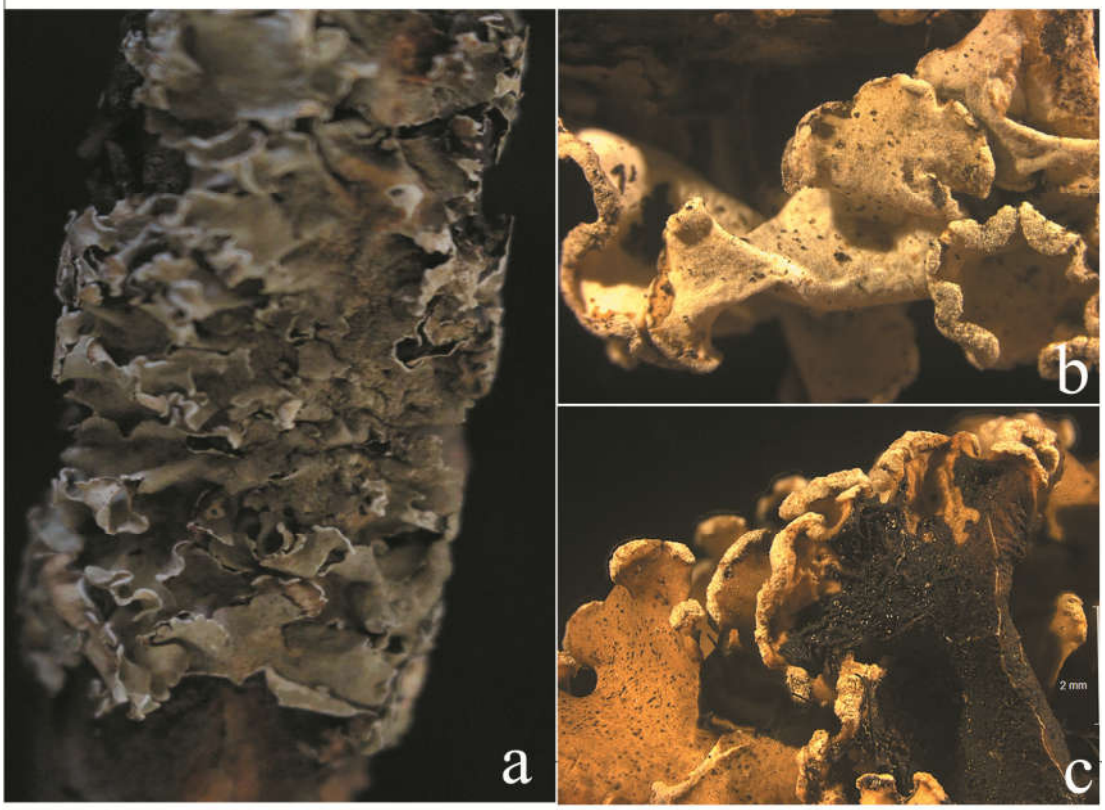


Fig.3. *P.austrosinense (Zahlbr.)* Hale*:* a.Habitat; b.Lobe margins; c.Soralia

Atranorin and lecanoric acid are present in TLC

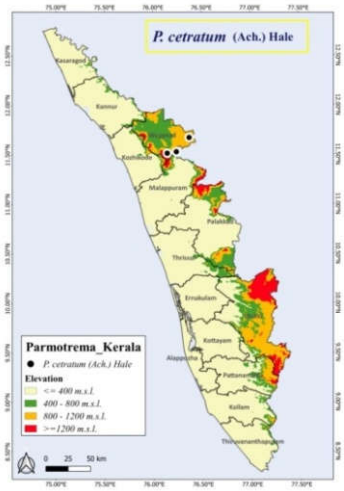
Distribution: This taxon is found in elevations above 700m msl.

3. Parmotrema cetratum (Ach.) Hale

Phytologia 28: 335. 1974; — *Parmelia cetrata* Ach., Syn. Meth. Lich.: 198. 1814.

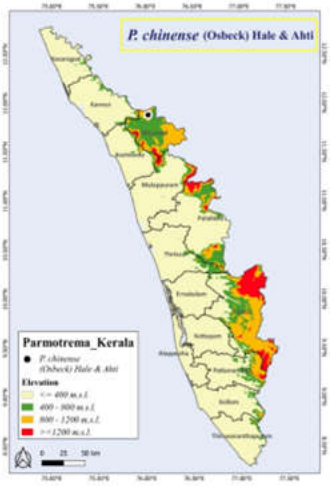
Index Fungorum Registration Identifier: 343018

Thallus pale green colour, foliose, corticolous or saxicolous, loosely adnate to the substratum (Fig.4a), 7 to 20 cm across; lobes rotund, 5 to 10 mm wide, margin ciliate; cilia black with tapering end, simple to furcated, 1 to 3 mm long; upper side grey to darker green, densely white-maculate; maculae reticulate and fissured into network (appearing as pseudocyphellae); isidia and soredia absent (Fig. 4b),; lower side centrally black, marginal narrow zone ciliate; sparsely rhizinate; rhizines restricted to the central part of the thallus, simple, black, 0.5 to 1 mm long; medulla white. Apothecia up to 10 mm in diameter, perforate; ascospores, colourless, simple, 13–17 × 6–10 µm. Pycnidia not seen.



Chemistry: Cortex K+ yellow; medulla K + yellow then red, C—, KC + red, P+ orange; atranorin, salazinic and consalazinic acids are present in TLC.

Distribution: In Kerala, this taxon is reported from Wayanad only.



4. *Parmotrema chinense* (Osbeck) Hale & Ahti

Taxon 35:133. 1986; *Lichen chinensis* Osbeck, Dagb. Cstind. Resa: 221. 1757.

Index Fungorum Registration Identifier: 114859

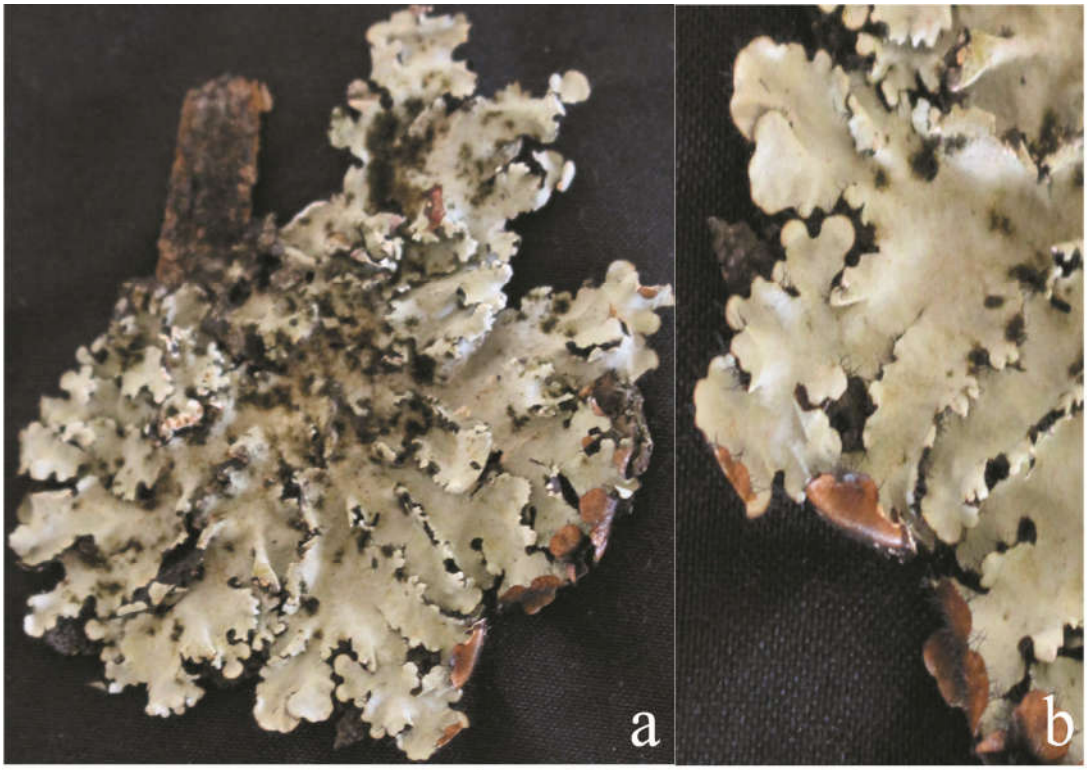


Fig.4. *P. cetratum* (Ach.) Hale: a. Habit;b.Thalus with mariginal cilia.

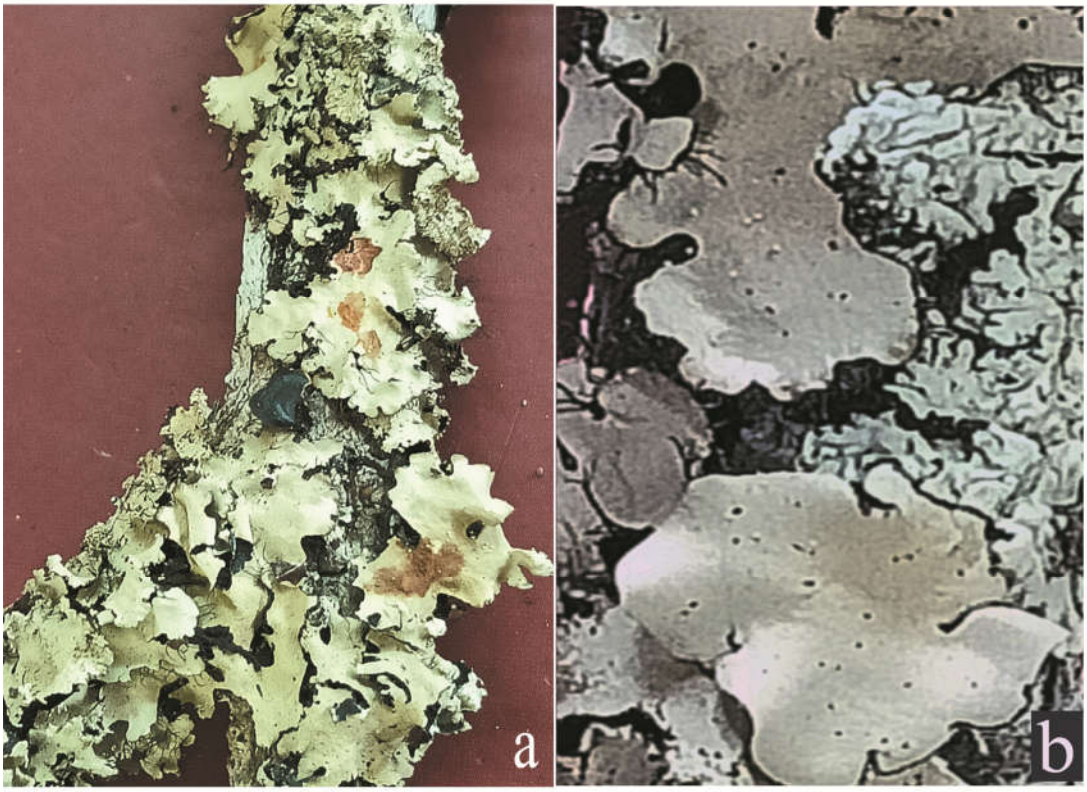


Fig.5. *P. chinense* (Osbeck) Hale: a. Habit;b.Thalus with mariginal cilia.

Thallus pale green in colour, corticolous, loosely attached to the substratum (Fig. 5a), 5 to 7 cm across; lobes irregular, routund, 5 to 8 mm wide, margins entire to crenate, ciliate; cilia simple to branched, 0.5 to 3 mm long (Fig. 5b); upper side pale grey to grey, dull, emaculate, smooth, sorediate; soralia marginal to submarginal; sorediate lobes often revolute; lower side shiny, centrally black; marginal zone brown to tan, erhizinate marginal zone; rhizines moderately dense simple, upto 2 mm ling; medulla white. Apothecia absent.

Chemistry: cortex K+ yellow; medulla K+ yellow, C—, KC—, P+ pale orange; stictic acid, atranorin, and constictic acids present in TLC.

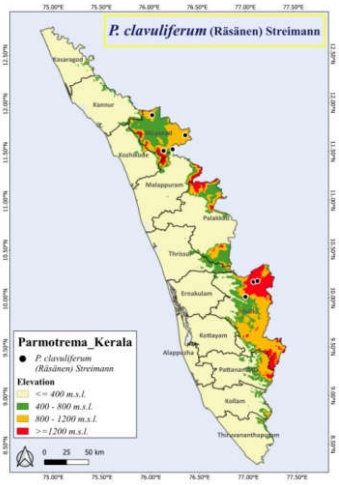
Distribution: The taxon was collected from Thirunelli (wayanad)

5. *Parmotrema clavuliferum* (Räsänen) Streimann

BiblothcaLichenol. 22: 93 (1986); - *Parmelia clavulifera* Räsänen,

Ann. bot.soc. Zool-Bot.fenn.Vanamo 20(no. 3): 4 (1944)

Index Fungorum Registration Identifier: 129346



Thallus pale green, foliose, corticolous, loosely attached with the substratum (fig. 6a) , 15-20 cm across; lobes dichotomously branched 5 -15 mm wide,lobe margins ciliate rotund, margins entire ciliate, 0.3–1.5 mm long, black; upper side pale green or whitish gray, dull to shiny, reticulatly maculate and cracked (Fig.16a); soralia capitates and stalked, marginal, present in the laciniate lobes which appears as palmate (Fig. 6c), the lower side of the lacciniae white in colour; soredia found in large cluster, granular, rotund; lower side centrally black narrow marginal zone, 2 mm wide, brown erhizinate; rhizines abundant, up to 1 mm; medulla white. Apothecia up to 8 mm in diameter (Fig. 6b), perforation are present in disc; ascospores, colourless, simple, 12–20 × 6–10 µm. Pycnidia not seen.

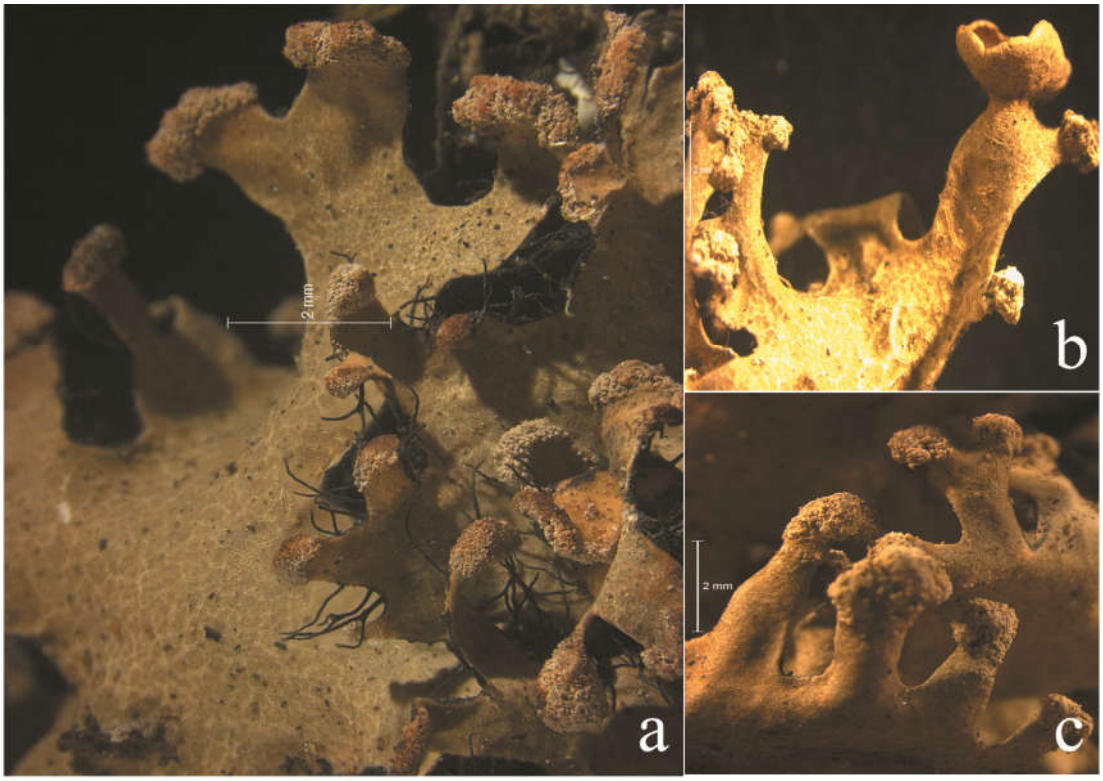


Fig.6. *P. clavuliferum* (Räsänen) Streimann: a.Thallus; b.Appothecia; c.Soralia.

Fig.7. *P. cooperi* (Steiner & Zahlbr.) Hale: a.Habit; b.Dimorphic lobes.

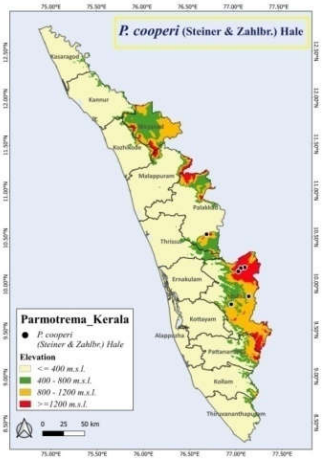
P.clavuliferum and *P. reticulatum* are similar in cortical and medullary chemistry in colour test and reticulate cracked upper side and differs in having palmate and elongate laciniae with capitates and stalked soralia; typicallyerhizinate and white lower side, lacking pigmentation by the former.

Chemistry: Cortex K+ yellow, KC–, C–, P+ yellow; medulla K + yellow then soon turning blood-red, C—, KC + red, P+ orange or deep yellow.

Distribution: In India this taxon is reported from Wayanad and Idukki districts only

6) ***Parmotrema cooperi*** (Steiner &Zahlbr.) Hale

Index Fungorum Registration Identifier: 107097



Thallus ash green clour, foliose, lobate, corticolous or saxicolous, loosely attached with the substratum, membraneous,largeupto 15 cm across (Fig. 7a), upper suface grey or pale green, emaculate, irregularly cracked in older parts of the lobes, lobes dimorphic (Fig. 7b), margins rotund or crenate and convoluted or incised; cilia present, cilia coarse or slender, black, simple to once or twice bifurcate, upto.4mm long (fig 7b); sorediate, soredia granular; soralia marginal and linear and sorediatemargins involute; isidia absent; lower side blackened throughout or becoming dark brown towards the margin, lower margins erhizinate, and present at the centre. 1 to 3 mm long; medulla white; Pycnidia and apothecia are not seen in specimen.

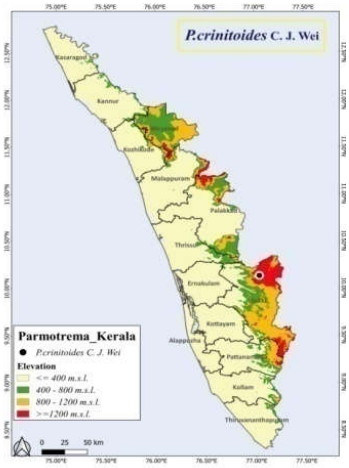
Chemistry:cortex K+ yellow; medulla K, C+ red, KC+ red, P—; lecanoricacidandatranorinpresentin TLC.

Distribution: this taxon found above 800 MSL.in kerala this taxon found in Idukki and Palakkad districts

7. *Parmotrema crinitoides* J.C. WeiEnum.

Lich. China: 177. 1991.

Index Fungorum Registration Identifier: 354727



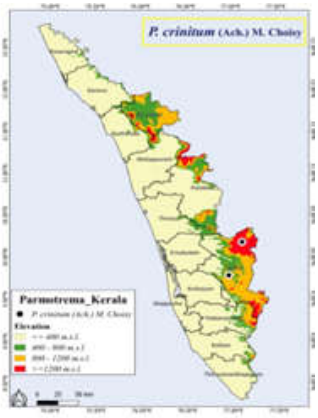
Thallus light green colour in mansoon and changes to ash green colour during summer, corticolous, adnate to the substratum, upto 7cm across; lobes subirregular, imbricate, 4 to 6 mm wide,margin crenate, eciliate; upper side pale grey, smooth,emaculate (Fig. 8a), isidiate; isidia laminal and marginal, cylindrical,simple to coralloid branched (fig. 8b), cilia short upto 5 mm long, eciliate at apices; lower side black to brown, erhizinate marginal zone, sparsely rhizinate; rhizines present in the centre, simple, slender, upto 1 mm long, medulla white. Apothecia and pycnidia are not seen in specimens.

Chemistry: cortex K+ yellow; medulla K+ yellow, KC—, C—, P+orange; sticticacid, atranorinandconstictic acids presentin TLC.

Distribution: this taxon distributed in the idukki district of Kerala

8) ***Parmotrema crinitum*** (Ach.) M. Choisy

Index Fungorum Registration Identifier: 119691



Thallus pale green in colour, foliose, corticolous, loosely attached with the substratum, the thallus often appear as a rosette of diameter up to 6-8 cm in diameter (Fig. 9a), irregular rotund lobes often reaches upto 10 mm width, lobe margins ciliate, cilia black, simple or forked at the tip; upper side grey or pale green in color and emaculate, lowersurface black except margins;isidiate; Isidia present in laminal as well as marginal, isidia simple or branched, branched isidia often appear as coralloid and apically ciliate; lower side dark and brown, shiny, nude marginal zone. Medulla white; apothecia and pycnidia are anot found.

Fig.8. *P. crinitoides* J.C. WeiEnum.: a. Habit; b. Lobes with marginal isidia.

Fig.9. *P. crinitum* (Ach.)M. Chisy: Habitat showing lobes with forked cilia.

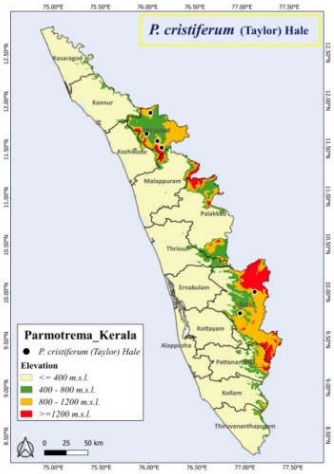
Chemistry: cortex K+ yellow; medulla K+ yellow, C—, KC—, P+ orange; consticticacidatranorin, stictic acid, present in TLC.

Distribution: this taxon collected from Idukki district of Kerala.

9) Parmotrema cristiferum (Taylor) Hale

Phytologia 28: 335. 1974; — *Parmelia cristifera* Taylor, London J. Bot. 6: 165. 1847.

Index Fungorum Registration Identifier: 34303



Thallus ash green colour, foliose, corticolous rarely saxicolous, loosely attached to the substratum,very large, spreading, 10 to 25 cm across (Fig. 10a); lobes rotund, laterally ascending, sinuous, 10-15 mm wide, emaculate; axils incised; margins entire, eciliate (Fig.10b); upper side grey to pale grey, centrally brownish, cracked, soralia marginal on lateral lobules in central part, crescent shaped or confluent; soredia marginal to submarginal, rounded to confluent, sinuous and revolute, granular (Fig.19c); lower side centrally black, wide marginal zone, 3-5 mm wide, brown, nude; rhizines sparse in the central part, short, corse, upto 1 mm long; medulla white. Apothecia rare and pycnidia were absent in specimens.

Chemistry: cortex K+ yellow; medulla K + yellow turning red, KC—, C—, P+ orange-red.TLC: salazinic acid, atranorin, and consalazinic acids.

Distribution: This taxon is found in elevations above 600 m msl.

This taxon was collected from Wayanad and Idukki districts.

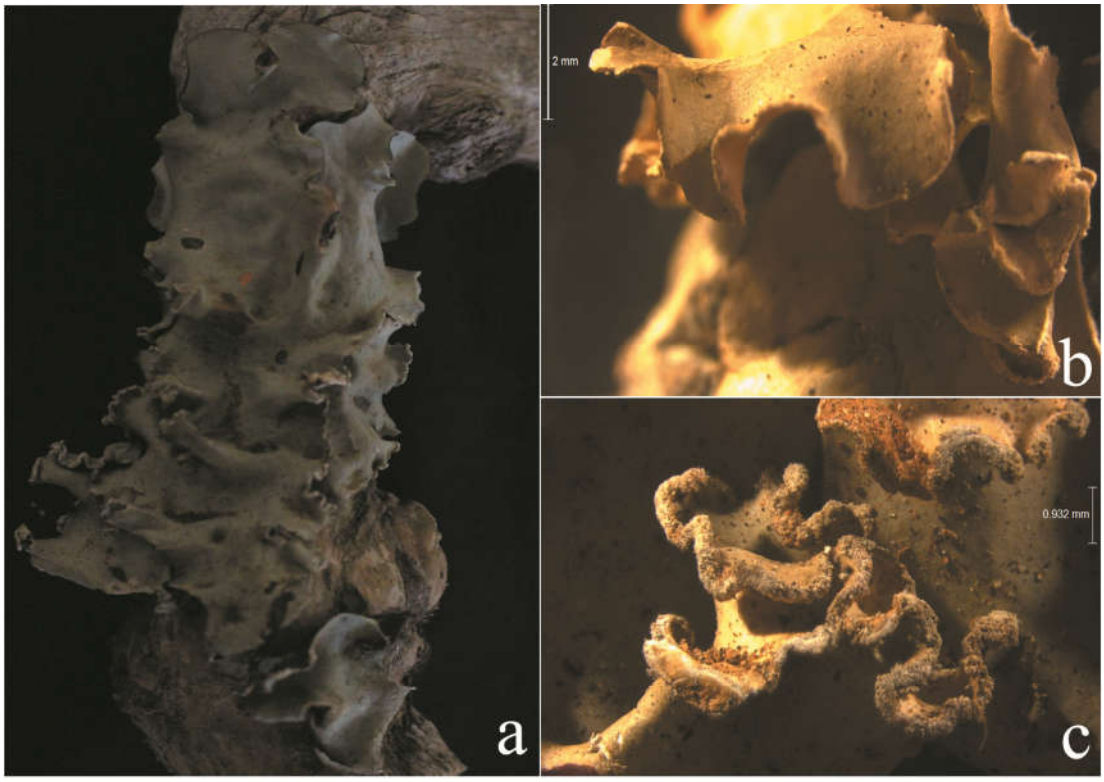


Fig.10. *P. cristiferum* (Taylor) Hale: a. Habit; b. Lobe margins; c. Soralia.

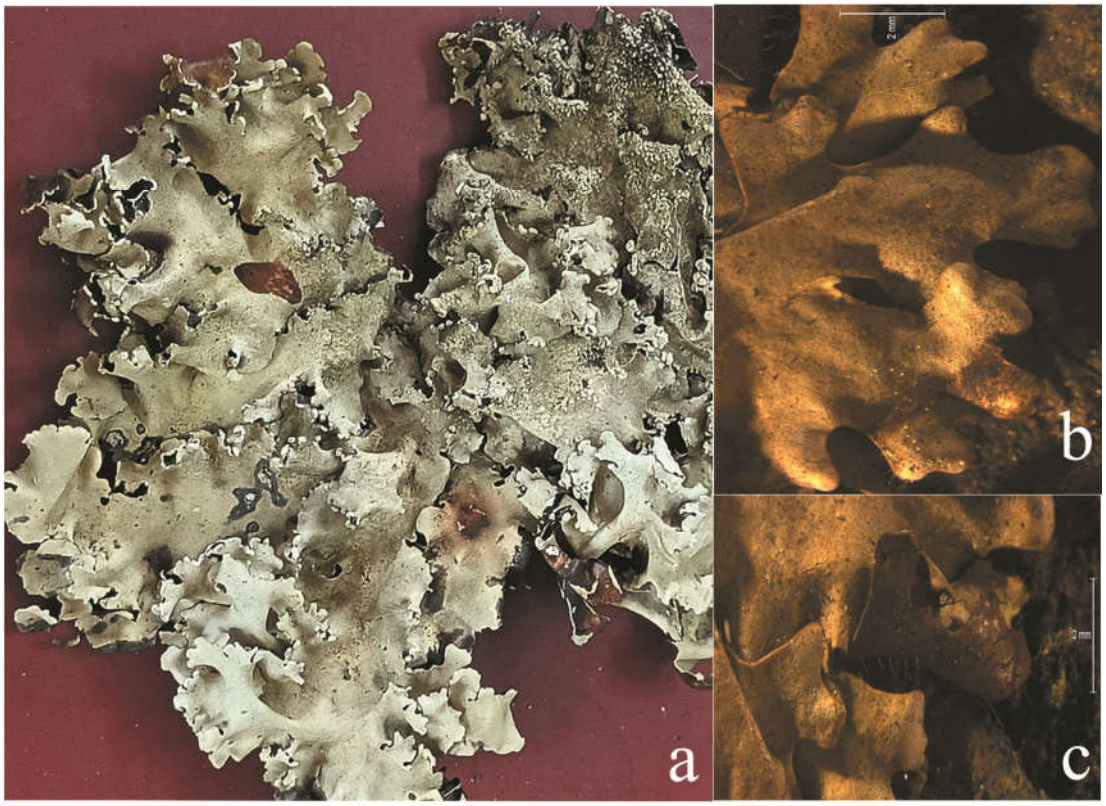
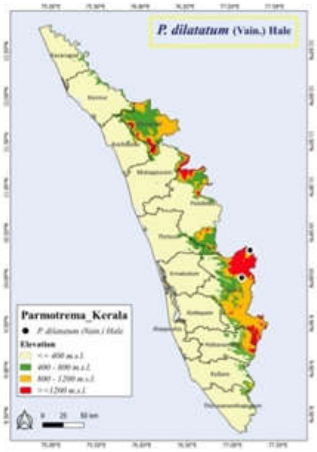


Fig.11. *P. dilatatum* (Vain.) Hale: a. Habit; b. Lobules with soralia; c. Rhizines.

10) *Parmotrema dilatatum* (Vain.) Hale

Phytologia 28: 335. 1974.

Index Fungorum Registration Identifier: 343038

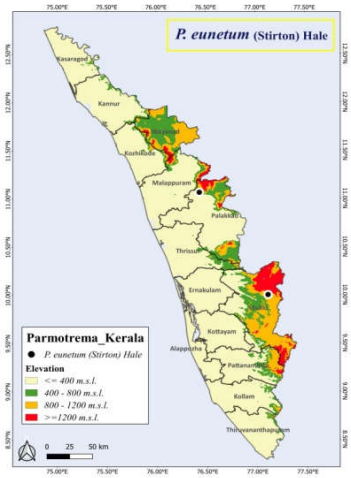


Thalluswhitish gray to bright white in colour saxicolous; upper surface, shiny,epruinose, emaculate and not cracked; thallus densely lobulate (Fig.11a), lobules breaking apart into cauliflower-like lobes, soralia with granular coarse soredia (Fig.11b); lobes small to moderate-sized, 2–7 mm wide, ±rotund, eciliate, distinctly delimited along the lobe edge by a conspicuous black rim; lower surface with a ±narrow, deep brown, erhizinate, ~ 1–2.5 mm wide margin, blackening and rhizinate towards the thallus center; rhizines short, stout, black, mostly simple, rarely sparsely branched (Fig.11c); medulla white. Apothecia and pycnidia not observed among the specimens.

Chemistry cortex-P+ yellow, K+ yellow, KC–, C–,P+ yellow turning orange, medulla K+ dirty yellowish brown, KC+ rosé to orange, C–Cortex with atranorin; medulla with atranorin, protocetraric and echinocarpic acid

Distribution: this taxon is found in the Idukki district of Kerala.

11. *Parmotrema eunetum* (Stirt.) Hale



Phytologia 28: 336. 1974; — *Parmelia euneta* Stirt., Scott. Naturalist (Perth) 4: 298. 1877-78.

Index Fungorum Registration Identifier: 343049

Thallus green colour, corticolous, loosely attached to the substratum, coriaceous, upto 10 cm across (fig: 12a) ; lobes rotund, 9to 15 mm wide, margin entire to

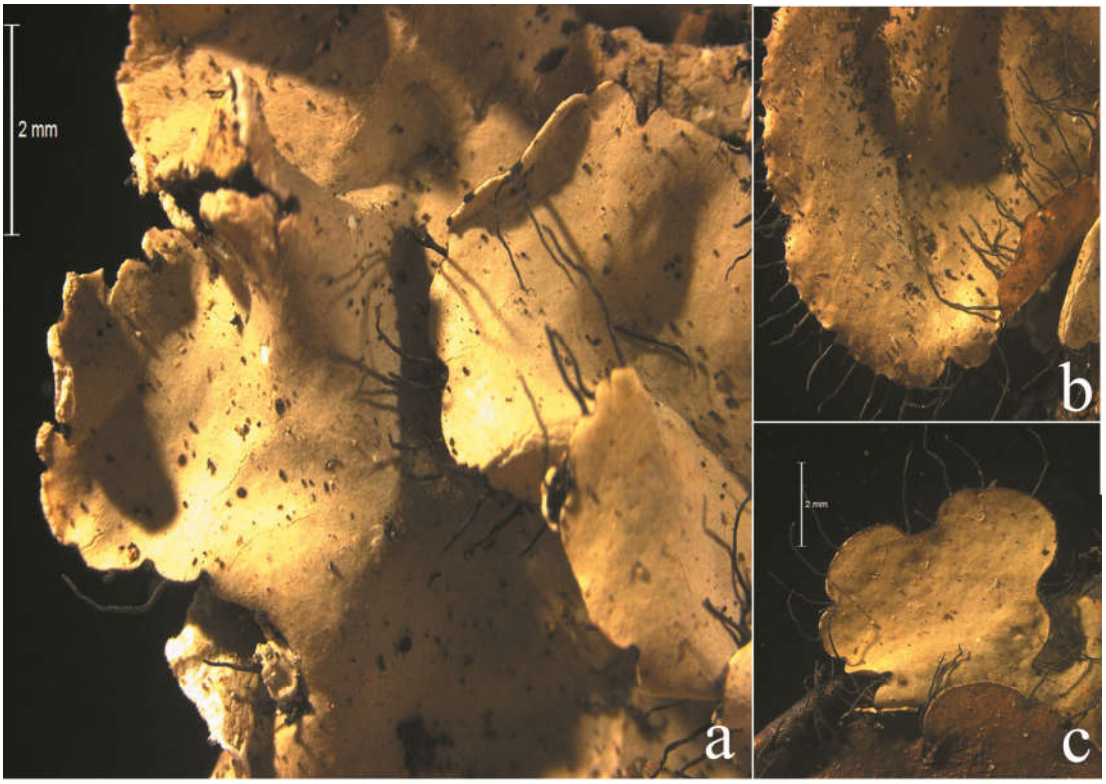


Fig.12. *P. eunetum* (Stirt.) Hale: a. Habit; b & c. Lobe margins

Fig.13. *P. greyanum* (Hue) Hale: a. Habit; b. Thallus with soralia.

crenate, ciliate; cilia black, tapering, 1–3 mm long (Fig.12b); upper side glaucous grey to grey, smooth, white-maculate, cracked with age, lacking isidia and soredia (Fig. 12c); lower side centrally black, wide marginal zone brown to white mottled, nude (Fig.23c); rhizines in the centre, black, simple, 1–2 mm long; medulla white. Apothecia and pycnidia are not seen in specimens.

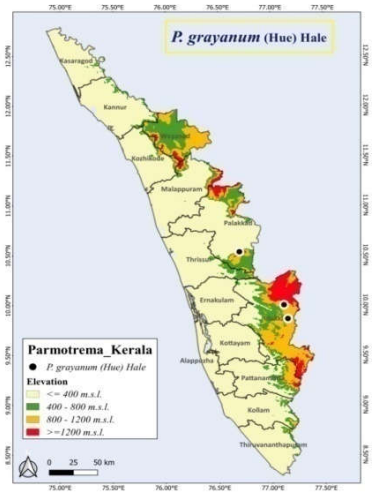
***Chemistry*:** cortex K+ yellow; medulla K—, C+ rose-red, KC+ red, P—; atranorin and gyrophoric acids present in TLC.

Distribution: this taxon is found in Idukki and Palakkad districts of Kerala.

12. *Parmotrema grayanum* (Hue) Hale

Phytologia 28: 336. 1974; — *Parmelia grayana* Hue, Nouv. Arch. Mus. Hist. Nat., ser. 4, 1: 184. 1899.

Index Fungorum Registration Identifier: 343059



Thallus pale green colour, saxicolous, corticolous, rarely terricolous, closely adnate to the substratum (Fig. 13), 4 to 8 cm across; lobes irregular, rotund, subimbricate to croweded, rather narrow, 4–8 mm wide, margins ascending, crenate or dentate, ciliate; cilia dense, 0.5–3 mm long(Fig. 13a); upper side pale green to brownish grey, smooth, epruinose or pruinose near apices, emaculate; soralia marginal or sub marginal (Fig.13b); in the central part of thallus, capitate; soredia brownish grey; lower side densely wrinkled, centrally black, marginal zone brown, erhizinate; rhizines at the centre, rhizines sparse, black in colour, simple, 1–2 mm long; medulla white. Pycnidia rare, present towards the periphery of lobes, immersed, conidia 8–9 um long.

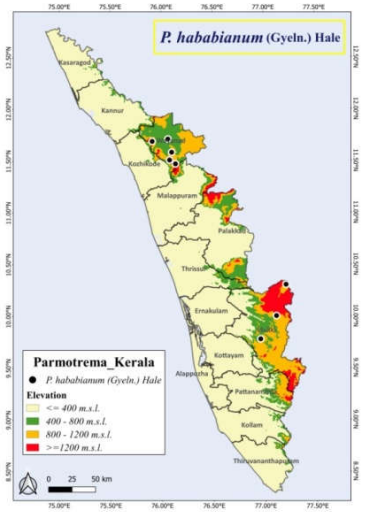
Chemistry: cortex K+ yellow; medulla K—, C—, KC—, P— ; atranorin and proto-lichesterinic acids and unknown fatty acids present in TLC.

Distribution: this taxon is present in the idukki and Palakkad districts of Kerala.

13. Parmotrema hababianum (Gyeln.) Hale

Phytologia 28: 336. 1974; — *Parmelia hababiana* Gyeln.Repert. Spec. Nov. Regni Veg. 29: 288. 1931.

Index Fungorum Registration Identifier: 343060



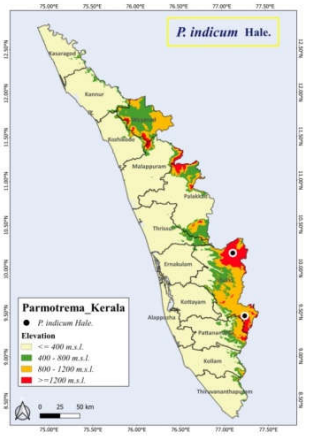
Thallus pale green, foliose, corticolous, loosely attached to the substratum (Fig. 14a), 8 to 10 cm across; lobes rotund, 5 to 15 mm wide, margin crenate, sparsely ciliate; cilia simple, 0.5–2 mm long (Fig. 14 b); upper side grey to brownish grey, smooth, faintly white-maculate to emaculate, sorediate; soralia marginal or submarginal; sorediate lobes revolute (Fig. 14c); lower side centrally brown-black; wide marginal zone ivory to brownish mottled, nude; rhizines sparse, uneven, present in scattered groups, simple, 1–2 mm long; medulla white. Apothecia and pynidia are not seen in specimens.

Chemistry: cortex K+ yellow; medulla K—, C—, KC+ reddish or purple, P— atranorin and protolichesterinic acids are present in TLC.

Distribution: This taxon is found in elevations above 750m msl

14. *Parmotrema indicum* Hale

Index Fungorum Registration Identifier:.343067



Thallus corticolous or saxicolous, loosely attached to the substratum, coriaceous, upto 20 cm across; lobes rotund, 7 to 16 mm wide (Fig.15a), margins crenate, ciliate; cilia sparse, simple to furcated, 0.5-2 mm long (Fig. 15b) ; upper side pale grey to greenish grey, emaculate, smooth at periphery, rugose and irregularly cracked towards the centre, sorediate; soralia marginal, coarse, ascending lateral lobes, sodredia granular; lower side minutely wrinkled,

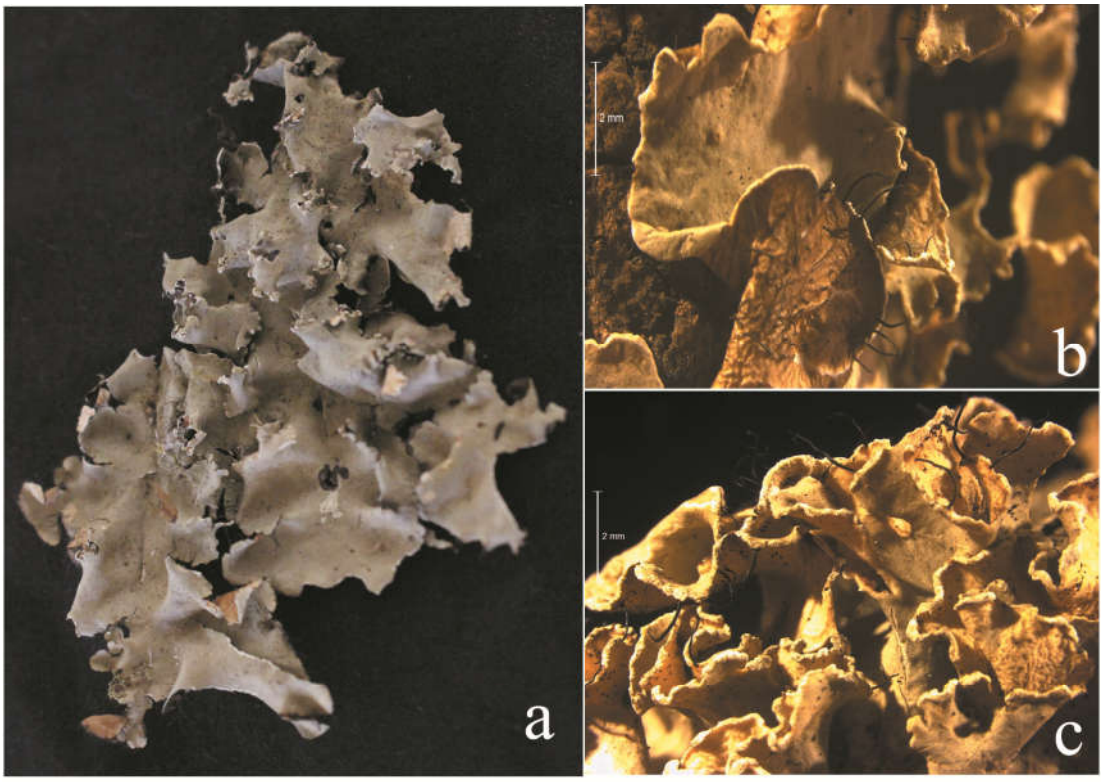


Fig.14. *P. hababianum* (Gyeln.) Hale: a. Habit; b. Lobe margins; c. Soralia.

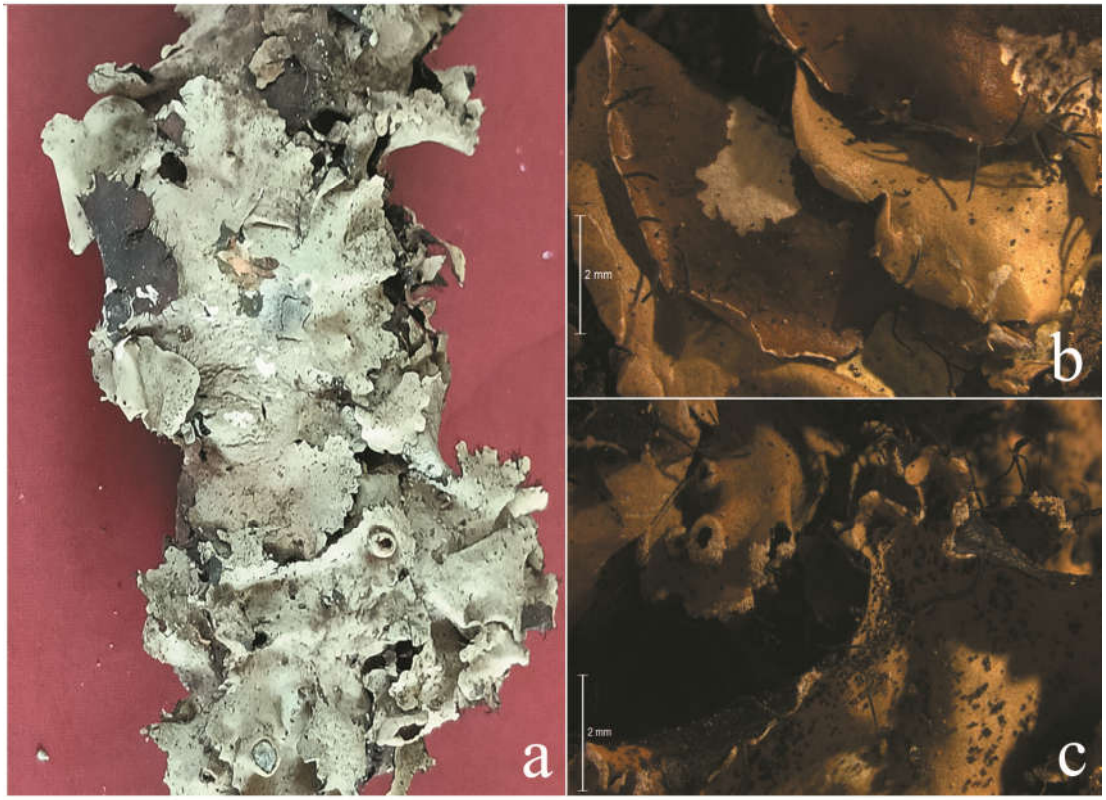


Fig.15. *P. indicum* Hale: a. Habit; b. Lobe margins; c. Appothecia.

centrally black, marginal zone brown or mottled, nude; rhizines sparse in the centre, simple, upto 1 mm long; medulla white. Apothecia up to 6 mm in diameter,disc imperforate,colourless; ascospores, colourless, simple, 12–20 × 6–10 µm (Fig. 15c). Pycnidia not seen

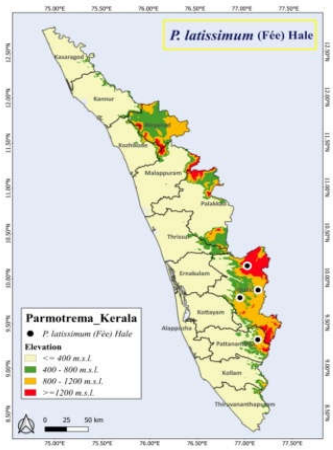
Chemistry: cortex K+ yellow; medulla K—, C+ rose, KC+ reddish, P-;

Gyrophoric acid, atranorin and norlobaridone present in TLC

Distribution: this taxon is found in the Idukki district of Kerala

15. *Parmotrema latissimum* (Fée) Hale

Phytologia 28: 337. 1974; — *Parmelia latissima*Fée, Essai. Crypt. Ecorc. Suppl.: 119. 1837.



Index Fungorum Registration Identifier:.343071

Thallus pale green in colour, corticolous, large, expanded, loosely attached to the substratum, 10 to 25 cm across; lobes rotund, 8-20 mm wide (Fig. 16a), margin entire, eciliate; upper side grey to pale grey, plane, dull, continuous or becoming cracked with age, emaculate, lacking isidia and soredia (Fig. 16b); lower side centrally black; wide marginal zone tan, nude; rhizines sparse in the centre; medulla white. Apothecia upto 16 mm in diam., disc imperforate, dark brown; Asci 8 spored, ascospores simple, colourless, ovoid 16–29 × 8–18 µm Pycnidia black, in submarginal area, conidia not seen.

Chemistry: cortex K+ yellow; medulla K + yellow turning red, KC—, C—, P+ orange-red; atranorin and salazinic acids present in TLC.

Distribution: This taxon is found in the Idukki district of Kerala (Fig.32)

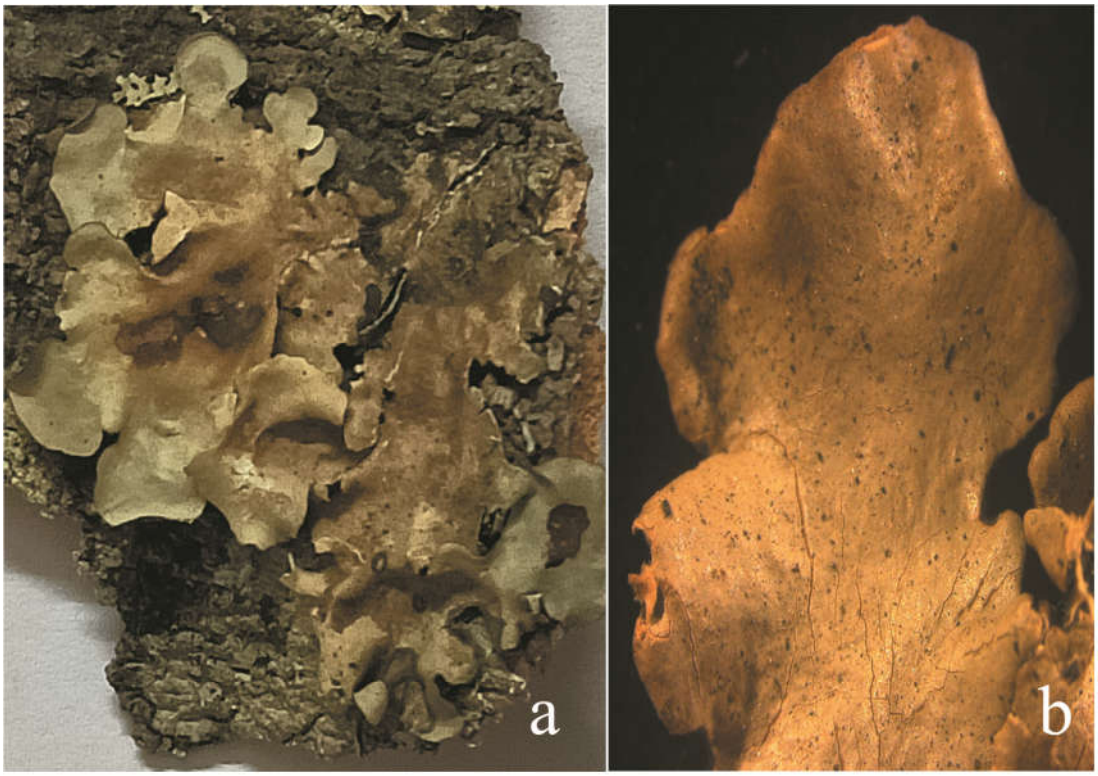


Fig.16. *P. latissimum* (Fée) Hale: a. Habit; b. Lobe margin lacking soredia.

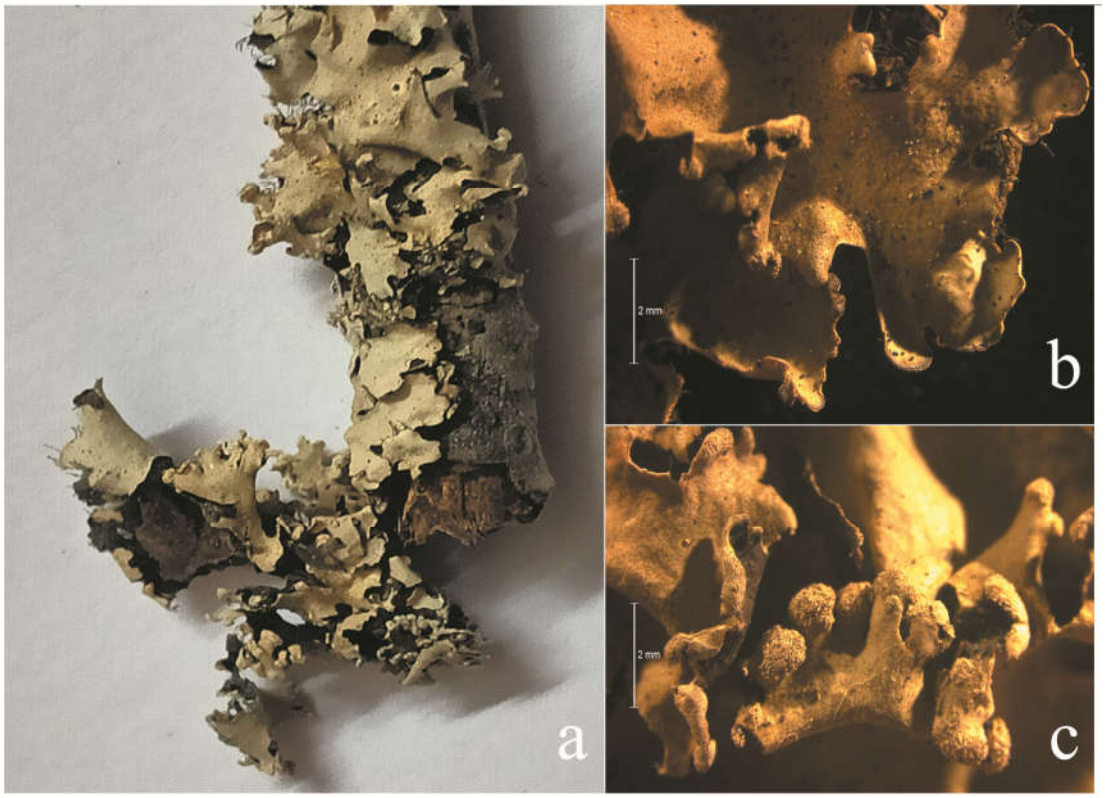
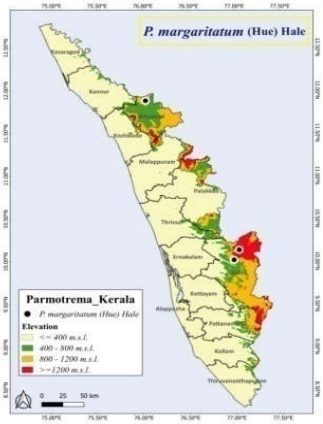


Fig.17. *P. margaritatum* (Hue) Hale: a. Habit; b. Lobe margins; c. Soralia.

16. Parmotrema margaritatum (Hue) Hale

Index Fungorum Registration Identifier:343081

Thallus pale green colour,corticolous, loosely attached to the substratum, coriaceous to crisp, upto 5 cm across; lobes rotund, upto 10 mm wide (Fig.17a), with several ascending, simple to dichotomously branched, upto 2 mm wide lacinules(Fig. 17b), irregularly developed from the margins in



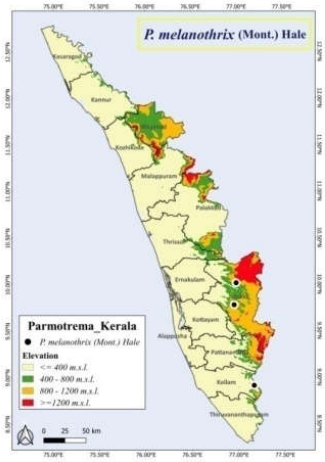
the central part, margins ciliate; cilia simple or dichotomously divided, 1–2 mm long; upper side grey, smooth, dull, densely white- maculate, sorediate; soralia orbicular, on apices of lacinules; sorediate lobes usually revolute (Fig. 17c); lower side centrally black, marginal zone brown to white mottled; rhizines sparse, in the central part; medulla white. Apothecia and pycnidia not seen.

Chemistry: cortex K+ yellow; medulla K + yellow turning red, C—, KC—, P+ orange-red; atranorin and Salazinic acids present in TLC

Distribution: this taxon is distributed in the Idukki district of Kerala.

17. *Parmotrema melanothrix* (Mont.) Hale

Phytologia 28: 337. 1974; —*Parmelia ureceolata* var. melanothrix Mont., Ann. Sci. Nat., Bot., ser. 2, 2: 372. 1834.



Index Fungorum Registration Identifier:.398619

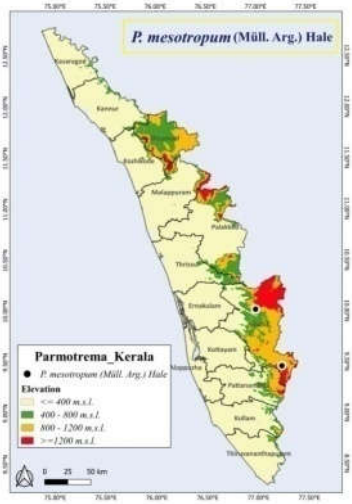
Thallusgrey-greencolour,corticolous, loosely attached to the substratum, 5–20 cm across; lobes rotund, 6–12 mm wide (Fig. 18 a); margins ciliate, dentate-laciniate at the centre; cilia short, simple, 0.5–2 mm long; upper side grey, plane shiny but sull rugose in the entre, white-maculate, lacking isidia and soredia (Fig. 18 b); lower

side centrally black, wide marginal zone tan to white mottled, nude; rhizines in the centre, simple, up to 2 mm long; medulla white. Apothecia substipitate, up to 10 mm in diam., disc imperforate, concave, brown; asci 8 spored, broadly clavate, 50–60 × 30–35 µm. Ascospores simple, hyaline, oval-ellipsoid, 20–26 × 10–16 µm; epispore 2–3 µm thick.

Chemistry: cortex K+ yellow; medulla K—, C—, KC—, P—; protolichesterinic acids and atranorin present in TLC.

Distribution: this taxon distributed in the Idukki distric of Kerala.

18. *Parmotrema mesotropum* (Müll. Arg.) Hale



Phytologia 28: 337. 1974; —*Parmelia mesotropa* Müll. Arg., Rev. Mycol. (Toulouse) 10:55. 1888.

Index Fungorum Registration Identifier:.343086

Thallus grey colour, corticolous or saxicolous, thickly adnate to the substratum

(Fig. 19), 4 to 6 cm across; lobes rotund 4 to 8 mm wide, margins entire, eciliate; upper side grey, plane, shining, emaculate, cracked in older parts, lacking isidia and soredia; lower side centrallyblack, a narrow zone along margin brown, nude, rhizines sparse distributed centrally, simple, upto 1 mm long; medulla white. Apothecia and pycnidia are absent.

Chemistry: cortex K+ yellow; medulla K—, C—, KC—, P—; atranorin and caperatic acids present in TLC.

Distribution: this taxon is distributed in the Idukki district of Kerala.

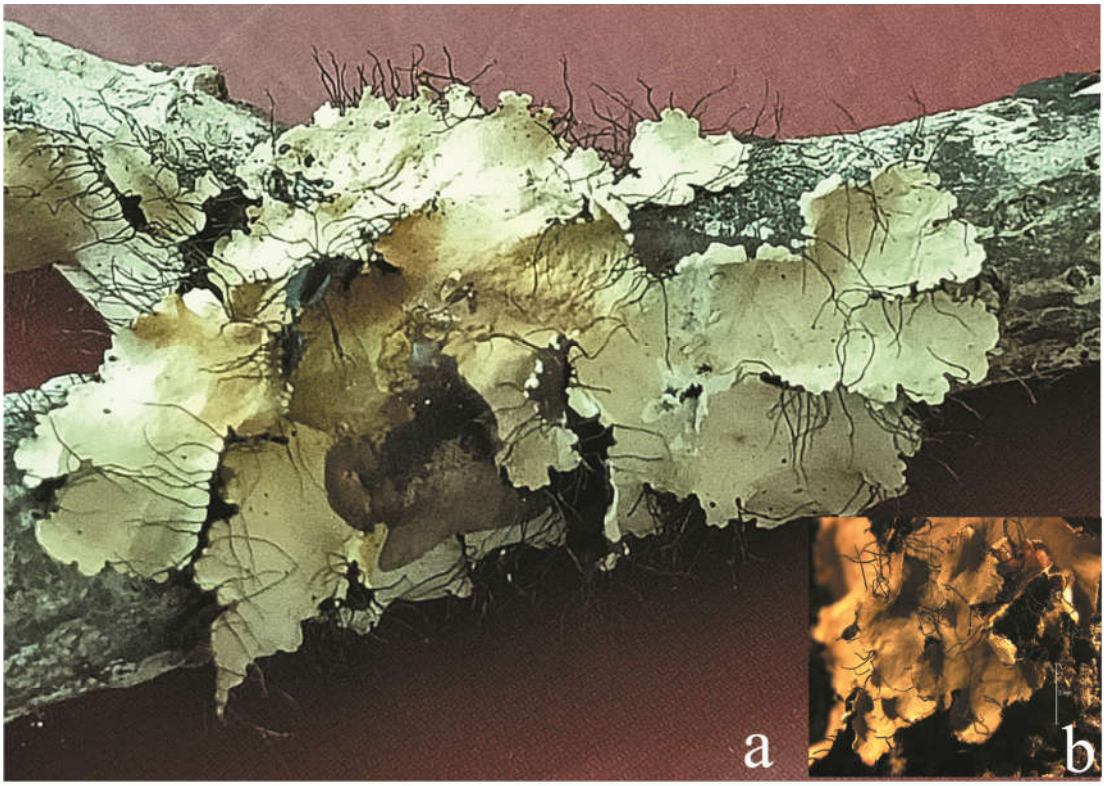


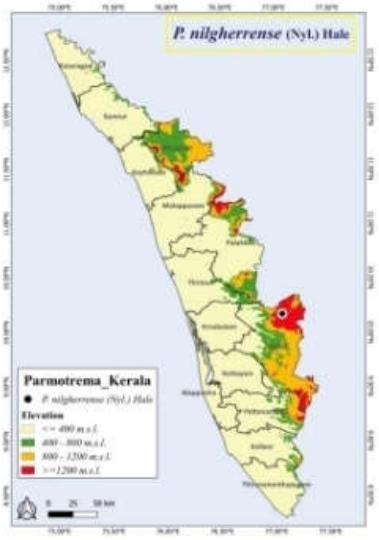
Fig.18. *P. melanothrix* (Mont.) Hale: a. Habit; b.Thallus lacking soredia.

Fig.19. *P. mesotropum* (Mull. Arg.) Hale: a. Habit with esorediate thallus.

19. *Parmotrema nilgherrense* (Nyl.) Hale

Phytologia 28:338. 1974; — *Parmelia nilgherrensis* Nyl., Flora 52: 291. 1869.

Index Fungorum Registration Identifier:.343094



Thallus pale green color, corticolous, terricolous or saxicolous, adnate to loosely attached to the substratum, coriaceous, 8 to 16 cm or more across; lobes plane to convoluted, rotund, 8–30 mm wide, margins ascending imbricate, entire to crenate or dentate(Fig. 20a), ciliate; cilia simple to furcated, 1–4 mm long(Fig. 20 b); upper side pale grey or darker, smooth, sometimes rugose in older parts, more or less shining, densely white-maculate, lacking isidia and soredia; lower side minutely wrinkled, centrally black, wide marginal zone brown, nude; rhizines simple to furcated, 1–4 mm long; medulla white. Apothecia common, stipitate, large, upto 20 mm in diam., often perforated or imperforate, dark brown Plate (Fig.20 c); concave; asci

clavate 70–85 × 25–30 µm, ascospores hyaline, simple, oval to ovoid 12–30 m thick. Pycnidia laminal to sub laminal 4µ× 10–19 µm, ostiole black, and 65–72 µm wide. Conidia sublageniform, 10–16 × 0.5 µm.

Chemistry: cortex K+ yellow; medulla K—, C—, KC+ pink -collatolic acidsαor red, P—; atranorin, and alectorilic are present.

Distribution: this taxon distributed in the Idukki distric of Kerala.

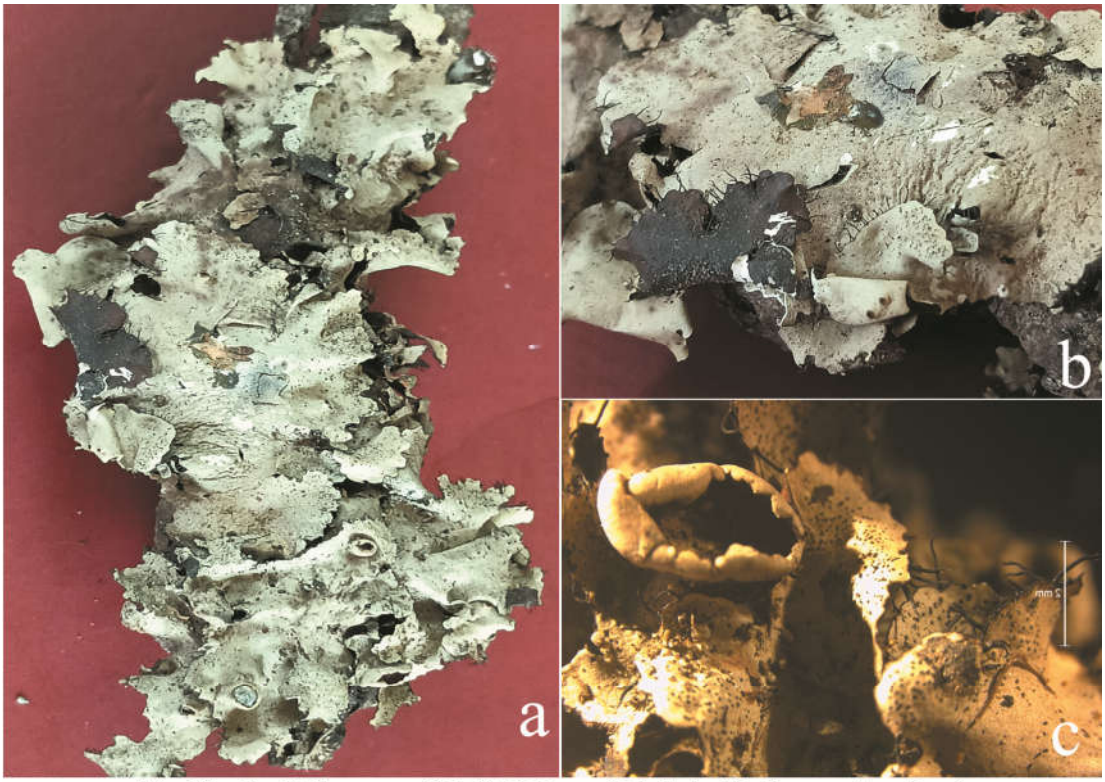


Fig.20. *P. nilgherrense* (Nyl.) Hale: a. Habit; b. Thallus; c. Apothecia.

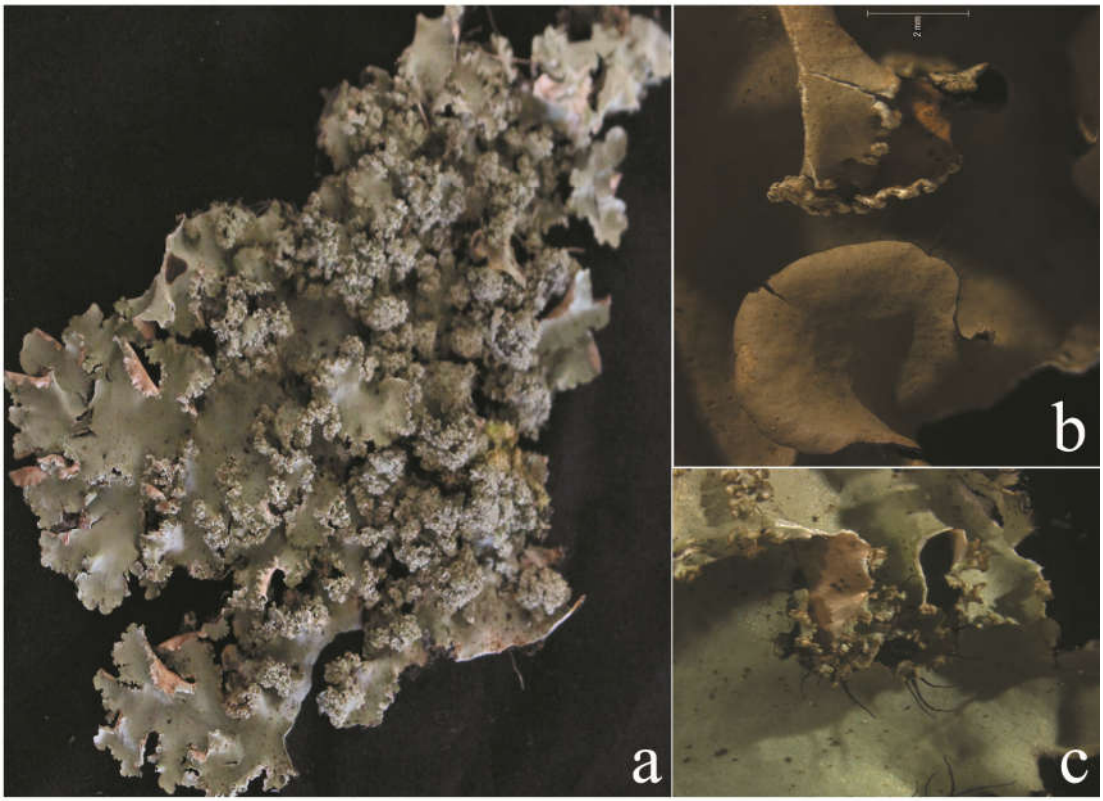
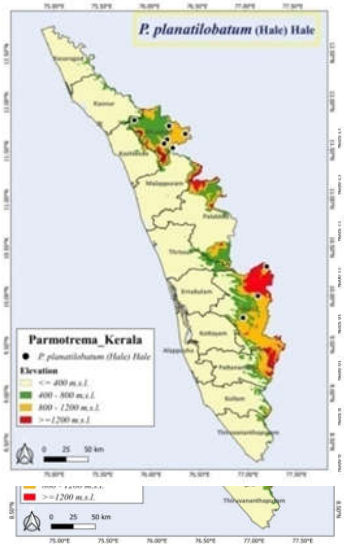


Fig.21. *P. planatilobatum* (Hale) Hale: a. Habit; b. Lobe margins; c.Isidia.

20. *Parmotrema planatilobatum* (Hale) Hale

Phytologia 28: 338. 1974; — *Parmelia planatilobata* Hale, J. Jap. Bot. 40: 200. 1965.

Index Fungorum Registration Identifier: 343105

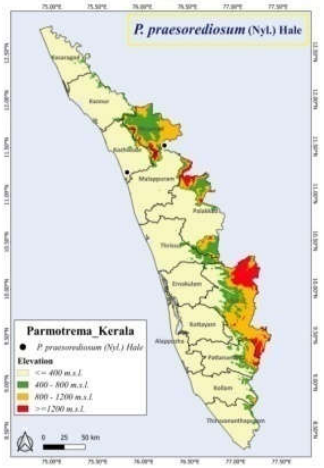


Thallus green colour, foliose, corticolous or saxicolous, closely to loosely attached to the substratum, 5 to 10 cm across; lobes rotund, 5–10 mm wide, apical margin entire or crenate, convolute (Fig. 21a), secondary lobules arise from the primary lobes (Fig.21 b), ciliate; cilia black, simple, 1–1.5 mm long; upper side grey, smooth, shiny, emaculate, with laminal to marginal isidia lacinulate thallus (Fig. 21c), lower side centrally black, marginal zone brown, nude; rhizines abundant, black, simple, 1–2 mm long; medulla white, with patches of K + purple pigment. Apothecia rare, up to 6 mm in diameter, disc imperforate, concave, pale brown; asci broad clavate, 38–42 × 20–25 µm, ascospores colourless, ellipsoid 15–18 × 7–9 µm. Pycnidia not seen.

Chemistry: cortex K+ yellow; medulla K—, C+ faint rose, KC+ red, P— ;TLC: atranorin, gyrophoric acids and skyrin.

Distribution: This taxon is found in elevations above 750m msl.

21. *Parmotrema praesorediosum* (Nyl.) Hale



Phytologia 28: 338. 1974; — *Parmelia praesorediosa* Nyl., Sert. Lich. Trop.: 18. 1891.

Index Fungorum Registration Identifier:343106

Thallus ash colour, saxicolous or corticolous, adnate attached to the substratum (Fig. 22a), 3 to 10 cm across;

Fig.22. *P. praesorediosum* **(Nyl.) Hale**: a. Habitat; b. Soredia; c. Apothecia.

Fig.23. *P. pseudotinctorum* (Abbayes) Hale: Habit with isidiated margins.

lobes rotund, 5-10 mm wide, margins entire or crenate, sub erect and sorediate, eciliate; upper side grey to darker, emaculate, smooth, becoming slightly rugose and cracked in older parts, sorediate; soralia usually marginal, linear or crescent shaped; soredia granular (Fig. 22 b); lower side centrally black, narrow marginal zone lighter tan, nude; rhizines sparse, simple, short, 1–2 mm long; medulla white. Apothecia rare, short stalked, 2 to 4 mm in diameter, disc imperforate (Fig.22c), dark brown; asci clavate, 40–45 × 16–19 µm, ascospores simple, colourless, 15–21 × 7–10 µm. Pycnidia not seen.

Chemistry: cortex K+ yellow; medulla K—, C—, KC—, P—

TLC:atranorin, proto praesorediosic acid, praesorediosic and fatty acids.

Distribution: This taxon isfound in elevations above 750m msl

22. *Parmotrema pseudotinctorum* (Abbayes) Hale

Phytologia 28:338. 1974. —*Parmelia pseudotinctorum* Abbayes, Bull. Inst. Franç. Afrique. Noire, A, 13: 973. 1951.

Index Fungorum Registration Identifier: 343113

Thallus ash colour, saxicolous, rarely corticolous, loosely adnate to the substratum, upto 6 cm across(fig 23: a); lobes rotund, 2–6 mm wide, margins entire to crenate, eciliateupper side grey, dull, isidiate, isidia thick, irregularly inflated, branched; lower side centrally black; wide marginal zone brownish, nude; rhizines sparse distributed in the centre, upto 1 mm long; medulla white. Apothecia and pycnidia are not seen.

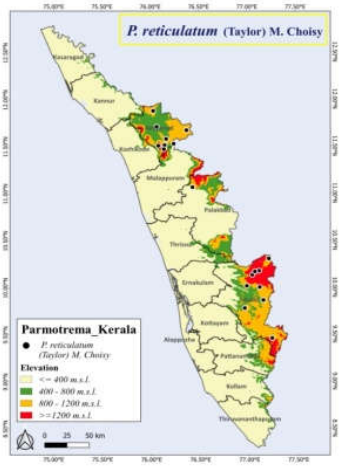
Chemistry: cortex K+ yellow; medulla K—, C+ blood-red, KC+ red, P—; atranorin and lecanoric acids present in TLC.

Distribution: this taxon is distributed in the Idukki district of Kerala .

23. *Parmotrema reticulatum* (Taylor) M. Choisy

Bull. Mens. Soc. Linn. Soc. Bot. Lyon. 21:175. 1952; -*Parmelia reticulata* Taylor, Fl. Hibern. 2:148. 1836.

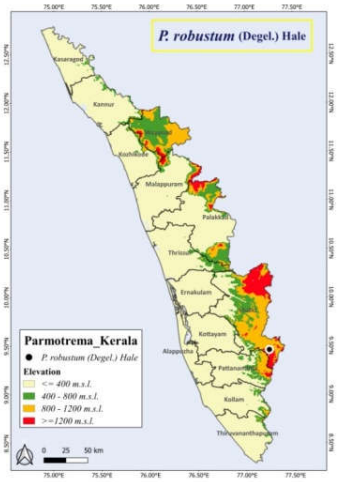
Index Fungorum Registration Identifier: 357464



Thallus brown green in colour, foliose, corticolous or saxicolous, adnate loosely attached to the substratum, up to 10-20 cm across (fig 24.a); lobesrotund, 5–15 mm wide, margin ciliate; cilia simple, black, 1– 1.5 mm long; upper side grey to darker, smooth, densely white maculate; maculae eventually reticulately fissured (Fig. 24 b), sorediate; soralia either capitate or marginal to submarginal on rounded or involute lobes; lower side centrally black, marginal zone white mottled or brown and nude or lower side black, rhizinate up to the margin (Fig. 24 c);; rhizines black, simple, 1–2 mm long; medulla white. Apothecia rare, up to 5 mm in diam., disc perforate or imperforate, brown; clavate Asci; ascospores 8-spored, colorless, simple, 15–18 × 6–10 µm. Pycnidia not seen in specimens.

Chemistry: Cortex K + yellow; medulla K + yellow then red, KC—, C—, P+ orange-red; atranorin, salazinic and consalazinic acids are present in TLC.

Distribution: This taxon is found in elevations above 600m msl.



24. *Parmotrema robustum* (Degel.) Hale

Phytologia 28: 338. 1974; — *Parmelia robusta* Degel., Goteborgs Kungl. Vetensk. Samhälles Handl., ser. 3, 1(7): 33. 1941.

Index Fungorum Registration Identifier: 343120

Thallus brownish green, corticolous, loosely attached to

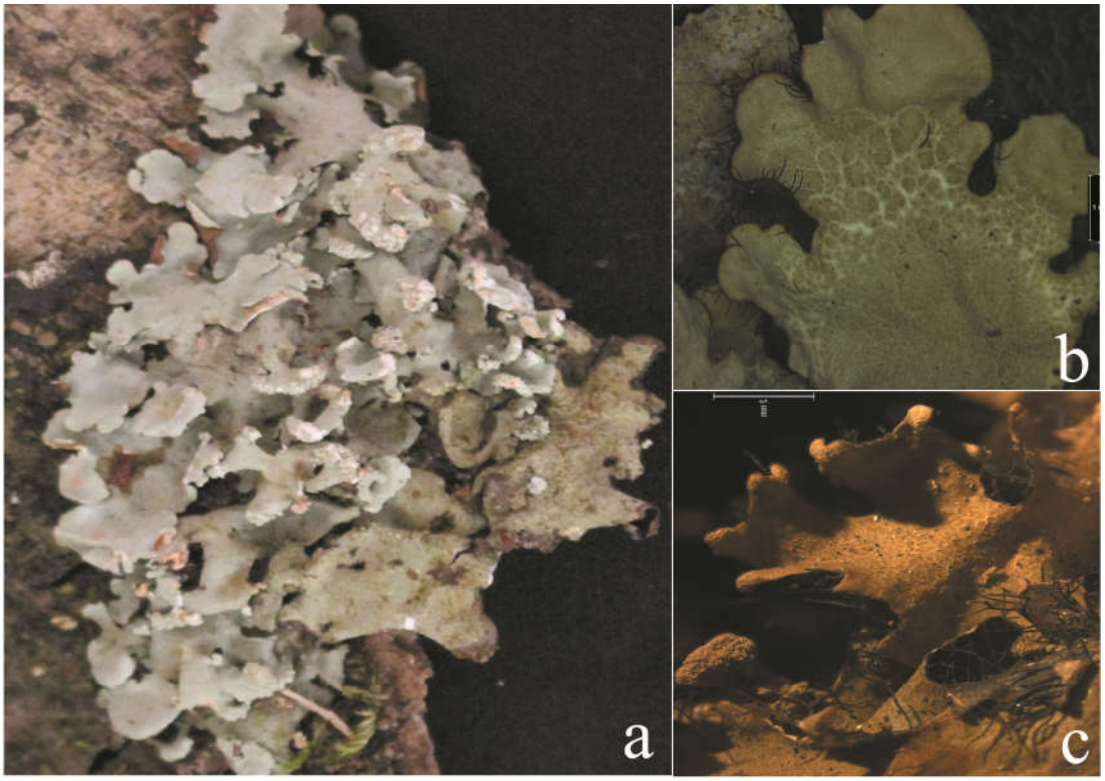


Fig.24. *P. reticulatum* (Taylor) M. Choisy: a. Habit; b. Thallus; c. Soredia.

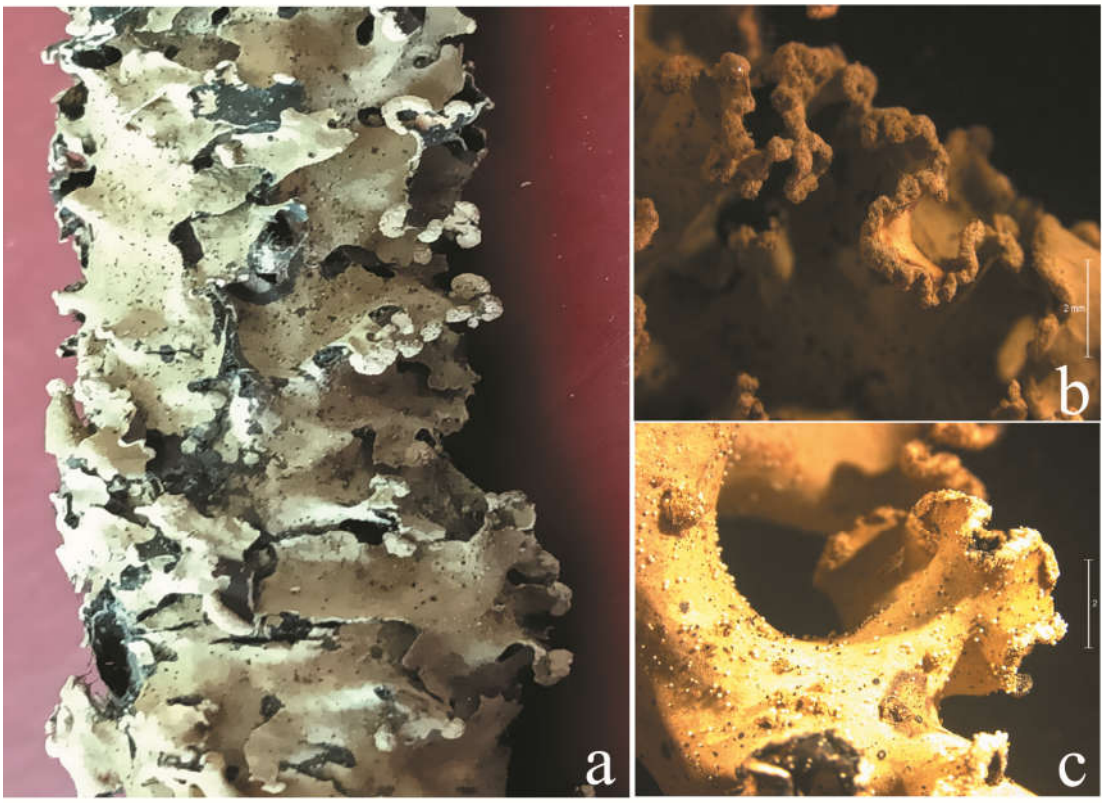


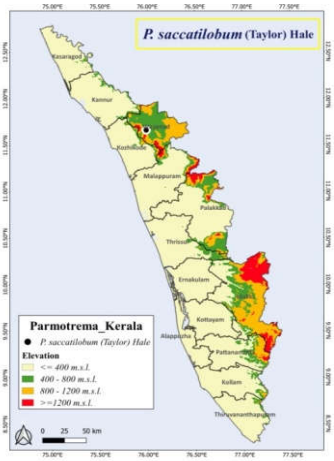
Fig.25. *P. robustum* (Degel.) Hale: a. Habit; b & c. Soredia.

the substratum, upto 10 cm across, membranaceous (Fig. 25a); lobes rotund, 5–10 mm wide, margins entire, sparsely ciliate, sometimes only in axils; cilia 0.5–1.5 mm long, black; upper side grey, smooth, dull, faintly white-maculate, soralia linear on margins of lacinules (Fig. 25b); soraliatelacinules convolute (Fig.25 c); lower side black, brown to tan, erhizinate marginal zone; rhizines sparse, present I n the centre of thallus, simple, black; medulla white. Apothecia and pycnidia are absent.

Chemistry: Cortex K + yellow; medulla K + yellowish or brownish, C—, KC—, P+ orange-red; atranorin and protocetraric acids with an unknown fatty acid present in TLC.

Distribution: this taxon is distributed in the Idukki district of Kerala.

25. *Parmotrema saccatilobum* (Taylor) Hale



Phytologia 28: 339, 1974; — Par*melia saccatiloba* Taylor in Hook.f., London J. Bot. 6: 174. 1847.

Index Fungorum Registration Identifier: 343122

Thallus corticolous, closely adnate to the substratum, 5 to 10 cm across; lobes rotund, 4–10 mm wide Kerala (Fig. 26a), involute tubular (saccate), eciliate; upper side grey, dull emaculate, cracked at centre, isidiate; isidia granular to filiform, simple, rarely branched (Fig. 26b); soralia either capitate or marginal to submarginal on rounded or involute lobes (Fig. 26c); lower side centrally black; marginal zone brown, nude; rhizines sparse, simple, upto 1 mm long; medulla white. Apothecia and pycnidia are not seen.

Chemistry: Cortex K + yellow; medulla K—, C— KC+ reddish, P+ red; atranorin and protocetraric acid present in TLC.

Distribution: this taxon distributed in the Wayanad distric of Kerala.

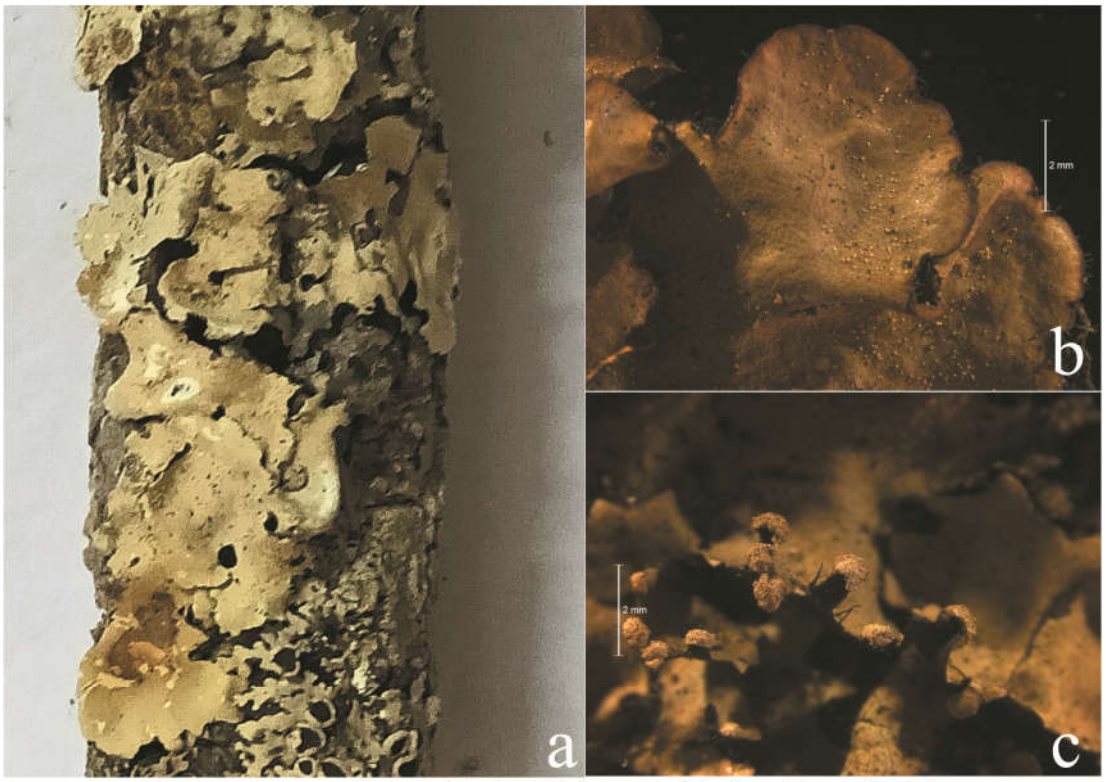


Fig.26. *P. saccatilobum* (Taylor) Hale: a. Habit; b. Isidia; c.Soridia.

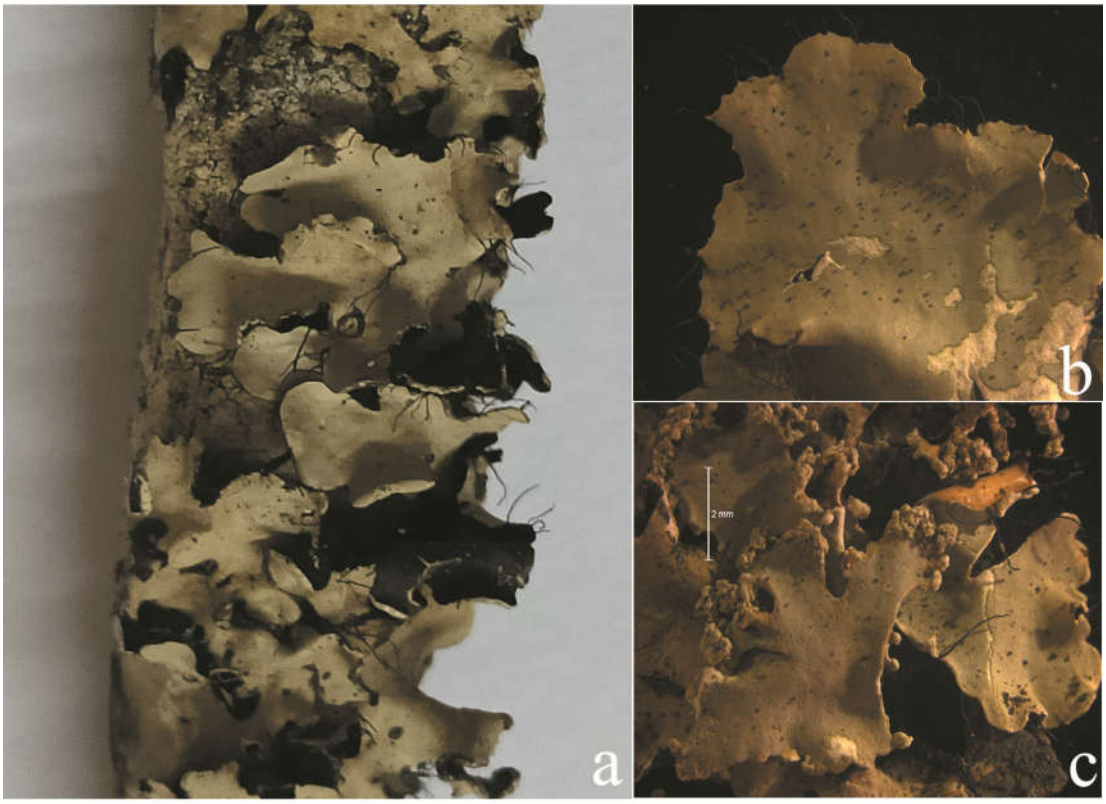
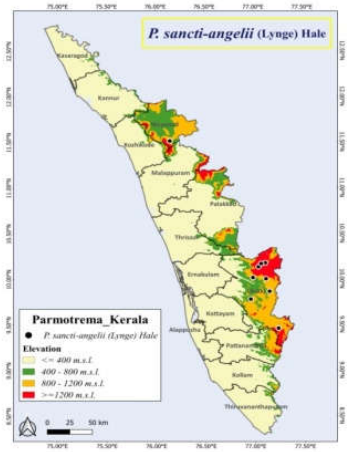


Fig.27. *P. sancti-angelii* (Lynge) Hale: a. Habit; b. Lobe margins c: Soralia.

26. *Parmotrema sancti-angelii* (Lynge) Hale

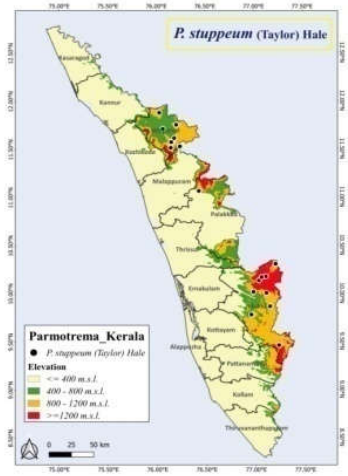
Index Fungorum Registration Identifier: 648356



Thallus pale green colour, foliose, lobate, corticolous, saxicolous, loosely attached with the substratum, membranous, and coriaceous and margins are less leathery, 5- 20 cm across (Fig.27a); lobes irregular 5-15 mm wide, lobe margins crenate, and separated apart, found ascending especially in fertile lobes, apices rotund and ciliate (Fig.27b). Cilia dense or sparse, simple or furcated, 1-4 mm long; Upper surface pale green to grey, emaculate and cracked in older parts; sorediate, soralia marginal to submarginal, confluent or discrete; soraliate lobes margins involute; soredia farinose (Fig.27c); lower side show wide erhizinate marginal zone, centrally black marginal zone shining brown or tan, rhizines distributed at the centre; 1–2 mm long; medulla white. Pycnidia and Apothecia are absent.

Chemistry: Cortex K + yellow; medulla K—, C+ rose-red, KC+ red, P—; atranorin and gyrophoric acids present with or without trace of lecanoric acid in TLC.

Distribution: this taxon seen above 800 MSL and this species has distributed through out Kerala.



27. *Parmotrema stuppeum* (Taylor) Hale

Phytologia 28: 339, 1974; — *Parmelia stuppea* Taylor, London J. Bot. 6: 174. 1847.

Index Fungorum Registration Identifier: IF343128

Thallus corticolous, rarely saxicolous, loosely adnate to the substratum, 10–15 cm across (fig:28a); lobes

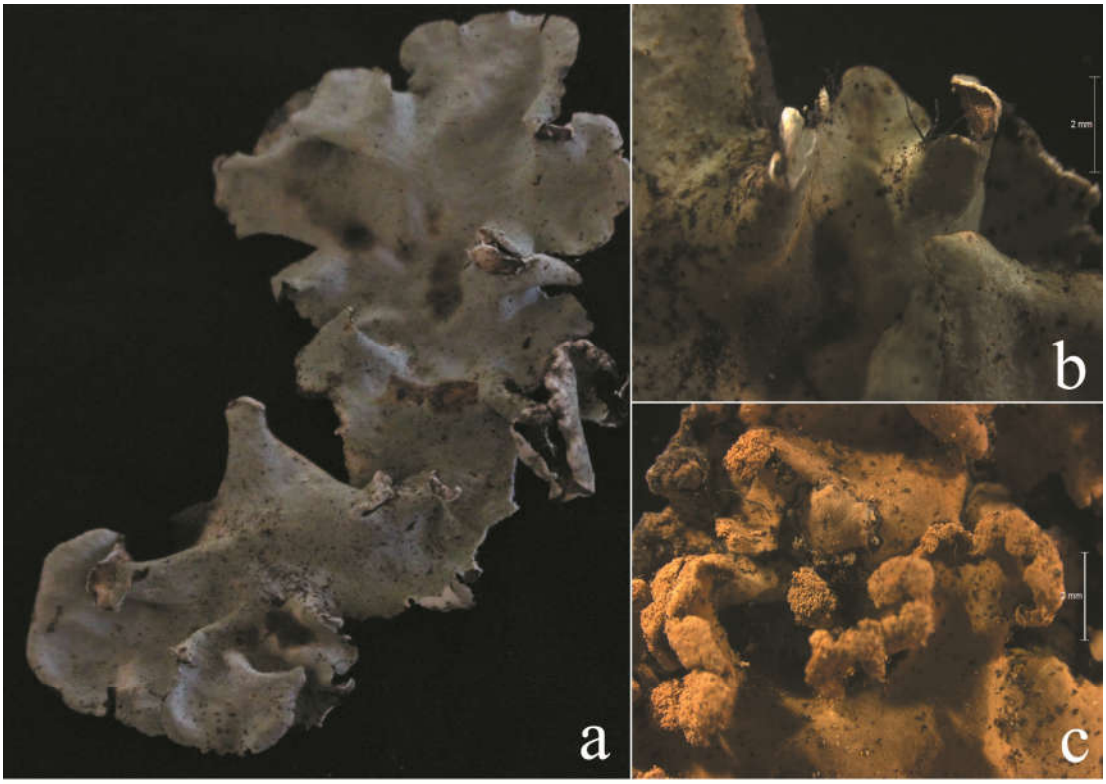


Fig.28. *P. stuppeum* (Taylor) Hale: a. Habit; b. Ciliated thallus; c. Soredia.

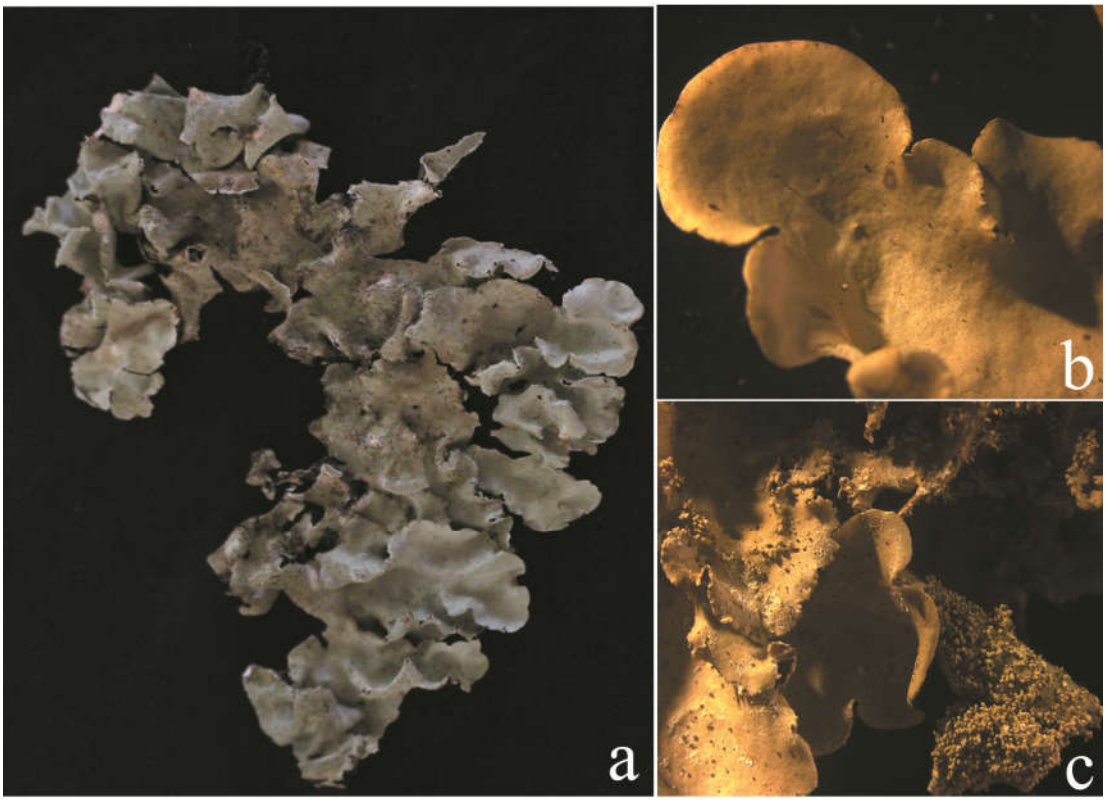


Fig.29. *P. tinctorum* (Despr. ex Nyl.) Hale: a. Habit; b. Thallus; c. Isidia.

rotund, 10-20 mm wide, crenate-dentate, ciliate; cilia sparse to dense, simple, 1–3 mm long (fig:28b); upper side grey, dull, smooth, emaculate, cracked in older parts, sorediate; soraliamarginal, on apices of dents in central part, often confluent and sub marginal; soraliatelobes involute; soredia farinose (fig:28c); lower side centrally black, wide marginal zone brown, nude or papillate; rhizines sparse, occur in patches in the central part, simple, 1–2 mm long; medulla white. Apothecia and pycnidia are not seen in Wayanad specimens.

Chemistry: Cortex K + yellow; medulla K + yellow turning red, C—, KC—, P+ orange-red; atranorin, salazinic and consalazinic acids present in TLC.

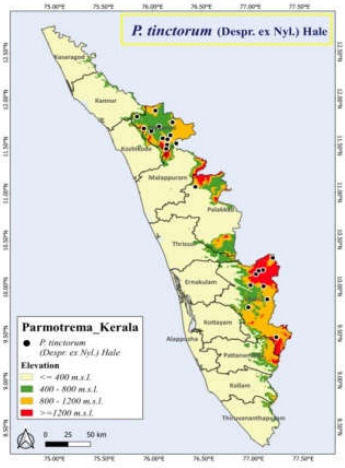
Distribution: This taxon is found above 750m msl and is distributed through the various lichen habitats of Kerala.

28. *Parmotrema tinctorum* (Despr. ex Nyl.) Hale

Phytologia 28: 339. 1974; — *Parmelia tinctoria* Despr. ex Nyl., Flora 55: 547. 1872.

Index Fungorum Registration Identifier: IF343140

Thallus ash grey, foliose, lobate, corticolous, saxicolous or terricolous, loosely attached to the substratum, membranaceous, 10–30 cm across; lobes irregular, 10–30 mm wide (Fig.29 a), apices rotund, margins entire to crenate, eciliate; upper side grey to pale green to mineral grey, emaculate(Fig.29 b); isidia granular to filiform becoming coralloid or rarely flattened (Fig. 29c); lower side centrally black, wide marginal zone, 3-6 mm, tan to brown, nude; rhizines sparse, dense at the centre, short 0.5–2.0 mm long; medulla white. Apothecia rare, not



present in the specimens examined, up to 10 mm in diam., disc imperforate; asci clavate, 8 spored, ascosporessimple, colourless, oval-ellipsoid 13– 18 × 6–10 µm, epispore 1.5 µm thick Pycnidia not present.

Chemistry: Cortex K + yellow; medulla K—, C+ red, KC+ red, P—

TLC: (plate 3, no.7) Atranorin, lecanoric acid and traces of orsellinic acid.

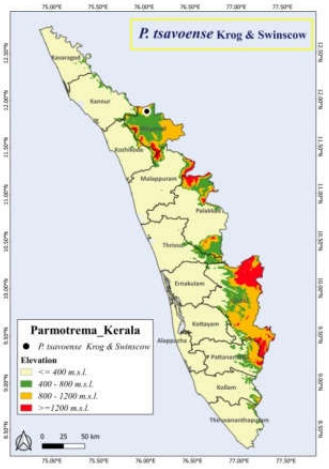
Distribution: This taxon is abundantly present in all sample sites of the study area.

29. *Parmotrema tsavoense* (Krog &Swinscow) Krog &Swinscow

Lichenologist 15:130. 1983; — *Parmelia tsavoensis* Krog &Swinscow, Bull. Brit. Mus. (Nat. Hist.), Bot. 9: 220. 1981.

Index Fungorum Registration Identifier: 109159

Thallus ash grey, saxicolous or corticolous, closely adnate to the substratum (Fig.30), upto 5 cm across; lobes rotund 2–3 mm wide, eciliate, black rimmed;upper side grey, smooth, shiny towards periphery, emaculate, dactyls short, c1aviform, apically breaking, not producing soredia; lower side centrally black, narrow marginal zone brown, nude; rhizines sparse, distributed in groups in the centre, simple, short, upto 1 mm long; medulla white. Apothecia and pycnidia were not seen.



Chemistry: Cortex K + yellow; medulla K—, C—, KC+ rose, P—; physodic acid, atranorin, and oxyphysodic acids present in TLC.

Distribution: this taxon distributed in the Thirunelli forest of Wayanad distric in Kerala.

Fig.30. *P. tsavoense* (Krog & Swinscow) Krog & Swinscow: Habit.

5.2 Biogeography

5.2.1. Biogeography of the genus *Parmotrema A. Massal.* In Wayanad

The analysis of species distribution across 31 sample sites in the Kerala, districts of Wayanad and Idukki revealed high species diversity and continuous distribution. Consequently, these two districts were selected for a detailed biogeographical assessment.

The different zones of the Wayanad district are given in Table 6. The number of each species reported from the sample sites and zone wise species distribution in the study areas of Wayanad district are given in Table 7 and 9. The occurrence of the species of *Parmotrema* in Wayanad district was mapped in Figure 31. Among the 16 sample sites in Wayanad, Neelimala exhibited the highest alpha diversity of the genus *Parmotrema* according to Shannon's index, with a value of 1.512, followed by Meppadi with an index of 1.285, and Irulam with an index of 1.213. The lowest Shannon's index was observed at Puthoorvayal, with a value of 0.60. Simpson's Index revealed that Chembra Village had the highest diversity index, with a value of 1.66, followed by Puthoorvayal with an index of 0.686. The greatest evenness of alpha diversity was recorded at Banasuramalai with an index value of 0.963, followed by Chembra Peak with an index value of 0.96 (Table 8.)

The species *Parmotreama tinctorum* has the highest IVI value in all the three zones of Wayanad district (Table10). The IVI value of *P. tinctorum*in zone 1 is 80.03, 71.34in zone two and 88.0 in zone three. *P. tinctorum* is the dominant species with the IVI values of 80.03, 71.346, and 88, respectively (Table 5). *P.reticulatum* is another species with high IVI and it is estimated as 24.25, 60.43, and 36.0 in zone 1, zone 2 and zone 3 respectively. *P.cetratum*is one species with a low IVI value, and from Kerala, it is reported from Wayanad district only. *P. praesorediosum* is another species with a low IVI value is (2.26), present only in zone 1, mainly due to its sensitivity towards pollution and it can be used as an ecological indicator of pollution.

SL. NO	Location	Zone	Elevation (m)	Lattitude(N)	Longitude(E)
1	Vaduvanchal	Zone 1 Altitude below 800 m	400	11.5210364	76.2393654
2	Chennalode		690	11.6631996	75.98487763
3	Arijermala		719	11.7146403	76.06218148
4	Neelimala		725	11.5327544	76.23645906
5	Kolagapara		753	11.61955001	76.17823095
6	Karappuzha		766	11.58407531	76.15903076
7	Puthoorvayal		766	11.58748825	76.09855323
8	Periya		769	11.81961661	75.83803087
9	Meppadi		785	11.57728355	76.14728895
10	ChooralMalai	Zone 2 Altitude between 800m-1200m	818	11.51799489	76.14309851
11	Thirunelli		851	11.88324358	76.02617202
12	Wayanad Wildlife Sanctuary		856	11.67626877	76.36811484
13	Irulam		890	11.75776861	76.19951054
14	Chembra	Zone 3 Altitude above 200m	1252	11.51220149	76.07799674
15	Vellarimala		1389	11.47523043	76.13555719
16	Banasuramalai		1911	11.69295873	75.90831713

Au- *P.austrosinense,* ***Ce-*** *P. cetratum,* ***Cl -*** *P. clavuliferum,* ***Ch-*** *P. chinense,* ***Cr*** *– P. cristiferum,* ***Ha*** *– P. hababianum,****Pl*** *– P. planatilobatum,* ***Pr-*** *P. praesorediosum,* ***Re*** *– P. reticulatum,****Sa –*** *P. saccatilobum****, Sn*** *-P. sancti-angelii,* ***St*** *– P. Stuppeum,* ***Ti-*** *P. tinctorum,* ***Ts-*** *P. tsavoense*

Table 6-Geographical zones of the study areas in Wayanad district

Sl. No.	Location	List of species and occurrence													
		Au*	**Ce***	**Ch***	**Cl***	**Cr***	**ha***	**Pl***	**Pr***	**Re***	**Sa***	**Sn***	**St***	**Ti***	**Ts***
1	Vaduvanchal	-	-	-	9	-	-	-	1	-	-	-	-	14	-
2	Chennalode	-	-	-	-	4	6	-	-	-	1	-	-	15	-
3	Arijermala	-	-	-	-	-	1	-	-	5	-	-	1	2	-
4	Neelimala	-	1	-	1	-	-	7	-	4	-	-	1	3	
5	Kolagapara	1	-	-	-	-	-	1	-	-	-		1	6	-
6	Karappuzha	-	-	-	-	-	-	-	-	8		-	4	9	-
7	Puthoorvazhal	-	-	-	-	1	1	-	-	-	-	-	-	9	-
8	Periya	3	-	-	-	-	-	6	-	-	-	-	-	9	-
9	Meppadi	4	-	-	-	-	-	4	-	-	-	-	1	4	-
10	Chooral Malai	-	1	-	4	1	-	-	-	6	-	1	1	11	-
11	Thirunelli	-	-	1	1	1	-	-	-	16		-	6	9	1
12	Wayanad Wildlife Sanctuary	-	1	-	-	-	-	3	-	9	-	-	-	8	-
13	Irulam	1	-	-	-	-	-	2	-	-	-	-	1	4	-
14	Chembra	-	-	-	-	-	1	-	-	2	-	-	-	2	-
15	Vellarimala	-	-	-	-	-	1	-	-	2	-	-	-	4	
16	Banasuramalai	4	-	-	-	-	3	-	-	-	-	-	-	6	-
	Total	13	3	1	15	7	13	23	1	52	1	1	16	115	1

Au- *P.austrosinense,* ***Ce***- *P. cetratum,* ***Cl*** - *P. clavuliferum,* ***Ch***- *P. chinense,* ***Cr*** – *P. cristiferum,* ***Ha*** – *P. hababianum,****Pl*** – *P. planatilobatum,* ***Pr***- *P. praesorediosum,* ***Re*** – *P. reticulatum,****Sa*** **–** *P. saccatilobum****, Sn*** **-** *P. sancti-angelii,* ***St*** – *P. Stuppeum,* ***Ti***- *P. tinctorum,* ***Ts***- *P. tsavoense*

Table 7 - List of species and its occurrence in the study areas of Wayanad district

Sl. No.	Location	Shannon Index (H)	Simpson's Index (D)	Evenness (E)
1	Chembra	1.055	1.66	00.96
2	Karappuzha	1.05	0.31	00.95
3	Meppadi	1.285	00.21	00.927
4	Kolagapara	1.003	00.375	00.723
5	Vaduvanchal	00.815	00.441	00.742
6	Neelimala	1.512	00.208	00.844
7	Vellarimala	00.956	00.291	00.87
8	ChooralMalai	1.4	00.264	00.781
9	Thirunelli	1.227	00.314	00.763
10	Banasuramalai	1.058	00.285	00.963
11	Chennalode	00.942	00.443	00.74
12	Puthoorvayal	0.60	00.686	00.546
13	Irulam	1.213	00.22	00.875
14	Arijermala	1.149	00.275	00.829
15	Periya	1.011	00.33	00.920
16	Wayanad Wildlife Sanctuary	1.154	00.304	00.832

Table 8 -Species diversity assessment of the study areas of Wayanad district

Sl. No.	Zone	Au*	Ce*	Ch*	Cl*	Cr*	Ha*	Pl*	Pr*	Re*	Sa*	Sn*	St*	Ti*	Ts*
1	Zone 1	8	1	0	10	*5*	*8*	18	1	17	1	0	8	71	0
2	Zone 2	1	2	1	5	2	0	5	0	31	0	1	8	32	1
3	Zone 3	4	0	0	0	0	5	0	0	4	0	0	0	12	0
	TOTAL	13	3	1	15	7	13	23	1	52	1	0	16	115	1

Au- *P.austrosinense,* ***Ce-*** *P. cetratum,* ***Cl -*** *P. clavuliferum,* ***Ch-*** *P. chinense,* ***Cr*** *– P. cristiferum,* ***Ha*** *– P. hababianum,****Pl*** *– P. planatilobatum,* ***Pr-*** *P. praesorediosum,* ***Re*** *– P. reticulatum,****Sa –*** *P. saccatilobum****, Sn****–P. sancti-angelii,* ***St*** *– P. Stuppeum,* ***Ti-*** *P. tinctorum,* ***Ts-*** *P. tsavoense*

Table 9 - zone wise species distribution in the study areas of Wayanad district

Sl. No.	Zone	Species	Number	Frequency	Density	Abundance	Relative Density	Relative Frequency	IVI
1	Zone 1	*Austrosinense*	6	30	0.4	1.33	5.4422	9.52	14.962
2		*P. cetratum*	1	5	0.05	1	0.7404	1.58	2.3204
3		*P. clavuliferum*	4	20	0.5	2.5	6.8027	6.34	13.14
4		*P. cristiferum*	4	20	0.25	1.25	3.40	6.34	9.74
5		*P. hababianum*	6	30	0.4	1.33	5.44	9.5	14.94
6		*P. planatilobatum*	8	40	0.9	2.25	12.24	12.69	24.93
7		*P. praesorediosum*	1	5	0.05	1	0.68	1.58	2.26
8		*P. reticulatum*	8	40	0.85	2.125	11.56	12.69	24.25
9		*P. saccatilobum*	1	5	0.05	1	0.68	1.58	2.26
10		*P. Stuppeum*	5	25	0.4	1.6	5.44	7.93	13.37
11		*P. tinctorum*	20	100	3.55	3.55	48.29	31.74	80.03
1	*ZONE 2*	*P. austrosinense*	1	5	0.05	1	1.16	2.43	3.59
2		*P. cetratum*	2	10	0.1	1	2.32	4.8	7.12
3		*P. chinense*	1	5	0.05	1	1.16	2.43	3.59
4		*P. clavuliferum*	3	15	0.25	1.66	5.81	7.31	13.12
5		*P. cristiferum*	2	10	0.1	1	2.32	4.8	7.12
6		*P. planatilobatum*	3	15	0.25	1.66	5.81	7.31	13.12
7		*P. reticulatum*	10	50	1.55	3.1	36.04	24.39	60.43
8		*P. sancti-angelii*	1	5	0.05	1	1.16	2.43	3.59
9		*P. stuppeum*	6	30	0.4	1.33	9.30	14.63	23.93

10		*P. tinctorum*	14	70	1.6	2.28	37.20	34.146	71.346
11		*P. tsavoense*	1	5	0.05	1	1.16	2.43	3.59
1	*ZONE 3*	*P. austrosinense*	2	10	0.2	2	16	13.33	29.33
2		*P. hababianum*	4	20	0.25	1.25	20	26.66	46.66
3		*P. reticulatum*	3	15	0.2	1.33	16	20	36
4		*P. tinctorum*	6	30	0.6	2	48	40	88

Au- *P.austrosinense,* ***Ce-*** *P. cetratum,* ***Cl -*** *P. clavuliferum,* ***Ch-*** *P. chinense,* ***Cr*** *– P. cristiferum,* ***Ha*** *– P. hababianum,****Pl*** *– P. planatilobatum,* ***Pr-*** *P. praesorediosum,* ***Re*** *– P. reticulatum,****Sa –*** *P. saccatilobum****, Sn****–P. sancti-angelii,* ***St*** *– P. Stuppeum,* ***Ti-*** *P. tinctorum,* ***Ts-*** *P. tsavoense*

Table 10- determination of I V I value of *Parmotrema* lichens in Wayanad district

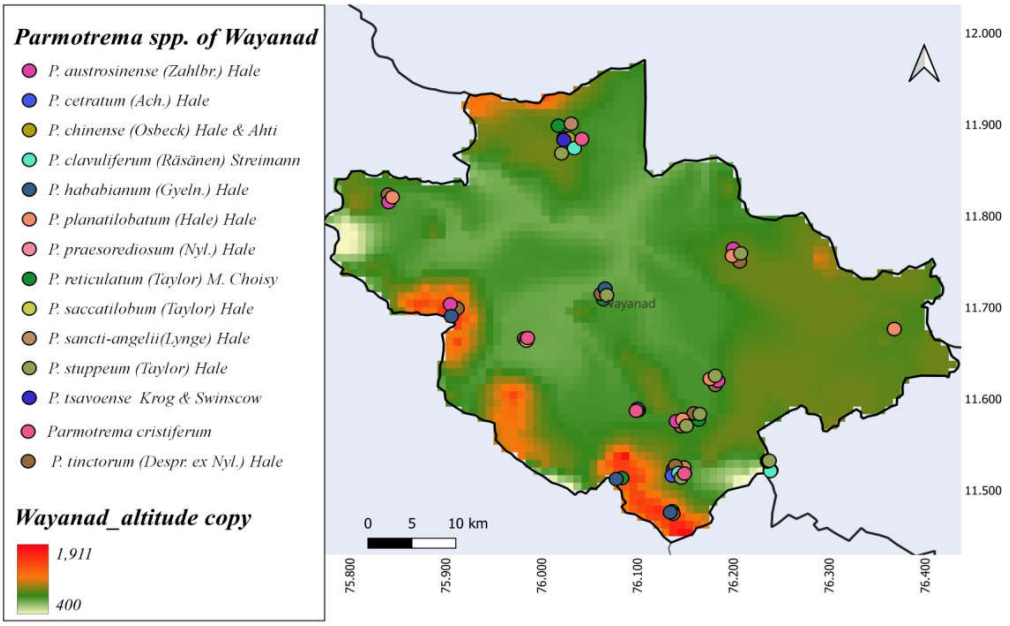


Figure 31– Map showing the distribution of the genus *Parmotrema* in Wayanad

5.2.2. Biogeography of the Genus *Parmotrema* Idukki

The alpha diversity assessment of Idukki district has estimated the species diversity of the genus *Parmotrema* in the study areas of Idukki district and found Gundumalai region has the highest value of Shannon index. The value was recorded as 2.221, and the Simpson index was maximum at Anamudi Shola with an index value of 2.06, and maximum evenness in the alpha diversity of the genus *Parmotrem*a was observed at Periyar Wildlife Sanctuary with an index value of 0.936. The detailed summary of the alpha diversity of the Idukki district is summarized in Table 13. The geographical factors of the district are given in Table 11, and observations of the quadrant study are tabulated in Table 12. Zone-wise representation of (no.) species in the areas of Idukki district and occurrence was given in Table 14. Based on these data, the IVI of each species recorded in various quadrets (Table 12) was calculated and tabulated in Table 15. The occurrence of the species of *Parmotrema* in the Idukki district was mapped in Figure 32.

*P. tinctorum*hasthe highest IVI Value in zones two and three of the district, with index values of 31.90 and 36.12, respectively. *P. reticulatum* has the highest IVI

value in zone one, recorded as 61.53, *P. tinctorum* has an index value of 46.15, and *P. reticulatum* has the index value of 26.42 and 29.22 in zone 2 and zone 3. The study has recorded certain species with critically low index values, such as *P. flavomedullosum* with 2.28, *P. pseudotinctorum* with an index value of 2.28, and *P. abnuens* having an index value of 2.73.

Sl. No	Location	Zone	Elevation (m)	Lattitude (N)	Longitude (E)
1	ChinnarWildlife Sanctuary	Zone 1 Altitude below 800m	600	10.307230	77.206821
2	Idukki WLS	Zone 2 Altitude between 800m-1200m	800	9.781676	76.957735
3	Bison valley.		900	10.005237	77.112427
4	Pettimudi		950	10.017922	76.980379
5	Cardamom hills		1000	9.866387	77.148758
6	Periyar WLS	Zone 3 Altitude above 1200m	1200	9.4622	77.2368
7	Gundumalai		1400	10.177959	77.105693
8	Eravikkulam		1600	10.1354	77.0366
9	Anamudi Shola NP		2200	10.2083	77.0833

Table 11 –Geographical factors of the study areas of Idukki district

No.	Name of species	Chinnar wildlife sanctuary	Idukki wildlife sanctuary	Bison valley	Petti mudi	Cardamom Hills	Periyar wildlife sanctuary	Gundu malai	Eravi kkulam	Anamudi Shola national park
1	*P. abnuens*	0	0	0	0	0	0	0	0	1
2	*P.austrosinense*	0	2	0	4	0	5	0	3	1
3	*P. clavuliferum*	0	0	0	1	0	0	1	0	1
4	*P.cooperi*	0	1	0	0	1	0	2	3	3
5	*P. crinitoides*	0	0	0	0	0	0	0	1	0
6	*P.crinitum*	0	2	0	0	0	0	2	0	0
7	*P.cristiferum*	0	1	4	0	0	0	0	0	0

8	*P.dilatatum*	1	0	3	0	0	0	0	0	0
9	*P.eunetum*	0	1	0	1	0	0	1	2	1
10	*P.flavomedullosum*	0	1	0	0	0	1	1	0	0
11	*P.grayanum*	0	0	1	0	1	0	0	0	0
12	*P.hababianum*	0	6	4	0	0	0	0	0	0
13	*P. indicum*	0	0	0	0	0	5	2	0	0
14	*P. latissimum*	0	1	0	0	1	0	0	1	0
15	*P.margaritatum*	0	0	0	3	0	0	0	1	0
16	*P.melanothrix*	0	1	0	3	0	0	0	0	-
17	*P.mesotropum*	0	0	0	2	0	1	0	0	0
18	*P. nilgherrense*	0	0	0	0	0	0	0	0	1
19	*P.planatilobatum*	1	1	1	0	0	0	0	0	0
20	*P.praesorediosum*	1	0	1	1	0	0	1	0	0
21	*P.pseudotinctorum*	0	0	0	1	0	0	0	0	0
22	*P.reticulatum*	6	6	3	4	4	3	5	3	2
23	*P.robustum*	0	0	0	0	0	1	0	0	0
24	*P. sanctae–angeli*	0	13	2	2	2	2	1	3	1
25	*P.stuppem*	1	3	1	1	1	3	2	1	1
26	*P. tinctorum*	6	4	6	6	4	3	4	4	8
Elevation		600m	800 m	900m	900m	1000m	1200m	1000 m	1800 m	2200m

Table 12– List of species and occurrences in the study areas of Idukki district

Sl.No	Location	Zone	Shannon Index (H)	Simpson's Index (D)	Evenness (E)
1	ChinnarWildlife Sanctuary	Zone 1 Altitude below 800m	1.778	00.195	00.914
2	Idukki WLS	Zone 2 Altitude between 800m-1200m	2.216	00.152	00.84
3	Bison valley.		2.111	00.139	00.917
4	Pettimudi		2.218	00.125	00.925
5	Cardamom hills		1.748	00.204	00.898
6	Periyar WLS	Zone 3 Altitude above 1200m	1.946	00.157	00.936
7	Gundumalai		2.221	00.128	00.926
8	Eravikkulam		2.087	00.134	00.95
9	Anamudi Shola		1.873	00.206	00.852

Table 13- Species diversity assessment of the study areas of Idukki district

No.	Name of Species	Zone 1	Zone 2	Zone 3
1	*P. abnuens*	0	0	1
2	*P.austrosinense*	0	6	9
3	*P. clavuliferum*	0	1	2
4	*P.cooperi*	0	2	8
5	*P. crinitoides*	0	0	3
6	*P.crinitum*	0	2	2
7	*P.cristiferum*	0	5	0
8	*P.dilatatum*	1	3	0
9	*P.eunetum*	0	2	4
10	*P.flavomedullosum*	0	1	2
11	*P.grayanum*	0	2	0
12	*P.hababianum*	0	10	0
13	*P. indicum*	0	0	7
14	*P. latissimum*	0	2	1
15	*P.margaritatum*	2	3	1
16	*P.melanothrix*	0	4	3
17	*P.mesotropum*	0	2	1
18	*P. nilgherrense*	0	0	1
19	*P.planatilobatum*	1	2	0
20	*P.praesorediosum*	1	2	1
21	*P.pseudotinctorum*	0	1	0
22	*P.reticulatum*	4	17	13
23	*P. robustum*	0	0	1
24	*P. sanctae - angeli*	0	19	7
25	*P.stuppeum*	1	6	7
26	*P. tinctorum*	3	20	19
	Total	13	111	87

Table 14- Zone-wise representation of (no.) species in the areas of Idukki district and occurrence

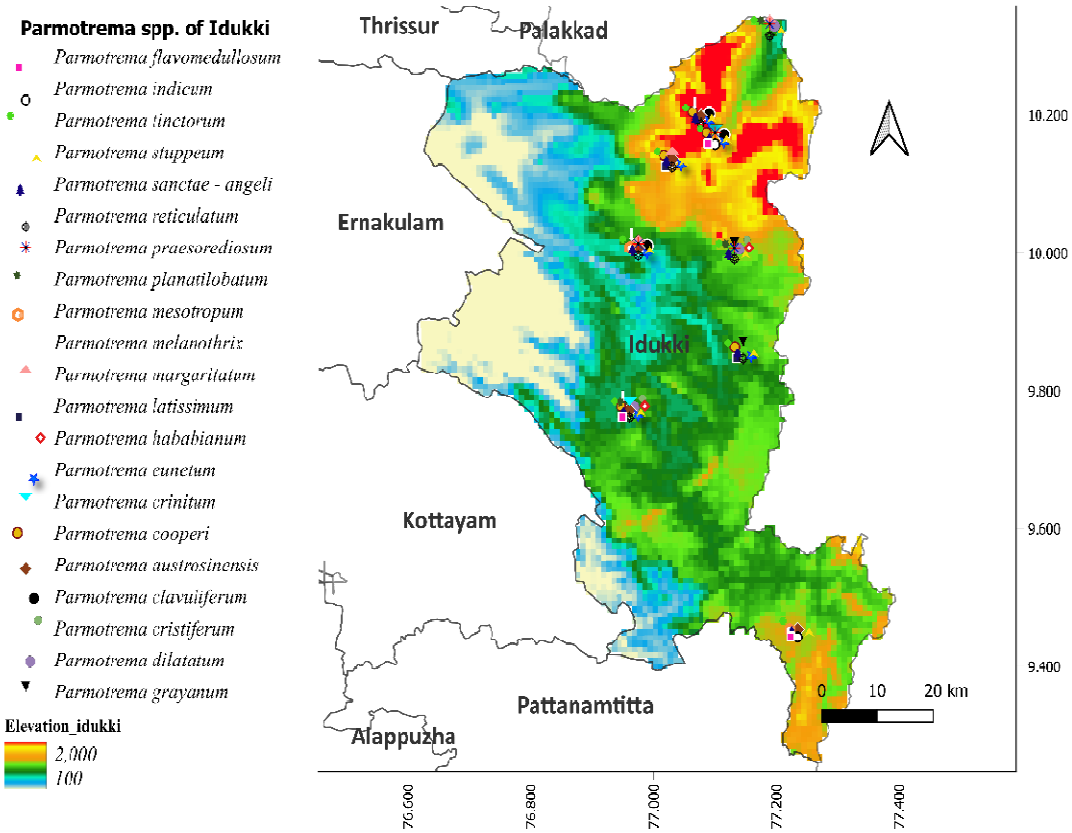


Figure 32 – Map showing the distribution of the genus *Parmotrema* in Idukki.

Sl.No.	Zones	Species	Number of Quadrats in species occurred	Frequency	Density	Abun dance	Relative density	Relative frequency	IVI
1	ZONE 1	*P.dilatatum*	1	0.1	0.1	1	7.692308	7.692308	15.38462
2		*P.margaritatum*	2	0.2	0.2	1	15.38462	15.38462	30.76924
3		*P.praesorediosum*	1	0.1	0.1	1	7.692308	7.692308	15.38462
4		*P.reticulatum*	4	0.4	0.6	1.5	30.76923	30.76923	61.53846
5		*P.stuppem*	1	0.1	0.1	1	7.692308	7.692308	15.38462
6		*P. tinctorum*	3	0.3	0.6	2	23.07692	23.07692	46.15384
7		*P.planatilobatum*	1	0.1	0.1	1	7.692308	7.692308	15.38462
1	*ZONE 2*	*austrosinense*	4	0.4	0.6	1.5	5.405405	5.555556	10.96096
2		*P.cooperi*	2	0.2	0.2	1	1.801802	2.777778	4.57958
3		*P.crinitum*	2	0.2	0.2	1	1.801802	2.777778	4.57958
4		*Cristiferum*	3	0.3	0.3	1	4.504505	4.166667	8.671172
5		*P.dilatatum*	3	0.3	0.3	1	2.702703	4.166667	6.86937
6		*P.eunetum*	2	0.2	0.2	1	1.801802	2.777778	4.57958
7		*P.flavomedullosum*	1	0.1	0.1	1	0.900901	1.388889	2.28979
8		*P.grayanum*	2	0.2	0.2	1	1.801802	2.777778	4.57958
9		*P. latissimum*	2	0.2	0.2	1	1.801802	2.777778	4.57958
10		*P.margaritatum*	3	0.3	0.3	1	2.702703	4.166667	6.86937

11		*P.melanothrix*	3	0.3	0.4	1.3	3.603604	4.166667	7.770271
12		*P.mesotropum*	2	0.2	0.2	1	1.801802	2.777778	4.57958
13		*P.praesorediosum*	2	0.2	0.2	1	1.801802	2.777778	4.57958
14		*P.pseudotinctorum*	1	0.1	0.1	1	0.900901	1.388889	2.28979
15		*P.reticulatum*	8	0.8	1.7	2.12	15.31532	11.11111	26.42643
16		*P. sanctae - angeli*	8	0.8	1.9	2.34	17.11712	11.11111	28.22823
17		*P.stuppem*	6	0.6	0.6	1	5.405405	8.333333	13.73874
18		*P. tinctorum*	10	1.0	2.0	2	18.01802	13.88889	31.90691
19		*P.planatilobatum*	2	0.2	0.2	1	1.801802	2.777778	4.57958
20		*P.hababianum*	6	0.6	1.0	1.6	9.009009	8.333333	17.34234
21		*P. clavuliferum*	1	0.1	0.1	1	0.900901	1.388889	2.28979
1	*ZONE 3*	*P. abnuens*	1	0.1	0.1	1	1.149425	1.587302	2.736727
2		*austrosinense*	6	0.6	0.9	1.5	10.34483	9.52381	19.86864
3		*P.cooperi*	4	0.4	0.8	2	9.195402	6.349206	15.54461
4		*P. crinitoides*	3	0.3	0.3	1	3.448276	4.761905	8.210181
5		*P.crinitum*	2	0.2	0.2	1	2.298851	3.174603	5.473454
6		*P.eunetum*	4	0.4	0.4	1	4.597701	6.349206	10.94691
7		*P.flavomedullosum*	2	0.2	0.2	1	2.298851	3.174603	5.473454
8		*P. indicum*	4	0.4	0.7	1.75	8.045977	6.349206	14.39518
9		*P. latissimum*	1	0.1	0.1	1	1.149425	1.587302	2.736727
10		*P.margaritatum*	1	0.1	0.1	1	1.149425	1.587302	2.736727
11		*P.melanothrix*	3	0.3	0.3	1	3.448276	4.761905	8.210181

12		*P.mesotropum*	1	0.1	0.1	1	1.149425	1.587302	2.736727
13		*P. nilgherrense*	1	0.1	0.1	1	1.149425	1.587302	2.736727
14		*P.praesorediosum*	1	0.1	0.1	1	1.149425	1.587302	2.736727
15		*P.reticulatum*	9	0.9	1.3	1.4	14.94253	14.28571	29.22824
16		*P.robustum*	1	0.1	0.1	1	1.149425	1.587302	2.736727
17		*P. sanctae - angeli*	7	0.7	0.7	1	8.045977	11.11111	19.15709
18		*P.stuppem*	7	0.7	0.7	1	8.045977	11.11111	19.15709
19		*P. tinctorum*	9	0.9	1.9	2.1	21.83908	14.28571	36.12479
20		*P. clavuliferum*	2	0.2	0.2	2	2.298851	3.174603	5.473454

Table 15- Ecological assessment of species occurred in the three zones of Idukki district

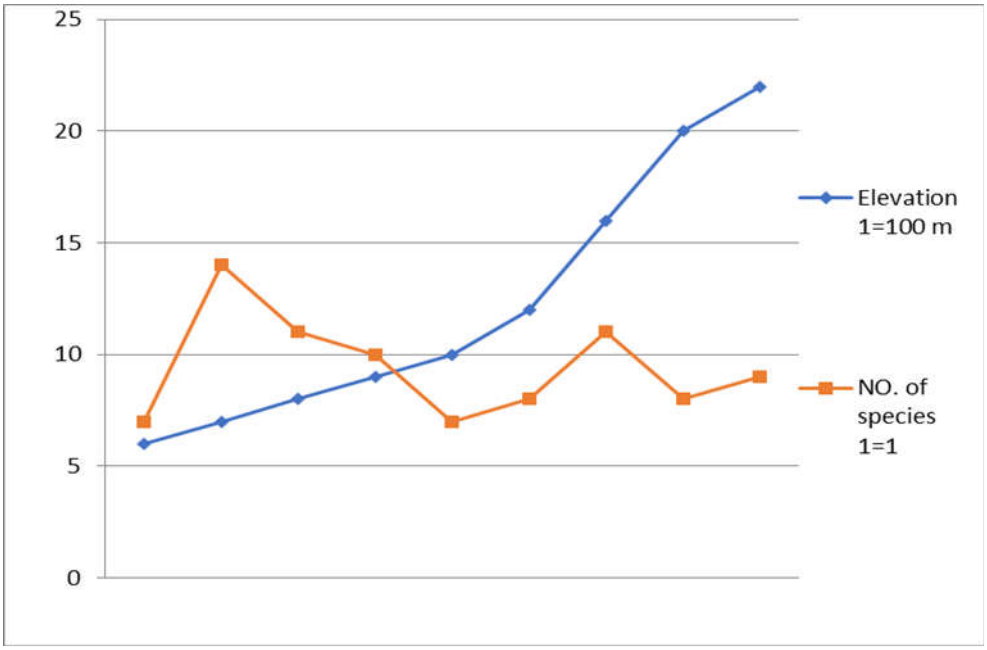


Figure 33- Species diversity along with elevation in the study area

The correlation between elevation and species diversity is given in figure 33. The graph shows a positive correlation between species diversity and elevation. The species diversity shows two peaks at the sample sites in Idukki Wildlife Sanctuary and the Gundumalai region with elevations of 700 m and 1600m, respectively. Both the regions are protected areas and regions with the least pollution and anthropogenic activities. The graph has two declining peaks at Cardamom Hills and Eravikkulam National Park at elevations of 1000 m and 2000 m, respectively. This is mainly due to the increased environmental pollution arising due to the large-scale mining and construction work. Environmental pollution is the chief detrimental factor for the lichens to a large extent.

5.3. Bioprospection

5.3.1. Active Principles of the Genus *Parmotrema* A. Massal.

The thin layer chromatography using the solvent system 'a' was carried out to determine the active principles found in the genus *Parmotrema* .The Major compounds are Atranorin, Salazinic acid, Leconoric acid, Gyrophoric acid and protocetraricacid.Depsides, Depsidones, Dibenzofurans, and Depsonesare the compound class in which these compounds belong. The depside and depsidone

series compounds of polyketide origin accumulate in lichen thalli's cortical or medullary layers. Despite lichen chemistry's taxonomic and ecological significance, many of these natural products are of medical, industrial, and agricultural importance. The parmaloid lichens are significant in the production of these compounds.The lichen substances identified using thin-layer chromatography and the TLC chromatogram is given in figure 34. The Rf class 1 contain consalazinic acid, Rf class 2 contain salzinic acid which appear as yellow orange spot, Rf class 3 contain gyrophoric acid and lecanoric acid and the Rf class 7 contain atranorin appear as yellow orange spot .

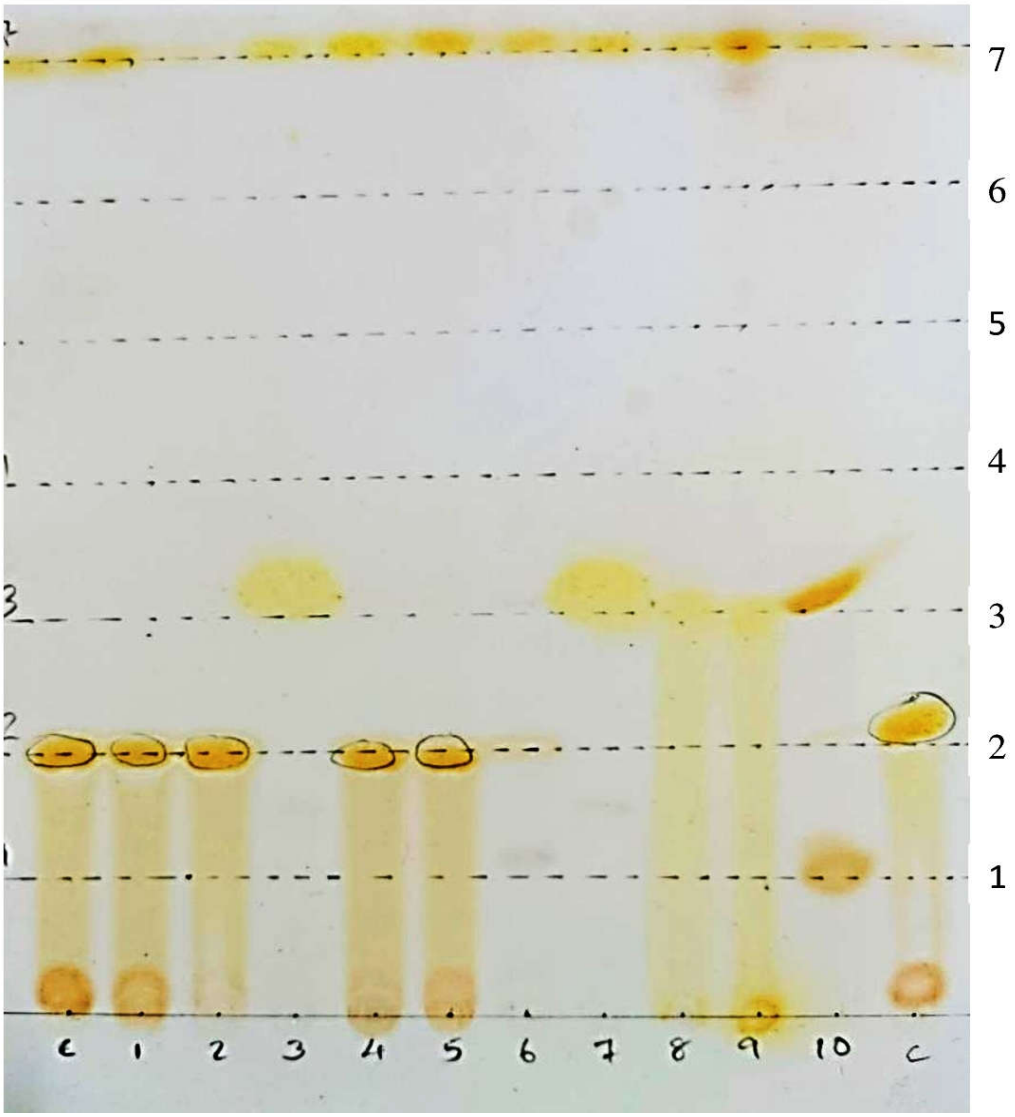


Figure 34 - TLC chromatogram acetone extract of the genus *Parmotrema* using solvent system A.

5.3.2. GC-MS analysis of selected species of the genus *Parmotrema* A. Massal

The GCMS analysis of ethyl acetate extracts of seven lichens yielded 78 different compounds. *Parmotrema tinctorum* has 27 compounds, the highest number, followed by *P. stuppeum* with 26 compounds. Table 16 lists potential pharmaceutically important compounds. The graph showing the retention time and peak area % is shown in Figures 35-41.

Benzoic acid and Benzaldehyde, 2,6-dihydroxy-4-methyl-are ubiquitous compounds in the genus *Parmotrema*. They are aromatic food additives with antimicrobial properties. In *P. tinctorum,* Benzoic acid contributes 34.64 % of the area of the spectrumand Benzaldehyde, 2,6-dihydroxy-4-methyl contributes 23.01%. In addition tothe benzoic compounds, it contains n-hexadecanoic acid with a retention time of 29.339 and Octadecanoic acid with a retention time of 33.105. Both the compounds constitute 5.91% and 2.27% of the Phytochemical composition of *P. tinctorum*. Phthalic acid is another compound present in *P. tinctorum*, having a retention time of 29.55 and contributing 2.06 % of the Phyto-chemical profile of the species. This compound is also present in *P.crinitoides* and *P.austrosinense*. This compound is good repellent and larvicidal properties hencethe compound has industrial relevance.

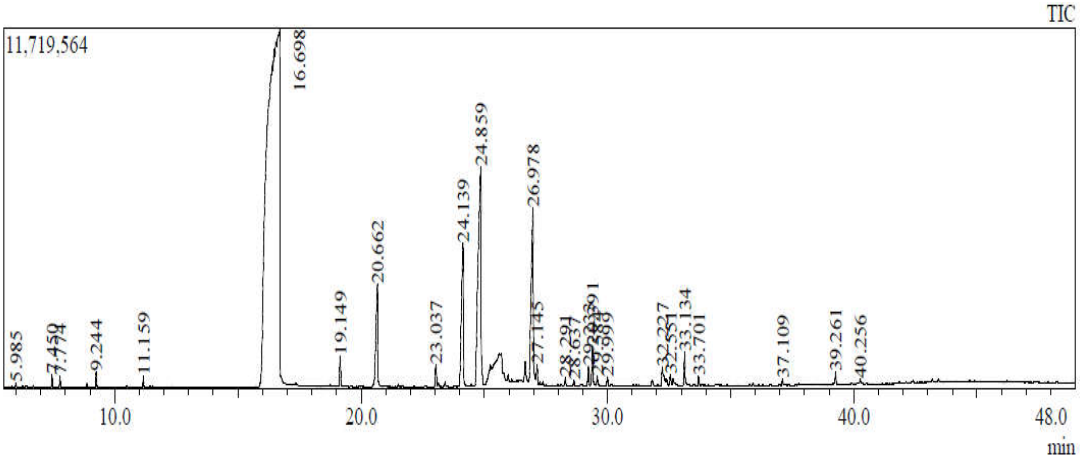
Orcinol is a pharmaceuticaly relevant phyto chemical that is found in *P. reticulatum, P.clavuliferum*and*P.austrosinense*. The former two areclosely related species.This compound represented species contains 80.18% area in *P.reticulatum*,70.67% in *P.austrosinenseis*and 37.5% in *P.clavuliferum.*These species can be used as the source of orcinol for chemical and pharma industries. Squalene is another phytosterol with medicinal properties. Squalene is found only in *P. stuppeum* with a concentration of 4.01%,and it has anti-carcinogenic and anti-microbial properties.

The studied species of lichenscontain several saturated fatty acids. n-Hexadecanoic acid is a proven anti-inflammatory compound, and it is the common phytochemical constituent present in all the members of this genus, Parmotrema. In all the studied samples, the compound is present with an absorbance area ranging from 0.77 to 5.91 percent.

Sl No	Compound name	R.time	Area
Parmotrema austrosinense			
1	Benzoic acid, 2,4-dihydroxy-3,6-dimethyl-, methyl ester	24.054	*34.64*
2	Benzaldehyde, 2,6-dihydroxy-4-methyl-	*20.478*	23.01
3	n-Hexadecanoic acid	*29.339*	5.91
4	Dibutyl phthalate	*29.196*	4.05
5	Ethyl 4-acetoxybutanoate	11.171	*1.04*
6	3-Chloro-2,6-dihydroxy-4-methylbenzaldehyde	19.109	*4.79*
7	Octadecanoic acid	33.105	*2.27*
8	Benzyl Benzoate	25.409	*0.66*
9	Docosanoic acid, ethyl ester	40.260	*1.13*
	Orcinol	16.698	*70.6*
10	Phthalic acid	29.584	*0.15*
Parmotrema clavuliferum			
10	Orcinol	16.026	*37.5*
11	Benzoic acid, 2,4-dihydroxy-3,6-dimethyl-, methyl ester	24.037	*26.9*
12	Benzaldehyde, 2,6-dihydroxy-4-methyl-	20.450	*11.1*
13	Propyl 2,4-dihydroxy-6-methylbenzoate	25.395	*2.93*
14	Octadecanoic acid	33.084	*0.82*
15	n-Hexadecanoic acid	29.307	*1.71*
16	1-Heneicosanol	25.955	*1.14*
Parmotrema crinitoides			
17	Benzoic acid, 2,4-dihydroxy-3,6-dimethyl-, methyl ester	24.038	*55.12*
18	Benzaldehyde, 2,6-dihydroxy-4-methyl-	20.459	*27.59*
19	3-Chloro-2,6-dihydroxy-4-methylbenzaldehyde	19.116	*6.81*
20	n-Hexadecanoic acid	29.318	*2.15*
21	Phthalic acid, 5-methyl-2-yl butylester	29.555	*1.15*
Parmotrema tinctorum			
22	*Benzoic acid*	*24.05*	*34.6*
23	*Benzaldehyde, 2,6-dihydroxy-4-methyl-*	*20.478*	*23.01*
24	*n-Hexadecanoic acid*	*29.339*	*5.91*
25	*Phthalic acid*	*29.55*	*2.06*
26	*Octadecanoic acid*	*33.105*	*2.27*

Parmotrema stuppeum			
27	Pentacosane	40.362	*9.86*
28	Bis(2-ethylhexyl) phthalate	39.254	*9.03*
29	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	42.409	*8.87*
30	Squalene	43.438	*4.01*
31	Octadecane	26.117	*0.76*
32	Benzyl Benzoate	25.406	*3.68*
33	Dotriacontane	41.841	*6.93*
Parmotrema reticulatum			
34	*Orcinol*	*16.653*	*80.18*
35	*Benzoic acid, 2,4-dihydroxy-6-methyl-, methyl ester*	*23.085*	*2.61*
36	*Benzoic acid*	*24.121*	*3.26*
37	*n-Hexadecanoic acid*	*29.338*	*0.23*
38	*Benzaldehyde, 2,6-dihydroxy-4-methyl*	*20.618*	*1.17*
39	n-Hexadecanoic acid	29.289	*1.68*
40	Pentacosane	37.135	*9.12*
Parmotrema cristiferum			
41	*n-Hexadecanoic acid*	31.929	*0.29*
42	*Chloratranol*	19.048	*1.33*
43	*Pentacosane*	37.135	*9.12*
44	*Benzaldehyde, 2,6-dihydroxy-4-methyl-*	20.543	*7.46*
45	*Tetradecanoic acid*	25.147	*0.18*

Table 16– Pharmaceuticaly important compounds identified from GCMS analysis



. **Figure 35** - GC-MS Chromatogram of *Parmotrema austrosinensis*

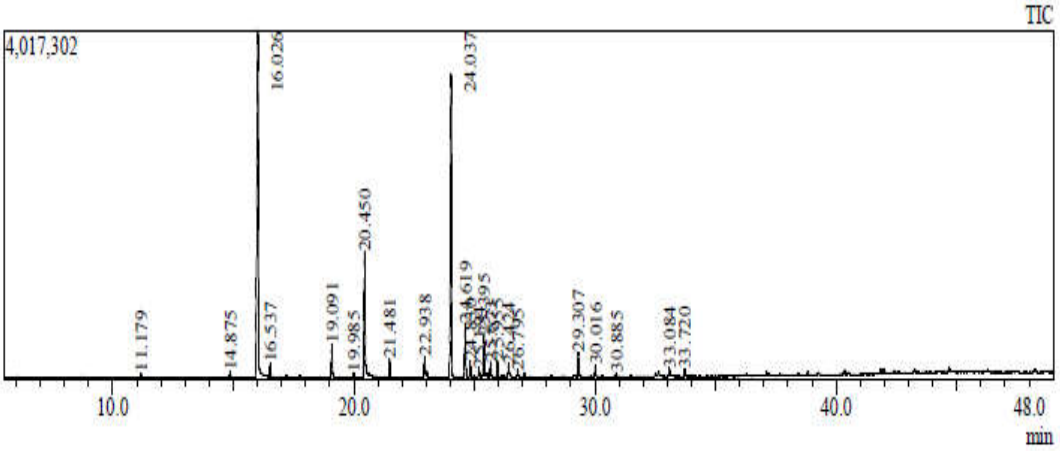


Figure 36 - GC-MS Chromatogram of *Parmotrema clavuliferum*

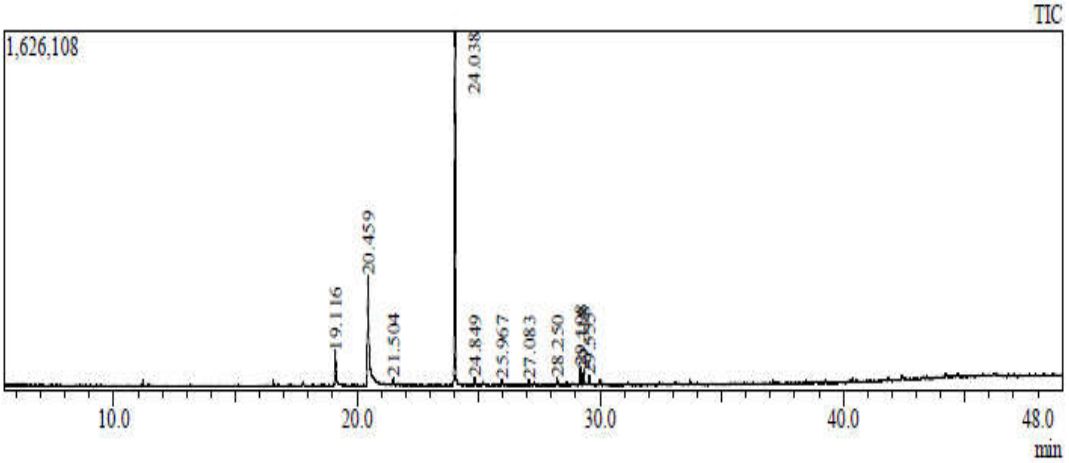


Figure 37 - GC-MS Chromatogram of *Parmotrema crinitoides*

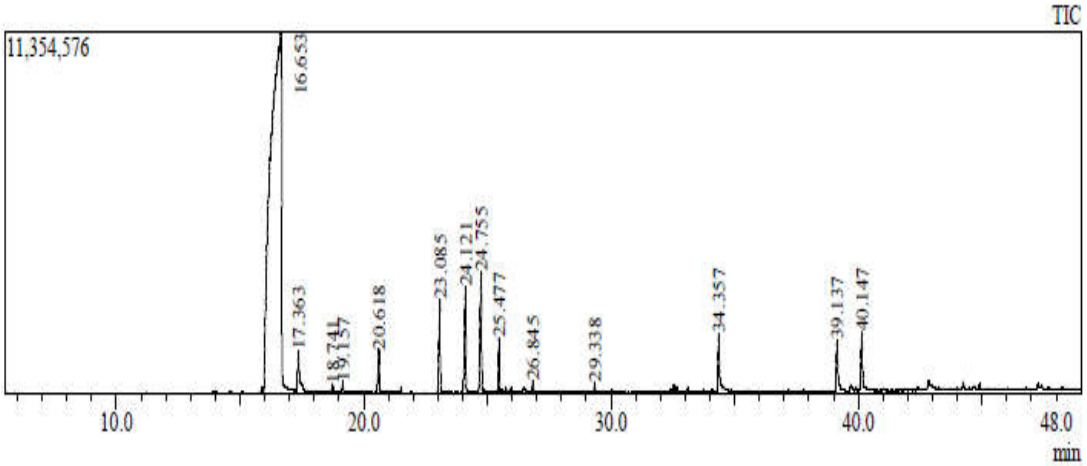


Figure 38 - GC-MS Chromatogram of *Parmotrema reticulatum*

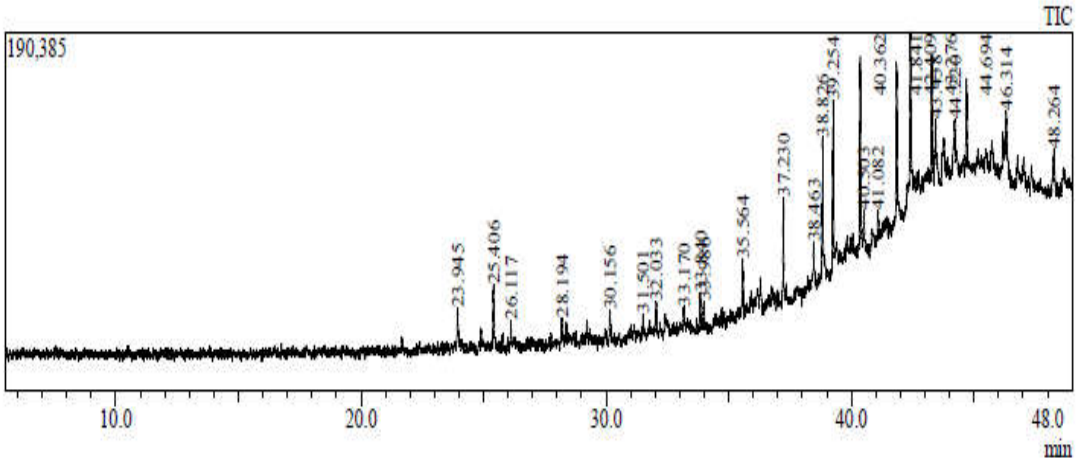


Figure 39 - GC-MS Chromatogram of *Parmotrema stuppeum*

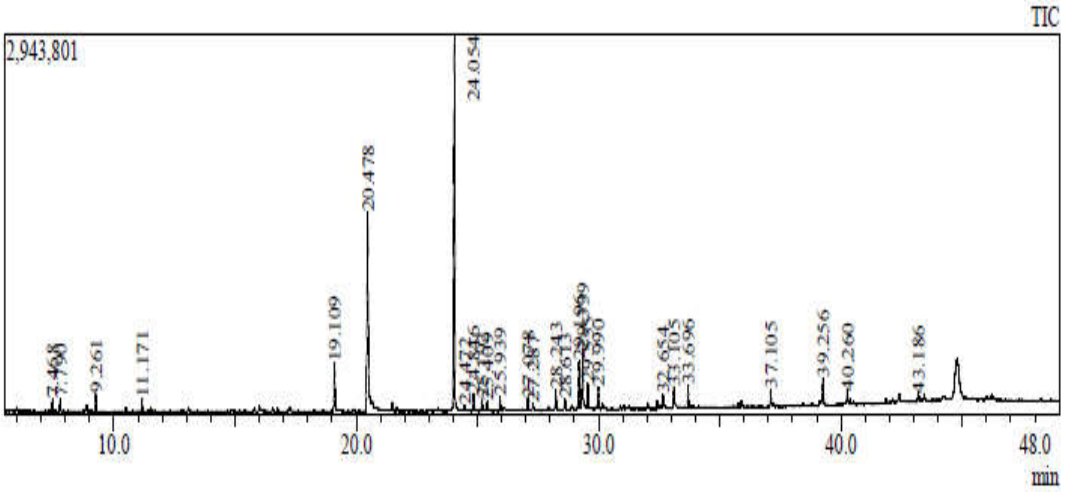


Figure 40 - GC-MS Chromatogram of *Parmotrema tinctorum*

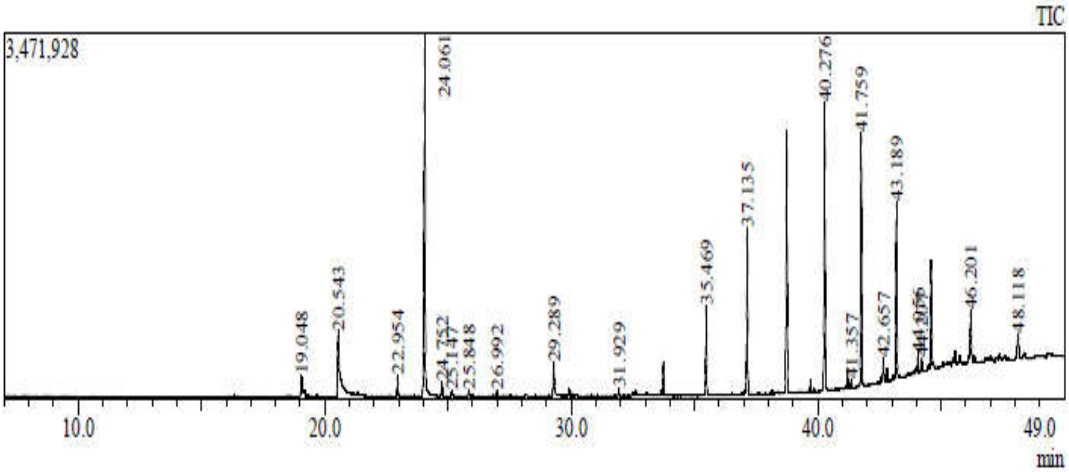


Figure 41 - GC-MS Chromatogram of *Parmotrema cristiferum*

5.3.3. Determination of Total Phenolic Content in Selected Lichens of the Genus *Parmotrema* A. Massal.

The phenolic content in the ethyl acetate extract of selected Parmotrema species was quantified using a standard gallic acid curve (Figure 42). Absorbance readings for standard gallic acid at various concentrations were recorded (Table 17), and the slope and intercept were determined (y = 5.925x + 0.079). The phenolic content of the samples was calculated using this equation and expressed as Gallic Acid Equivalent (GAE/g). *Parmotrema clavuliferum* exhibited the highest phenolic content with172.32±1.55mg GAE/g. The lowest phenolic content was found in *Parmotrema stuppeum*, with a value of 63.62±0.94mg GAE/g

Sl No	Concentration	MeanAbsorbance	Slope	Y-Intercept
1	0.1	0.6645	5.925	0.079
2	0.2	1.3094		
3	0.3	1.7882		
4	0.4	2.4871		
5	0.5	3.0386		

Table 17 – Absorbance at 760 nm against various concentrations of gallic acid.

Parmotrema cristiferum and *P. tinctorum* also showed significant phenolic content, with values of 131.81±1.55mg GAE/g and 122±1.18 mg GAE/g, respectively

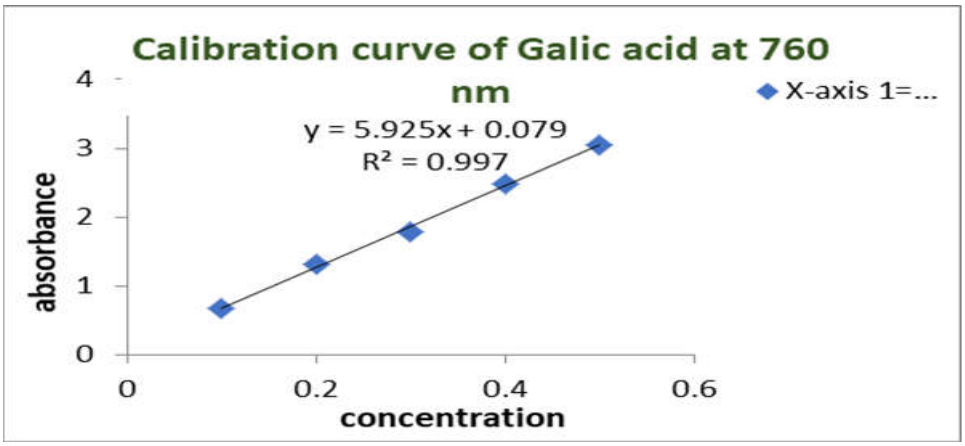


Figure 42- Standard graph of galic acid ploted concentration against sbsorbance.

Sl. No	Name of the Species	OD Value	Total Phenolic Content
1	*P. tinctorum*	0.802±0.007	122±1.18 mg GAE/g
2	*P. reticulatum*	0.643±0.011	95.18±1.9mg GAE/g
3	*P. crinitoides*	0.663±0.013	98.56±2.3mg GAE/g
4	*P. austrosinense*	0.573±0.013	83.64±2.23mg GAE/g
5	*P. clavuliferum*	1.10±0.009	172.32±1.55mg GAE/g
6	*P. stuppeum*	0.456±0.005	63.62±0.94mg GAE/g
7	*P. cristiferum*	0.860±0.009	131.81±1.55mg GAE/g

Table 18- Quantification of total phenols in selected lichens of the genus *Parmotrema*

5.3.4. Determination of Total Flavonoid Content in Selected Lichens of the Genus *Parmotrema A. Massal*

The total flavonoid content of the ethyl acetate extract from selected Parmotrema species was quantified using Quercetin as the standard, with absorbance measurementstaken across various Quercetin concentrations to create a standard graph (Table 19). The absorbance of the samples was plotted against the slope equation of the standard graph: y = 1.0725x - 0.023(Figure 43) and estimated total flavanoids as the Quercetine equivalent. Among the studied species, *Parmotreama clavuliferum* has the highest flavanoid content and the concentration of flavanoid is 82.8±1.3mg QE/g. It is followed by *P. tinctorum* with a value of 79±0.98mg QE/g.The least value of flavanoid concentration was recorded in *P. cristiferum* with 28±1.4mg QE/g as the value. The concentreation of flavanoid among the studied species was given in Table 20.

Sl. No	Concentration	Mean Absorbance	Slope	Y-Intercept
1	25	0.0824	**0.004**	**-0.023**
2	50	0.1950		
3	100	0.3032		
4	150	0.4060		

Table 19- : Absorbance at 415 nm against various concentrations of Quercetin

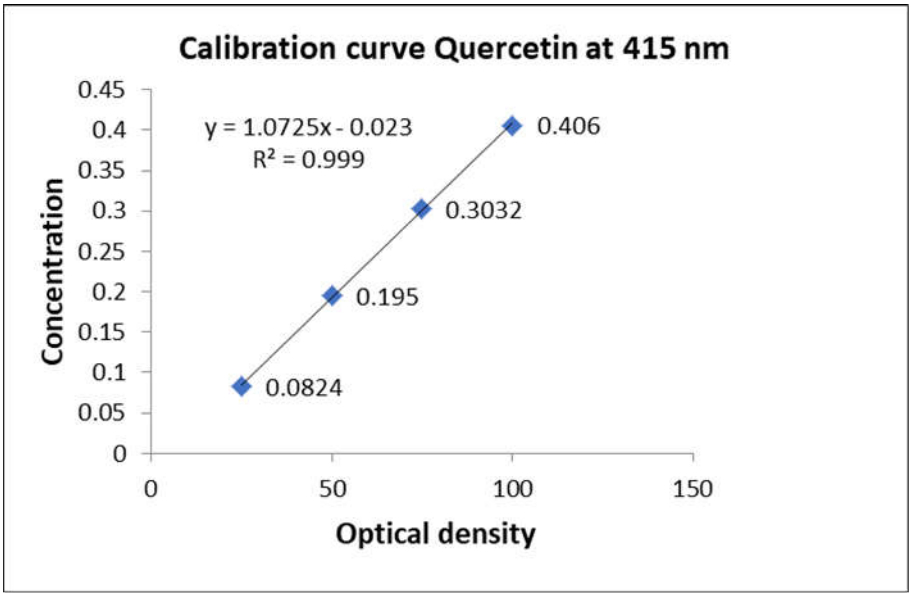


Figure 43- Standard graph of Quercetin, plotted concentration against absorbance.

Sl. No.	Name of the Species	OD at 415 nm	Total flavonoid Content
1	*P. tinctorum*	0.062±0.001	79±0.98mg QE/g
2	*P. reticulatum*	0.020±0.0011	40±1.1mg QE/g
3	*P. austrosinense*	0.04135±0.0013	60±1.24mg QE/g
4	*P. clavuliferum*	0.0658±0.0014	82.8±1.3mg QE/g
5	*P. stuppeum*	0.0032±0.0011	24.46±1.1mg QE/g
6	*P. crinitoides*	0.0334±0.0012	52.6±1.13mg QE/g
7	*P. cristiferum*	0.0070±0.0014	28±1.4mg QE/g

Table 20-Quantification of total flavanoids in selected lichens of the genus *Parmotrema.*

5.3.5. Anti-oxidant Properties of Lichens of the genus *Parmotrema* A. Massal.

5.3.5.1 DPPH Assay

The free radical scavenging activity of the ethyl acetate extract of lichens was estimated by DPPH assay. The percentage of discoloration of the DPPH solution was noted against various concentration of the lichen extract and tstandard graph was ploted (figure 44). Based on the linear graph equation thus obtained was used to determine IC 50 value of ech species. The inhibitory concentration of the standard

ascorbic acid was determined as 80.42±2.0µg/ml. Three species of lichens show 50% or more free radical scavenging activity compared with standard ascorbic acid. They are *P.tinctorum*, *P. clavuliferum*, and *P. reticulatum*, and their IC50 values were determined as 154.94±0.6µg/ml, 153.20±0.49µg/ml, and 146.58±0.53µg/ml respectively. *P. stuppeum* is the species with lowest free radical scavenging property among the seven species studied and the IC50 value was determined as 1353.27±1.36µg/ml.The results showing the anti-oxidant properties of the selected lichens were tabulated in Table 21.

Sl. No	**Name of the Species**	**Concentration**	**Absorbance**	**Percentage of discolorisation**	**IC_{50} Value**
1	*P. reticulatum*	50	0.9863±0.001	7.364±0.12	146.58±0.53µg/ml
2		100	0.823±0.007	22.645±0.69	
3		150	0.5351±0.009	49.742±0.9	
4		200	0.2651±0.009	75.101±0.88	
5	*P. clavuliferum*	50	1.0169±0.002	4.490±0.23	153.20±0.49µg/ml
6		100	0.8348±0.008	21.593±0.79	
7		150	0.6452±0.006	39.401±0.56	
8		200	0.3925±0.0073	63.135±0.69	
9	*P. crinitoides*	50	0.9819±0.003	7.777±0.29	940±2.4µg/ml
10		100	0.9397±0.002	11.740±0.09	
11		150	0.9169±0.001	13.882±0.12	
12		200	0.8967±0.001	15.779±0.1	
13	*P. stuppeum*	50	1.0325±0.007	3.024±0.75	1353.27±1.36µg/ml
14		100	1.0098±0.001	5.156±0.1	
15		150	0.9968±0.002	6.377±0.23	
16		200	0.9727±0.002	8.641±0.24	
17	*P. tinctorum*	50	0.8936±0.001	16.070±0.18	154.94±0.6µg/ml
18		100	0.6952±0.0003	34.705±0.03	
19		150	0.5232±0.0004	50.859±0.04	
20		200	0.4048±0.005	61.980±0.5	
21	*P. austrosinense*	50	1.0225±0.002	3.964±0.3	1011.69±7.4µg/ml
22		100	1.0177±0.007	4.414±0.7	
23		150	0.9957±0.007	6.481±0.69	
24		200	0.9424±0.003	11.487±0.35	
25	*P. cristiferum*	50	1.0191±0.007	4.283±0.69	638.80±4.8µg/ml
26		100	0.9578±0.002	10.040±0.26	
27		150	0.9145±0.005	14.107±0.46	
28		200	0.8975±0.009	15.704±0.9	
29	Standard	50	0.7031±0.01	33.963±0.9	80.42±2.0µg/ml
30		100	0.4223±0.01	60.336±0.9	
31		150	0.1548±0.004	85.461±0.4	
32		200	0.0736±0.001	93.087±0.10	

Table 21- Antioxidant property of lichens analysed using DPPH

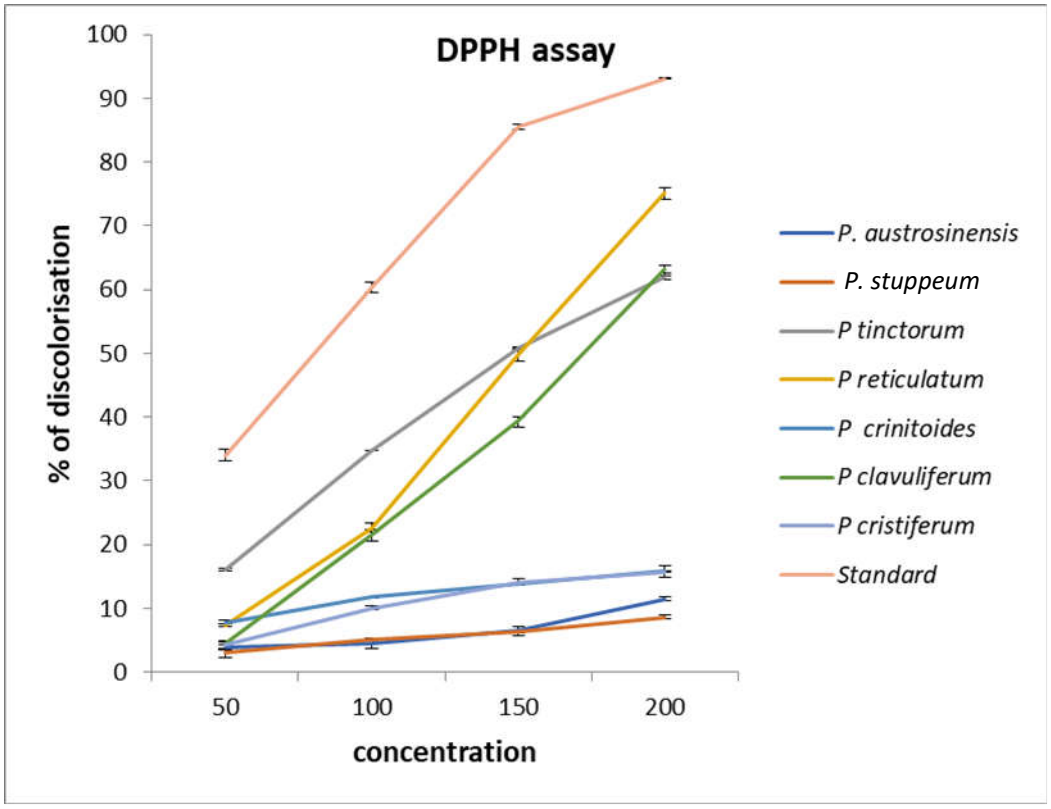


Figure 44– Anti oxidant preoperty of lichens analysed using DPPH assay.

5.3.5.2 ABTS Assay

The ABTS assay was used to analyze the free radical scavenging properties of the samples, expressed as IC_{50} values. The IC_{50} value for the standard ascorbic acid was determined to be 5.009 mg/ml. Among the selected lichens, *P. tinctorum* exhibited the highest antioxidant activity, with an IC_{50} value of 13.65 ±1.58mg/ ml, followed by *P. clavuliferum* with an IC_{50} value of 14.006±0.8 mg/ml. *P. cristiferum* displayed the lowest antioxidant activity, with an IC_{50} value of 25.32 ±0.8 mg/ml. Table 22 presents the antioxidant properties of the selected lichens, and a comparative analysis with the standard is shown in Figure 45.

Sl. No	Name of the Species	Concentration mg/ ml	Absorbance	% of Decolorisaation	IC $_{50}$ value
1	*P.austrosinenense*	4	0.493425±0.003	26.900±0.5	21.020±01.47 mg/ml
2		3	0.50864±0.009	24.646±1.3	
3		2	0.513797±0.005	23.882±0.8	
4		1	0.51599±0.003	23.557±0.5	
5	*P.stuppeum*	4	0.502484±0.01	25.558±1.5	22.79 ±1.6mg/ml
6		3	0.510604±0.007	24.355±1.1	
7		2	0.521384±0.005	22.758±0.8	
8		1	0.528174±0.009	21.752±1.4	
9	*P. tinctorum*	4	0.472736±0.01	29.965±1.6	13.65 ±1.58mg/ ml
10		3	0.495173±0.001	26.641±0.2	
11		2	0.507593±0.003	24.801±0.6	
12		1	0.516571±0.001	23.471±0.2	
13	*P.reticulatum*	4	0.493425±0.007	26.900±1	24.37±0.62mg/ ml
14		3	0.50189±0.004	25.646±0.5	
15		2	0.51109±0.009	24.282±1.3	
16		1	0.51599±0.007	23.557±1	
17	*P.crinitoides*	4	0.49156±0.007	27.176±1	21.008 ±0.82mg/ml
18		3	0.501917±0.01	25.642±1.4	
19		2	0.511299±0.06	24.252±0.9	
20		1	0.518771±0.004	23.145±0.6	
21	*P.clavuliferum*	4	0.481511±0.01	28.665±1.5	14.006 ± 0.8mg/ ml
22		3	0.494498±0.007	26.741±0.6	
23		2	0.509537±0.005	24.513±0.7	
24		1	0.5243±0.009	22.326±1.3	
25	*P.cristiferum*	4	0.501161±0.005	25.754±0.9	25.32±0.8mg/ ml
26		3	0.506682±0.009	24.936±1.4	
27		2	0.516213±0.01	23.524±1.7	
28		1	0.523456±0.005	22.451±0.8	
29	Standard	4	0.378959±0.004	43.858±0.7	5.009±0.4mg/ ml
30		3	0.426256±0.004	36.851±0.5	
31		2	0.473411±0.005	29.865±0.8	
32		1	0.508511±0.004	24.665±0.7	

Table 22- Anti oxidant property of lichens analysed using ABTS assay

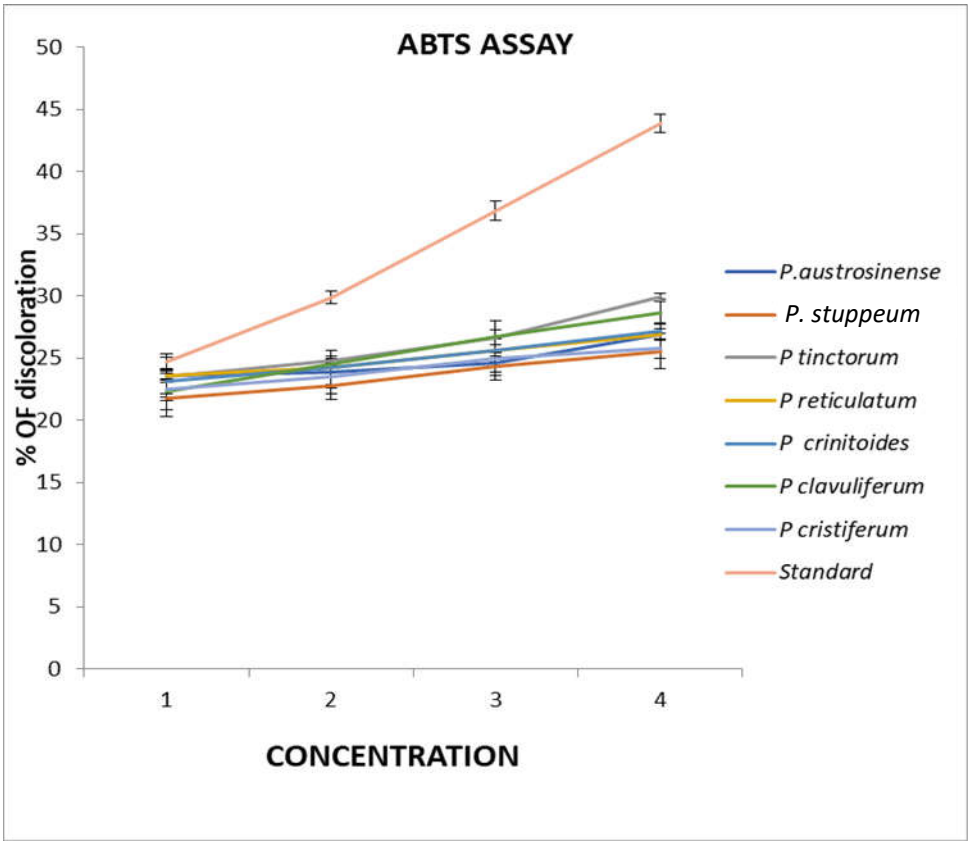


Figure 45 – ABTS assay-Anti oxidant properties of the genus *Parmotrema* A. Massal.

5.3.6. Anti-microbial Properties of the Genus *Parmotrema* A. Massal.

Figure 46- A, B,C,D and E shows the antimicrobial activity of the solvent Ethyl acetate (negative control) aganst the test bacteria *S. aureus, E. coli, K.pneumonia,* and the pathogenic fungi *Penicillium citrinum* and *Aspergillus niger* respectively, and found that the ethyl acetate has no inhibitory properties againt test organisms. Ampicillin is used as the positive control in anti bacterial studies and *Itraconazole* was used as the positive control in antifungal study.

Anti-bacterial properties of the genus *Parmotrema* A. Massal.

The antimicrobial properties of seven Parmotrema species were evaluated using pathogenic gram-positive and gram-negative bacteria. All lichens used in this study were available in large quantities from collection sites in Kerala. *P. clavuliferum* exhibited the highest inhibitory activity against *Staphylococcus aureus* (15±0.2 mm), while *P. tinctorum* demonstrated the strongest inhibition against *E. coli* (18±0.25 mm) and *Klebsiella pneumoniae* (20 ±0.5mm). *P. cristiferum* also showed significant inhibitory activity against *Klebsiella pneumoniae* (20±0.5mm), similar to *P. tinctorum*. The results are presented as the zones of bacterial growth inhibition around wells containing lichen extracts (Table 23 and Figure 47), with the anti-bacterial potential measured as the zone of inhibition shown in Figure 48.

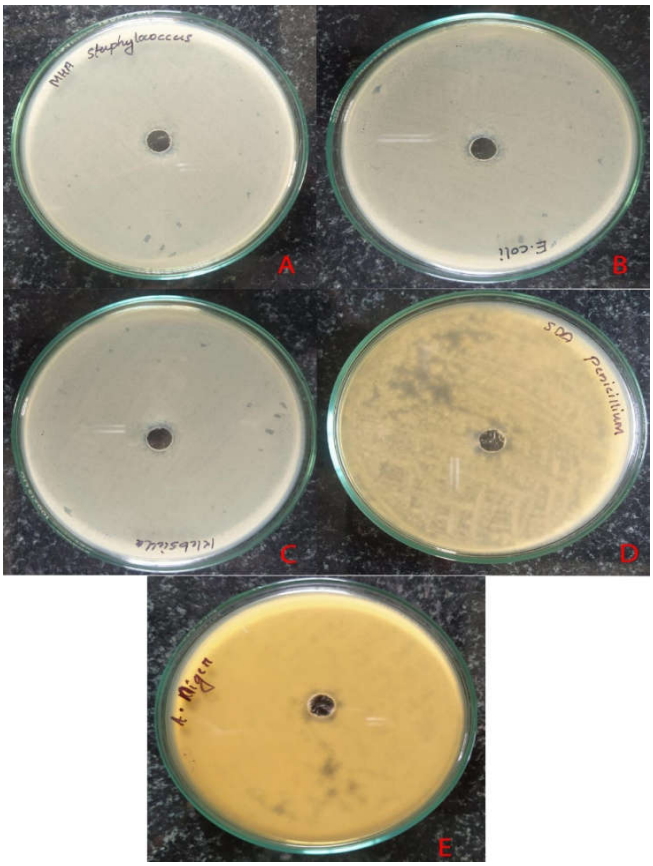


Figure. 46 Zone of inhibition by the negative control against the test organism.

Sl. No	Name of the Species	Zone of Inhibition in mm		
		E. coli	***Staphylococcus aureus***	***Klebsiella pneumoniae***
1	*P. reticulatum*	15±0.2	12±0.4	10±.03
2	*P. crinitoides*	16±0.5	15±0.2	12±0.25
3	*P. clavuliferum*	13±0.4	14±0.5	12±0.2
4	*P.austrosinense*	14±0.25	11±0.5	11±0.3
5	*P. stuppeum*	16±0.6	12±0.25	18±0.5
6	*P.cristiferum*	14±0.45	14±0.4	20±0.5
7	*P.tinctorum*	18±0.25	12±0.2	20±0.5
8	control	12±0.35	8±0	10±0.2

Table 23- Anti-bacterial properties of seven lichens against three pathogenic bacteria

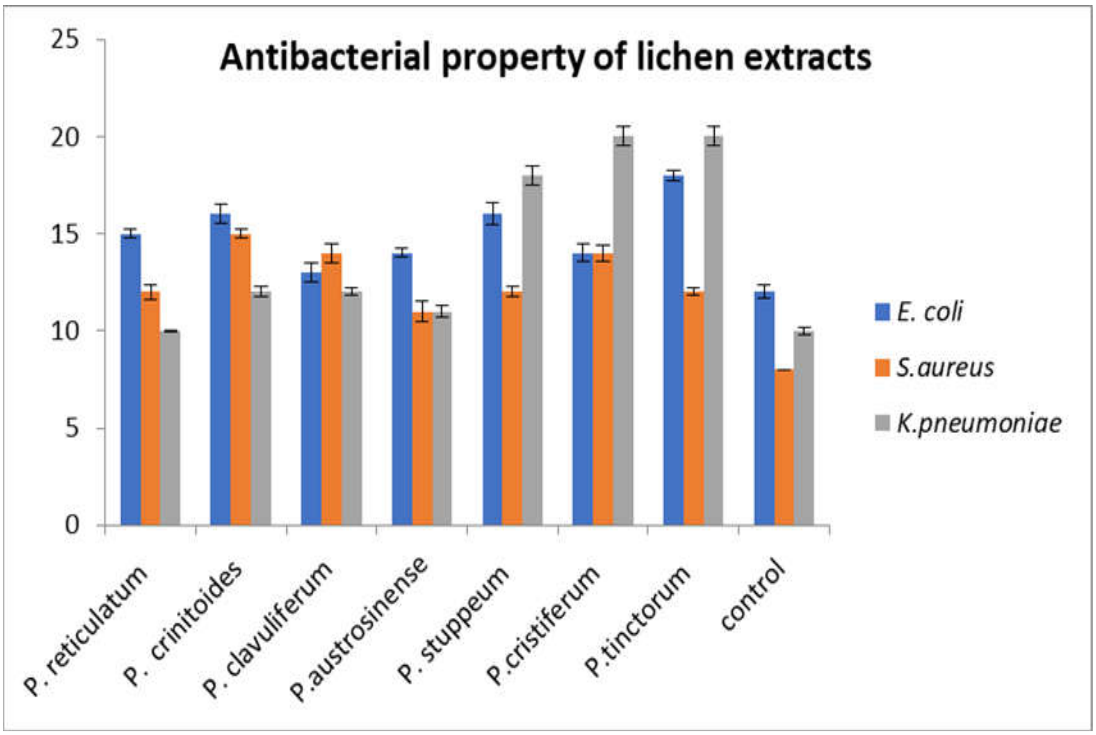


Figure 47–Antibacterial properties of the genus Parmotrema in Kerala

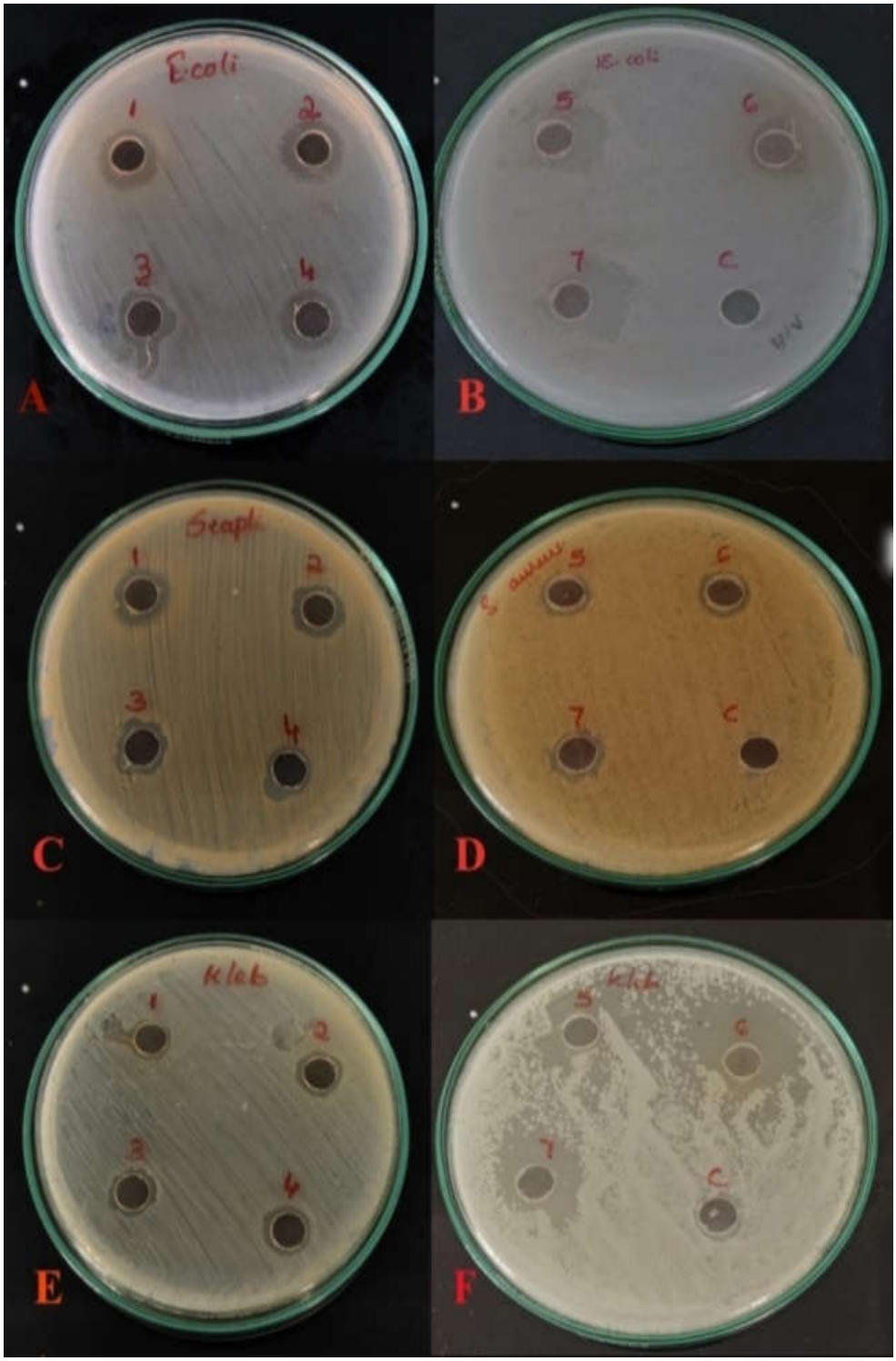


Figure. 48 Anti-bacterial properties of lichen; A1- Zone of inhibition by *P. reticulatum against E. coli, A2*- Zone of inhibition by *P. crinitoides against E. coli, A3- zone* of inhibition by *P. clavuliferum against E coli, A4*- Zone of inhibition by *P.austrosinse against E coli,*

B5 Zone of inhibition by *P. stuppeum against E. coli, B6 Zone* of inhibition by *P.cristiferum, B7* Zone of inhibition by *P.tinctorum against E. coli, BC - Zone* of inhibition by positive *Control*

C1- Zone of inhibition by *P. reticulatum against S. aureusC2-* Zone of inhibition by *P. crinitoides against S. aureus, C3- zone* of inhibition by *P. clavuliferum against S. aureus, C4-* Zone of inhibition by *P.austrosinse against S. aureus,*

D5 Zone of inhibition by *P. stuppeum against S. aureus, D6 Zone* of inhibition by *P.cristiferum, D7* Zone of inhibition by *P.tinctorum against S. aureus, DC - Zone* of inhibition by positive *Control*

E1- Zone of inhibition by *P. reticulatum against K. pneumoniae E2-* Zone of inhibition by *P. crinitoides against K. pneumoniae, E3- zone* of inhibition by *P. clavuliferum against K. pneumoniae, E4-* Zone of inhibition by *P.austrosinse against K. pneumoniae,*

F5 Zone of inhibition by *P. stuppeum against K. pneumoniae, F6 Zone* of inhibition by *P.cristiferum, F7* Zone of inhibition by *P.tinctorum against K. pneumoniae, FC - Zone* of inhibition by positive *Control*

Anti-Fungal properties of the Genus *Parmotrema* A. Massal.

The antifungal activity of ethyl acetate extracts from seven lichen species against various pathogenic fungi is detailed in Table 24. Figure 49 compares the antifungal properties of the selected lichens, while Figure 50 illustrates the zones of inhibition produced by *Parmotrema* lichens against two pathogenic fungi, *Penicillium citrinum* and *Aspergillus niger*.*P. cristiferum* showed the highest antifungal activity against *Penicillium citrinum*, with a zone of inhibition of 20± 1mm.*P. reticulatum* and *P. stuppeum* both exhibited significant inhibitory properties against *Penicillium citrinum*, with a zone of inhibition of 15± 0.95 mm., 15± 0.5 respectivly. For *Aspergillus niger*, *P. reticulatum*, *P. crinitoides*, and *P. tinctorum* demonstrated the highest antifungal activity, with a zone of inhibition of 13± 1 mm, 13± 0.6, and 13± 0.6 respectivly. *P. stuppeum* had the lowest antifungal activity, with a zone of inhibition of only 11± 1 mm. Ethyl acetate was used as the negative control, and

Itraconazole was used as the positive control the zone of inhibition by the positive control is estimated as 14± 0.4mm and 12± 1 mm against *Penicillium citrinum* and *Aspergillus niger* respectively.

Sl. No.	Name of the Species	Zone of Inhibition in mm	
		Penicillium citrinum	***Aspergillus niger***
1	*P. reticulatum*	15± 0.95	13± 1
2	*P. crinitoides*	13± 0.5	13± 0.6
3	*P. clavuliferum*	11± 0.6	12± 0.5
4	*P. austrosinese*	14± 0.6	12± 0.5
5	*P. stuppeum*	15± 0.5	11± 1
6	*P. cristiferum*	20± 1	12 ± 0.5
7	*P. tinctorum*	12± 0.5	13± 0.6
8	*control*	14 ± 0.4	12 ± 1

Table: 24 -Antifungal properties of the genus *P.*A. Massal.

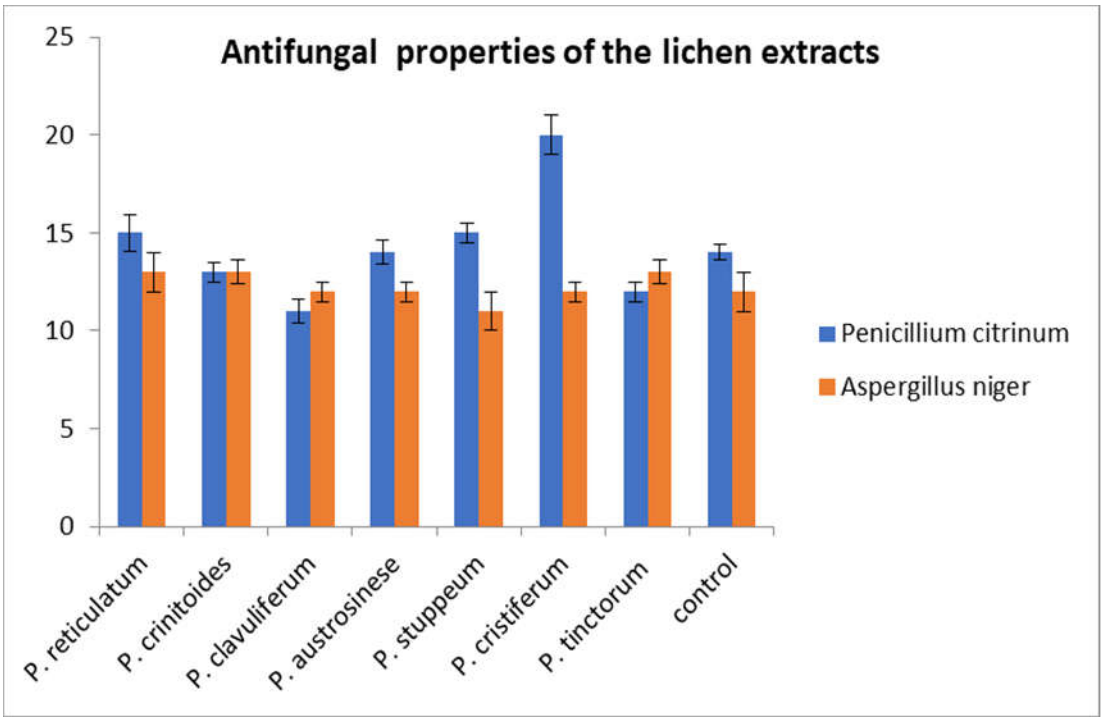


Figure 49 – Antifungal properties of the genus *Parmotrema*A. Massal.

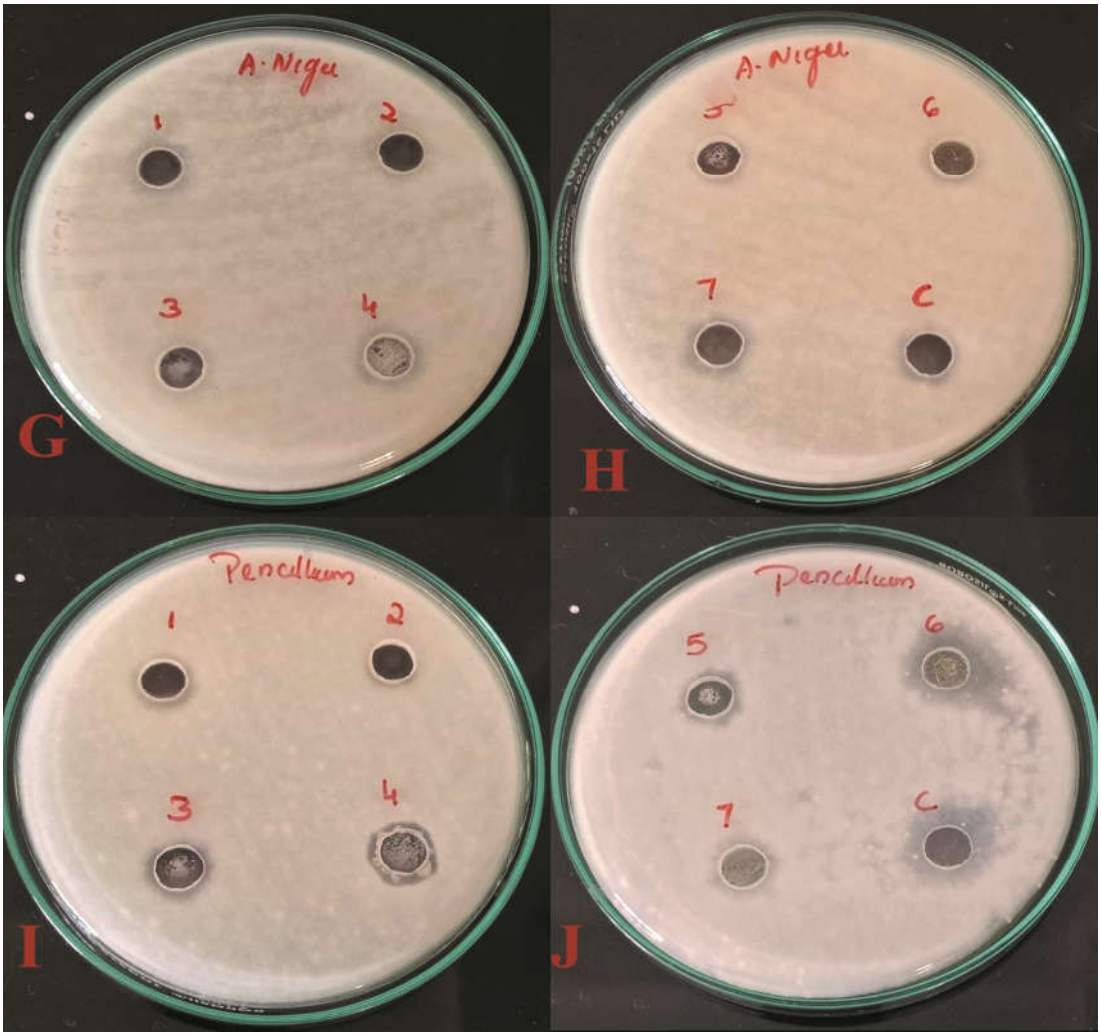


Figure. 50 - Anti Fungal properties of lichen; G1- Zone of inhibition by *P. reticulatum against A. niger, G2-* Zone of inhibition by *P. crinitoides against A. niger, G3- zone* of inhibition by *P. clavuliferum against A. niger, G4-* Zone of inhibition by *P.austrosinse against A. niger,*

H5 Zone of inhibition by *P. stuppeum against A. niger, H6 Zone* of inhibition by *P.cristiferum, H7* Zone of inhibition by *P.tinctorum against A. niger, HC - Zone* of inhibition by positive *Control*

I1- Zone of inhibition by *P. reticulatum against P. citrinum, I2-* Zone of inhibition by *P. crinitoides against P. citrinum, I3- zone* of inhibition by *P. clavuliferum against P. citrinum, I4-* Zone of inhibition by *P.austrosinse against P. citrinum,*

J5 Zone of inhibition by *P. stuppeum against P. citrinum, J6 Zone* of inhibition by *P.cristiferum, J7* Zone of inhibition by *P.tinctorum against P. citrinum, JC - Zone* of inhibition by positive *Control*

CHAPTER 6

DISCUSSION

6. Discussions

6.1. Systematic Treatment of the Genus *Parmotrema A. Massal.*

Lichens are the pioneers of xerarch succession, indicators of pollution, etc. The natural healthy lichen biota of a region indicates the ecological well-being and lesser environmental pollution of the region. The systematic assessment of the species involves the collection, identification, and preservation of the species. Thus, systematic study can be considered the most important part of ecological conservation. Information needs for biodiversity studies are many and varied. The systematic study also includes information about species diversity, challenges and threats, faunal and floral interaction, population studies, etc. The systematic assessment of various lichen habits of Kerala has yet to be studied, and the systematic of lichen in the fragile ecosystem of Kerala requires immediate action as the lichens of this region are facing challenges.

Study of Species

The present study focuses on the lichen diversity of the genus *Parmotrema* and elucidated the presence of 29 species in Kerala. In the comprehensive revisionary study of the lichen genus *Parmotrema* A. Massal. of India, Mishra and Upreti (2017) have provided a detailed morpho-taxonomic account of 53 species of *Parmotrema*. *P. clavuliferum* is a species excluded from the genus *Parmotrema* as this species made conspecific with *P. reticulatum* (Divakar *et al.,* 2001) and synonymized to *Rimelia reticulata* (Taylor) Hale & Fletcher (Divakar and Upreti, 2005). *P. clavuliferum,* with its unique characteristics; it is a notable addition to the genus *Parmotrema.* It resembles *P. reticulatum* in its white to whitish grey colour, densely reticulate-maculate, often cracked upper surface, abundantly sorediate margins, and simple cilia. In addition they share the same chemistry (cortex with atranorin, medulla with salazinic acid). *P. clavuliferum* can be distinguished from *P. reticulatum* with the following characters. The former having capitates soralia, sorediate lacinules at the lobe margins and the variegated lower side of the soredia (non pigmented and white). The distinctly stalked, capitates soralia protruding from long, slender, laciniate lobes are the characteristic feature of *P. clavuliferum* where

as in *P. reticulatum.* The soralia present in laminal to submarginal regions of the thallus (Moon *et al.,* 2001).This character can be used to segregate *P. clavuliferum* from *P. reticulatum*. Even though the erhizinate or nude and broad marginsare the key character of the genus *Parmotrema*, the broad lobes of *P. reticulatum* are typically densely rhizinate even close to the margin but the lacunae or clavulae of *P. clavuliferum* always devoid of rhizines (Bungartz and Spielmann, 2019).

The molecular studies of Ahn & Moon (2016) also provide evidence of the existence of *P. clavuliferum* as a separate species and not morphotypes. Spielmann and Marcelli (2009) reported *P. clavuliferum* from Brazil with filiform conidia and the presence of salazinic acid (K+ yellow turning blood red) as the secondary metabolite. Bungartz and Spielmann (2019) also recognize *P. clavuliferum* as a separate species in their comprehensive inventory of all Galapagos lichens. The comparative study of *P. clavuliferum* collected from different locations in Kerala with the description of *Rimelia reticulata* provided by the taxonomic description available, we can conclude both the species are different taxons bolongs to two different genera of the Parmeloid lichens. Both species have similarities in the foliose nature of the thallus and rotund or sub rotund lobes with densely white maculae with reticulate fissures and cilia. But *P. clavuliferum* has capitates soralia, sorediate lacinules at the lobe margins and the variegated lower side of the soredia. The distinctly stalked, capitates soralia protruding from long, slender, laciniate lobes are the characteristic feature of *P. clavuliferum.*

6.2. Biogeographic Pattern of Distribution of the Genus *Parmotrema* in different Lichen Niche.

The present study focuses on the species diversity of the genus *Parmotrema* in the study area and also analyses the ecological factors that determine their successful establishment in the habitat. It compares the pattern of distribution using the alpha diversity indices as well as the species' IVI value. For the comparative biogeographic pattern analysis, Idukki and Wayanad were selected.

Any data that deals with biodiversity information has to be geographically based and that must contain the precise location where the end user can collect these species.

Here GIS plays an important role. The role of GIS, a crucial tool, is to integrate and analyze large varieties of spatial and attribute data for biodiversity assessment and monitoring purposes. The GIS data can be used for todays and tomorrow's needs as a baseline data to understand the trends in biodiversity changes of lichens due to pollution, urbanization and climate change. The study has documented the Geo coordinates of the species from where they occur and compiled the data as GIS map using QGIS software.

6.2.1. Comparative Biogeography of the Genus *Parmotrema* in Wayanad and Idukki Districts of Kerala.

All the previous works add valuable additions to the lichen biota of Kerala and India, but none of these studies did not care about the pristine habitat of the species and the ecological aspects of each species, and hence, the biogeography and conservation status are undermined. The study of the lichen ecosystems of Wayanad and Idukki districts of Kerala has importance, as the regions are facing multiple threats, in the form of landslides and habitat loss will help the conservation of the biodiversity ofthe area. All the species were also facing threats due to pollution arising from automobile exhausts, and the acidic residues of sulphide and nitrite arising from various anthropogenic activities. The study of diversity and distribution patterns of lichens based on the geographical parameters shoe a general trend of decline of species diversity in the human settlements and high species diversity in the dedicated ecological conservation areas.

The species diversity of Idukki shows the highest values at the sample sites in Idukki Wildlife Sanctuary and the Gundumalai region with elevations of 700 m and 1600m, respectively. Both the regions are protected areas and regions with the least pollution and anthropogenic activities. The graph has two declining peaks at Cardamom Hills and Eravikulam National Park at elevations of 1000 m and 2000 m, respectively. This is mainly due to the increased environmental pollution arising due to the large-scale mining and construction work. Environmental pollution is the chief detrimental factor for the lichens to a large extent. Similar trends can observe

in Wayanad district as the Puthurvayal and Chennalode regions are semi urban regions of Wayanad district shows a declining tendency in the number of species.

The monsoon rains, the presence of coast line and the Western Ghats all together determine the climate of the state of Kerala. Even though seasonal changes in temperature, precipitation and relative humidity, Kerala has near uniform climate in different locations of the state (Mehta and Rai, 2023). The major factor that causes changes climatic and land form is the elevation. Hence we are analyzing the diversity of lichens of the genus Parmotrema on the basis of elevation as the variable and hence the zone wise analysis of diversity will give more insights.

The present study depicts the importance of the Wayanad and Idukki district as a pristine habitat for lichens. This study can be used as baseline data about the lichen diversity and bio-geographic pattern of foliose lichens, and it can be used to assess the impact of climate change and pollution on the biodiversity of the Wayanad and Idukki districts and the Western Ghats.

The study also provides insights to the threatened species of this region. *P. saccatilobum*, *P. abnuens*, *P. praesorediosum* and *P. tsavoense* are the four lichens of the genus *Parmotrema* that face threats either due to anthropogenic activities or habitat specificity. The identification of *P. tsavoense* from Wayanad indicates the undiscovered species wealth of this region. All the species with IVI value less than three requires protective measures in their habitat and close monitoring regarding their well being as the biodiversity is as important as the economic prosperity of the nation.

The general trend of lichen diversity of the study area is among the crustose lichens, Graphidacea is the dominant, foliose lichens *Parmotrema* is the dominant followed by *Heterodermia*, and among fruticose lichens, Usnea is the dominant family. The fruticose lichens belong to the genus *Ramalina*, and the dimorphic genus. *Cladonia* is also remarkably present in the study area (Kumar, 2000). Among the genus *Parmotrema, P. tinctorum* is a versatile species distributed evenly and abundantly through the study area and the species is highly sensitive to environmental changes and pollution.

The twenty-nine species reported from Kerala show the habitat suitability of the genus *Parmotrema* in the study area. The moist and humid climate in the high-altitude regions, especially the Idukki and Wayanad districts, is suitable for foliose lichens. Based on IVI value *P. tinctorum* is the dominant species except in zone 1 of Idukki district where IVI value of *P. reticulatum* is the highest. It is also noted that the presence of *P. reticulatum* is limited in the regions where atmospheric pollution is high. Hence these species can be further utilized as a standard pollution indicator.

The occurrence of 136 species belongs to 24 families and 45 genera of lichens were reported by Biju *et al.*, (2014) from the Idukki district of Kerala. They also report the dominance of the Parmeliaceae family with 47 species and *Parmotrema* as the dominant genera with 18 species. The present investigation also has similar findings and updates the lichen diversity of the genera *Parmotreama* as 26 species. Among the members of the genus *Parmotrema* several members requires immediate measures to conserve them as they were reported to have low IVI value and limited presence in the sample sites. Such species Include *P. robustum, P. margaritatum, P. mesotropum, P. praesorediosum, P. latissimum,* and *P. flavomedullosum.*

6.3. Bioprospection of the genus *Parmotrema* A. Massal.

Bioprospecting is the exploration of biodiversity for new resources of social and commercial value. It is carried out by a wide range of established industries, such as pharmaceuticals, manufacturing and agriculture, as well as a wide range of comparatively new ones, such as aquaculture, bioremediation, biomining, biomimetic engineering and nanotechnology. The benefits of bioprospecting have emerged from such a wide range of organisms and environments worldwide that it is not possible to predict what species or habitats will be critical to society or industry in the future. The benefits include an unexpected variety of products that include chemicals, genes, metabolic pathways, structures, materials and behaviors. Contemporary bioprospecting has multiple goals, including the conservation of biodiversity, the sustainable management of natural resources and economic development. Ecologists are involved in three vital ways: first, applying ecological principles to the discovery of new resources. In this context, natural history becomes

a vast economic database. Second, carrying out field studies, most of them demographic, to help regulate the harvest of wild species. Third, it emphasizes the profound importance of millions of mostly microscopic species to the global economy.

6.3.1. Active principles of the genus *Parmotrema A. Massal.*

The cortical substances produced by the lichens as a result of the polyketide synthase (PKS) pathway cause a zone of inhibition to the contagious organisms. The foliose lichens of the Parmeliaceae family appear as a rosette of the thallus in the substratum and have a delimited area in the substratum, free from fungal and bacterial pathogens observed with a distinct colour around the thallus. The cortical substances produced as secondary metabolites play a vital role in the formation of this zone. Due to their antibacterial, anticancer, and immune-suppressive characteristics, many polyketides are significant clinically. Additionally, they play a crucial role in developing organisms by promoting communication and competition for substrates (Khosla, 2009; Upreti *et al.*, 2005). Depsides, Depsidones, Dibenzofurans, and Depsones are among Lichens' distinctive polyketide secondary metabolites (Pizarro *et al.*, 2020). The depside and depsidone series compounds of polyketide origin accumulate in lichen thalli's cortical or medullary layers. Despite the taxonomic and ecological significance of lichen chemistry many of these natural products are of medical, industrial, and agricultural importance. The parmaloid lichens are significant in the production of these compounds. *Parmotrema austrosinensis* is a good biocontrol agent against phytopathogenic fungi *Fusarium oxysporum. Parmotrema grayanum*, *P. tinctorum*, and *P. reticulatum* can also used as a biocontrol agent against *Fusariumo xysporum* in commercial cultivation of various capsicum species (Furmanek *et al.,* 2022).

6.3.2. TLC Analysis for the Potential Bioactive Compounds Present in the Genus *Parmotrema A. Massal.*

Atranorin, Salazinic acid, Lecanoric acid and Gyrophoric acid are secondary metabolites commonly found in *Parmotrema* and belong to the compound class Depsides. Atranorin has analgesic, anti-inflammatory, antibacterial, antifungal,

cytotoxic, antioxidant, antiviral, and immune-modulatory properties (Studzinska-Sroka *et al.*, 2017). Gyrophoric acid inhibits topoisomerase 1 activity and cell cycle arrest, reduces cell survival, and encourages apoptosis, making it an excellent anticancer medication. Gyrophoric acid has cytostatic qualities, which means that its biological functions and potential medicinal value may go beyond its effects on cancer cells and be relevant to any process where cell proliferation and-differentiation are regulated (Mohammadi *et al.*, 2022). Lecanoric acid is an antioxidant categorized as a polyphenol and a didepside. Lecanoric acid may be a potential new candidate for anti-cancer therapy because it has anti-proliferative effects on cancer cell lines and does not affect primary immune cells (Luo *et al.*, 2010). Salazinic acid and protocetraric acid are classified as depsidone. Salazinic acid and protocetraric acid were found to have strong anticancer activity toward both cell lines with IC (50) values ranging from 35.67 to 60.18μg/ml, and salazinic acid had stronger antioxidant activity than protocetraric acid (Manojlović *et al.*, 2012).

Lichens are the largest producer of primary (intracellular) and secondary (extracellular) chemicals such as polyphenols, flavanoids in the fungal kingdom. These compounds have a variety of biological functions (Richardson, 1988; Hertog *et al.,* 1994; Manach *et al.*, 2004; Maleki *et al.,* 2019). However, compared to other fungus, our understanding of the biological potential of many lichens and their metabolites is quite limited. The species-level richness of the vast spectrum of potential lichen substances makes the genus *Parmotrema* a resourceful target for bioprospection. The determination of biodiversity hotspots of potential lichens and their niche are essential for the sustainable utilization of these lichens. The studies of lichen substances will provide leads to phytoremediation sustainable environment, novel merchandise, and medicine. The recent developments in the field of bioprospection will provide new opportunities in natural product research. The present study provides important baseline information and the presence of many important *Parmotrema*; this will help in the long-term monitoring of lichens, besides acting as an essential document in planning conservation efforts for the nation.

6.3.3. GC-MS Analysis of Selected Species of the Genus *Parmotrema A. Massal.*

GCMS analysis of the selected lichens of the genus *Parmotrema* has listed more than hundred compounds and many of them were reported with promising pharmaceutical applications. The GCMS analysis has listed the volatile compounds present in the genus *Parmotrema*. Orcinol is a major compound identified in these species. It found in *P. austrosinensis, P. clavuliferum* and *P. reticulatum.* Orcinol is a 5-alkylresorcinol in which the alkyl group is specified as methyl. It has a role as an Aspergillus metabolite. It is a 5-alkylresorcinol and a dihydroxytoluene. Orcinol is a strong anti-oxidant and has significant anti-microbial properties. Benzoic acid and Benzaldehyde are the ubiquities compounds in the entire presently studied GC MS spectrum. They are used as anti-microbial agent and as a food additive which can simultaneously impair the fresh aroma of food and also increase the shelf life of meat and cooked food .Based on consumption; benzaldehyde is the second most important molecule in the flavour industry. The annual world production of synthetic and natural benzaldehyde is 7000 tons and 100 tons, respectively (Brenna *et al.*, 2016). Benzaldehyde is one of the most important aromatic aldehydes, having considerable industrial use in perfumery and pigment processing (Haffenden *et al.*, 2001).It is also an essential intermediate for many pharmaceutical products (Chen *et al.*, 2014).This molecule has also been reported as the best repellent for driving bees (Townsend, 1963) and is also known for anti-tumour (MacEwen, 1986), antibacterial, and antifungal activities (Lee *et al.,* 2014). Moreover, the present supply of benzaldehyde is met by synthetic route and chemical reactions from bitter almond oil containing fruit kernels or natural cinnamon oil. This process often produces harmful by-products

Ethyl 2,4-dihydroxy-6-methylbenzoate is a compound only found in *P. austrosinensis*. It has anti-microbial properties and a natural aroma, hence widely used as a food additive and preservative. Squalene is only present in *P. stuppeum* which is an important phytosterol with remarkable anti-microbial properties. Squalene is a 30-carbonpolyprenyl compound including 6 isoprenoids. Squalene, which is structurally similar to beta-carotene, is an intermediate product in

cholesterol synthesis. Squalene and associated compounds, oxidosqualene and bis-oxidosqualene, are the precursors of approximately 200 triterpenes (Xu *et al.*, 2004). Squalene has high applications in the cosmetic industry. Squalene is one of the most important hydrating agents in nature (Huang *et al.*, 2009). It rapidly and effectively penetrates into the skin and provides healthy elasticity and regaining of flexibility without leaving a greasy residue.

6.3.4 Quantification of Total Phenols and Flavonoids in Selected Lichens of the Genus *Parmotrema*A. Massal.

Parmotrema clavuliferum is the species that shows the highest value of phenols and flavonoids in the genus *Parmotrema*, and that is 172.32mg GAE/g and 82.8 mg QE/g, respectively. Plant phenolics are generally involved in defense against ultraviolet radiation or aggression by pathogens, parasites and predators, as well as contributing to plants' colours. They are ubiquitous in all plant organs and are, therefore, an integral part of the human diet. Phenols are chemical compounds featuring one or more aromatic rings each with one or more hydroxyl groups. These compounds are widely found throughout the plant kingdom and are among the most prevalent secondary metabolites in plants. Over 8,000 distinct phenolic structures have been identified, ranging from basic molecules like phenolic acids to complex, polymerized forms such as tannins (Dai and Mumper, 2010). Phenol compounds in lichens play a crucial role in their biology and have a variety of practical applications. These compounds are involved in the lichen's interactions with its environment, and their unique properties make them valuable for several uses in research, medicine, and industry.

Although polyphenols have been widely distributed in lichens for a long time, their health impacts have only recently gained attention from nutritionists. Interest in these compounds has grown due to their strong antioxidant properties, their presence in many dietary sources, and their potential role in preventing diseases linked to oxidative stress. Research, including epidemiological studies as well as in vitro and in vivo experiments, supports the benefits of polyphenols in reducing the risk of cardiovascular diseases, neurodegenerative disorders, and cancer, leading to specific

dietary recommendations. Additionally, polyphenols have been found to influence the activity of various enzymes and cell receptors, offering a range of biological effects beyond their antioxidant capabilities, which may aid in both the prevention and treatment of various health conditions.

Phenolic compounds, such as usnic acid and atranorin, absorb UV light, protecting lichens from harmful solar radiation. This ability helps lichens survive in exposed environments with high UV exposure. Atranorin is a common secondary metabolite in the genera *Parmotrema*. These compounds are also responsible for the antimicrobial properties, protecting lichens from pathogens such as bacteria and fungi. Their antimicrobial properties help prevent infections and contribute to the lichen's resilience. In addition to this as a response to extreme conditions, such as drought or high temperatures, phenols help lichens adapt by stabilizing cellular structures and mitigating stress-induced damage. *Parmotrema reiculatum* is the species with highest concentration of Usnic acid and the Ecological assessment of *Parmotrema* in Wayanad district records *Parmotrema tinctorum* with highest IVI value.

Flavonoids are secondary metabolites commonly found in plants, fruits, and seeds, contributing to their color, fragrance, and flavor. In plants, flavonoids serve a variety of important roles, including regulating cell growth, attracting pollinator insects, and providing protection against both biotic (such as pests and diseases) and abiotic (such as UV radiation and drought) stresses. (Terahara, 2015; Rodríguez *et. al.,* 2020) For instance, plant flavonoids can operate as signal molecules, UV filters, and reactive oxygen species (ROS) scavengers and have several functional roles in drought, heat, and freezing tolerance. These roles may be modified in lichens and the flavonoids in lichens are mainly for regulating the symbiotic association by preventing the over growth of phycobiont, release oxidative stress and protect from UV rays. In lichens the production of phenols is often noticed as the inherent property of fungi as they do when they are out of the symbiosis.

6.3.5. Anti-oxidant Properties of the Genus *Parmotrema A. Massal*.

The genus *Parmotrema* is well-known for its diverse secondary metabolites and notable antioxidant properties. These metabolites are vital for the lichens' survival and have sparked interest due to their potential applications in health and industry. The secondary metabolites in *Parmotrema* lichens, such as usnic acid, various lichen acids (e.g., atranorin, protocetraric acid, salazinic acid), leprolone, orcein, vulpinic acid, evernic acid, polysaccharides, and glycoproteins, are key contributors to their antioxidant activity. This activity helps lichens defend against environmental stressors and oxidative damage.

Ongoing research into these compounds aims to better understand their mechanisms and explore their applications further. Seven *Parmotrema* species were selected for large-scale antioxidant assays, and their IC50 values were measured. The antioxidant properties of these lichens hold significant health benefits, potentially aiding in the prevention and management of chronic diseases related to oxidative stress, such as cardiovascular diseases, cancer, and neurodegenerative disorders. Additionally, the antioxidant capacity of lichens is utilized in food preservation and cosmetics, where it helps extend shelf life and protect the skin from oxidative damage.

Parmotrema lichens contain various secondary metabolites, including phenolic compounds, flavonoids, and terpenoids, which are crucial for their antioxidant properties. Phenolics neutralize free radicals through hydrogen atom donation or electron transfer, thereby reducing oxidative damage to cellular components like lipids, proteins, and DNA. Studies show that lichen-derived phenolics effectively scavenge reactive oxygen species (ROS) and prevent oxidative stress-induced cellular damage. Flavonoids, another important class of antioxidants in lichens, chelate metal ions and inhibit oxidative enzymes, further enhancing their antioxidant activity and modulating cellular signalling pathways involved in oxidative stress responses.

6.3.6. Anti-microbial Properties of the genus *Parmotrema A. Massal.*

Controlling the pathogenic microorganisms in the biological system is essential for the well-being and survival of mankind. The pharmaceutical industry has derived so many formulations to prevent diseases caused by pathogenic bacteria and fungi and these compounds are known as antibiotics. Most of the antibiotics are associated with side effects, and sometimes microbes become resistant (superbugs) to these therapeutic formulations and often become worthless. In contrast, the rising demand for and use of antibiotics has led to higher levels of these drugs in natural environments. This increased presence has driven natural selection and adaptation, resulting in mutations of antibiotic resistance genes within bacterial populations. The spread of these resistance genes in ecosystems has led to the development of antibiotic-resistant bacteria, which are causing a range of global antibiotic-resistant diseases. The solution lies in an ecological perspective on antibiotic resistance and emphasizes that are no longer effective against current treatments. (Chin *et. al*., 2023)

The results of our study indicate that the genera *Parmotrema* exhibits significant antimicrobial activity against *Staphylococcus aureus, E. coli,* and *Klebsiella pneumoniae*. These findings are suggesting that lichens are a promising source of bioactive compounds with potential therapeutic applications. The antimicrobial effects observed can be attributed to the presence of specific bioactive compounds in *Parmotrema*. Key compounds such as atranorin, salazinic acid and other secondary metabolites are known for their antimicrobial properties. Usnic acid, in particular, is a well-documented antimicrobial agent, effective against a range of microorganisms, including *S. aureus*. The inhibition of *bacterial* growth by *Parmotrema* likely results from these compounds disrupting bacterial cell membranes or interfering with metabolic processes. It is effective against a broad range of bacteria, fungi, and viruses. In comparison to traditional antibiotics, the antimicrobial activity of *Parmotrema* against *Staphylococcus aureus, E. coli, and Klebsiella pneumoniae* could provide alternative or complementary treatment options. With the increasing issue of antibiotic resistance, natural products such as those derived from lichens

offer a valuable reservoir of novel antimicrobial agents. The effectiveness of *Parmotrema* suggests that it could be considered for further development as a natural antimicrobial agent, potentially reducing reliance on synthetic antibiotics.

The anti fungal properties of Lichens of *Parmotrema* the highest zone of inhibition against the pathogenic fungi *Penicillium citrinum* was resulted by *P. cristiferum* and *P. reticulatum, P. crinoids and P. tinctorum* show the highest value of Antifungal properties against *Aspergillus niger.*

The anti bacterial and the anti fungal properties of the genus *Parmotrema* show almost similar results in case of the species that is showing the greatest inhibition in growth. It indicates the medicinal properties of the different species of the genera are the result of the secondary metabolites produced as a result of the symbiotic association of the fungal and algal components. Thus lichen substances have enormous pharmaceutical interest, which are the biological wealth associated with the biodiversity of the genera *Parmotrema*.

Secondary metabolites produced by *Parmotrema* lichens represent a promising source of novel drug leads. Despite their significant potential, the diverse biological activities of lichens—ranging from anticancer and antimicrobial to antioxidant, anti-inflammatory, analgesic, antipyretic, and anti parasitic effects—have not been fully explored or utilized. The beauty and importance of these compounds are often underappreciated, highlighting a need for further research to unlock their therapeutic potential. (Adenubi *et al*., 2022)

In conclusion, our study demonstrates that *Parmotrema* has substantial antimicrobial activity against *pathogenic microbes*, which may be attributed to its bioactive compounds. This supports the potential of lichens as sources of new antimicrobial agents, especially in the context of rising antibiotic resistance. However, further research is necessary to fully understand the mechanisms involved and to explore the untapped potential of *Parmotrema* in clinical level.

CHAPTER 7
SUMMARY AND CONCLUSIONS

7. Summary and Conclusion

The high-altitude regions of Kerala, part of the Western Ghats, are rich in biodiversity and species richness of the lichens thus considered suitable habitats for macrolichens. Even though a comprehensive study for the assessment of lichen diversity is lacking. The present study gathers the species of the genus *Parmotrema* A. Massal. of Kerala with the aim of bioprospection at phytchemical and species level. We have identified twenty-nine species come under the genus *Parmotrema.* The present study is the pioneer work to analyze the genus *Parmotrema*'s diversity and rediscover *Parmotrema margaritatum,* which was last reported by Awasthi (1976). This species was originally collected by Sidgwick in 1918 from Karnataka and this taxon was categorized as a rare species by Divakar and Upreti in the book parmelioid lichens of India. We have located the distribution of these threatened taxa from two locations in the Idukki district of Kerala: Pettimudi village and the Eravikulam National Park. *Parmotrema abnuens* is another species with ecological interest and is listed as vulnerable. This species was first reported in India in 1842, and in 2012, it was also reported from Uttarakhand. The present study helps add *Parmotrema abnuens* to Kerala's species list. *Parmotrema tsavoense* is another species listed as a rare species and reported from Rajamala hills of Munnar Kerala and these species became untraceable for decades from Kerala. We have rediscovered this species from Thirunelly forest In the Wayanad district of Kerala.

Parmotrema clavuliferum is an excluded taxon from the genus *Parmotrema* by Divakar and Upreti, and it was included in the genus Rimelia and synonymised with *Rimelia reticulata* (Taylor) Hale & Fletcher. The reticulate maculate condition of *Parmotrema clavuliferum* resembles *Rimelia reticulate,* but the former has an erhizinate marginal zone, densely ciliate margins and sorediate margins with a white lower surface. The latter one has soralia marginal to submarginal and shape linear or capitate, but the former has capitates soralia. The recent molecular studies also corroborate the reinstating of *Parmotrema clavuliferum* in the genus *Parmotrema.* The present study has reported the presence of *Parmotrema clavuliferum* in Kerala for the first time, and hence the species *Parmotrema clavuliferum* is a new report to

Kerala, and it is registered as the 54th species of lichen belongs to the genus *Parmotrema.* This species is found in the Idukki and Wayanad districts of Kerala. Based on the field-based assessment of the diversity of the genus *Parmotrema*, the Wayanad and Idukki districts of Kerala observed maximum diversity and continuous distribution of species with a diversity of the species. Both the districts were thoroughly studied to analyze the alpha diversity of the genus *Parmotrema* in various altitudinal zones and the Importance Value Index of each species in the study area. In the Wayanad district, in all three zones of altitude, *Parmotrema tinctorum* is the species with the higher IVI value. In the Idukki district, in zone 1, *Parmotrema reticulatum* has the maximum IVI value, followed by *Parmotrema tinctorum.* In zone 2, *Parmotrema tinctorum* has the maximum IVI value, and in zone 3, *Parmotrema tinctorum* has the maximum IVI value, followed by *Parmotrema reticulatum.* Neelimala and Puthoorvayal in Wayanad show the maximum alpha diversity of the genus *Parmotrema.* In the Idukki district, the Gundumalai region near Anamudi has maximum alpha diversity. The ultimate aim of the ecological and population study of the genus *Parmotrema* has the twin objectives of the conservation of biodiversity and the successful utilization of these bioresources sustainably. During the study, QGIS maps were prepared showing the natural habitat of each species in Kerala. These graphs will be suitable for future biodiversity assessment and bioprospection.

Lichens are the largest producer of primary (intracellular) and secondary (extracellular) chemicals in the fungal kingdom. These compounds have a variety of biological functions. However, our understanding of many lichens' biological potential and metabolites is quite limited compared to other fungi. The species-level richness of the vast spectrum of potential lichen substances makes the genus *Parmotrema* a resourceful target for bioprospection. Determining biodiversity hotspots of potential lichens and their niche is essential for the sustainable utilization of these lichens. The studies of lichen substances will lead to a phytoremediation and sustainable environment, novel merchandise, and medicine. The recent developments in the field of bioprospection will provide new opportunities in natural product research. The present study provides essential baseline information

and the presence of many important *parmotrema*; this will help in the long-term monitoring of lichens, besides acting as an essential document in planning the nation's conservation efforts.

The ethnomedicinal properties of the *Parmotrema* lichens were considered, and these ethnic practices were validated. The selected samples underwent various biochemical assays to determine the active compounds in these groups of lichens. The GCMS analysis shows the presence of Orcinol, Benzoic acid Benzaldehyde, etc. These compounds are industrially and pharmaceutically relevant. Benzoic acid is an Antimicrobial Food Additive (Lück and Jager, 1997). The anti-bacterial and anti-fungal properties of the genus *Parmotrema* were studied, and the application of this genus as an anti-microbial agent was proved. This group of foliose lichens is widely used in the traditional cuisines of the regions with the twin objectives, either flavoring agents or preservatives, where these lichens are traditionally found. In addition to this, the ethnic stakeholders of lichen habitats are using these lichens in their ethnic pharmacopoeia. The present study has validated these ethnic disease healing practices. This lichen contains active principles against pathogenic bacteria and fungi, significant anti-oxidant properties, anti-diabetic and anti-carcinogenic properties, and insect-repellant properties.

The antioxidant properties of *Parmotrema* lichens are closely linked to their secondary metabolites, including salazinic acid, lecanoric acid, gyrophoric acid, atranorin, and usnic acid and other bioactive compounds. These metabolites play a critical role in protecting the lichen from oxidative damage and contribute to its overall resilience. Understanding the correlation between antioxidant activity and secondary metabolites not only highlights the biological significance of these compounds but also opens avenues for their application in medicine, cosmetics, and food industries. Continued research into the antioxidant mechanisms of *Parmotrema* lichens will further elucidate their potential benefits and applications.

The pollution and habitat loss aided by large-scale construction and urbanization are causing a severe threat to the foliose lichens. As they are sensitive to environmental changes, anthropogenic activities will lead to the loss of lichens. This permanent

loss of lichens will affect the well-being of the ecosystem. Lichens cannot be considered as a negligible component in the ecosystem. They are more than competent to hold their own in the great struggle for existence. At the same time, they are so well adapted to the scorching sunlight and temperature. Indeed, many of the much-studied and much-praised higher plants owe their very existence to the lichens.

Let us obtain a better insight into these plants so that we may judge them more fairly and as-sign them to their proper position in the world of life and duly credit them with the grand work they are performing. A lichen deserves recognition as the coconut tree upon which it grows or the reindeer whose life it sustains.

CHAPTER 8
RECOMMENDATIONS

8. RECOMMENDATIONS

Parmotrema A. Massal. is the largest lichen genera of the family Parmeliaceae and is distributed throughout the tropical and subtropical regions of the globe. The genus comprises more than three hundred species, and 54 species so far reported from India. The taxonomic complexity arises due to all the members of the genus having green algae similar to phycobiont. The diversity of this genus arises mainly due to the diversity of the mycobiont. Due to this, the members are morphologically similar but possess significant diversity in the secondary metabolites or the high-value lichen substances and their physiology. This property often provides challenges and opportunities. Molecular techniques in species delimitation can effectively solve the challenges arising from morphological similarity. The molecular technique and the effective protocols for the isolation of the DNA of the fungal DNA without contamination by the algal DNA and the extraction of the DNA from the phenol and flavonoid-rich thallus are lacking. The techniques to isolate the lichen DNA can be applied in lichen taxonomy and biotechnology. The pollution-sensitive nature of lichens has ecological significance, and leafy lichen can be used to assess pollution; such a lichen-based technique was not yet standardized; lichens are actively monitored to provide inferences about pollution levels in the environment where they live. More than 700 lichen substances are known today; most are pharmaceutically and industrially important. The ethnobotanical therapeutic practices use these lichens for many diseases and other aspects of life. Validation of this time-tested wisdom requires evaluating the medicinal properties using in-vitro methods and identifying the active principle responsible for the particular property. Hence, the pharmacological studies on lichens with ethnobotanical significance will provide novel drugs. The conservation of lichens requires immediate attention. Conservation of lichen primarily is by protecting the lichen habitat free from atmospheric pollution beyond the permitted levels. The assessment of diversity and equipping the inhabitants for conservation by providing adequate knowledge about the lichens require dedicated lichen conservatories as a part of protected areas and national parks. In the scenario of frequent and catastrophic landslides and flash floods that devastated Kerala, the biogeographic data of lichens can be used as the

baseline data for comparing pre- and post-catastrophic ecosystems and for damage assessment and mitigation. Kerala Part of the Western Ghats is regarded as a suitable habitat for lichens of different growth forms. Still, a comprehensive lichen diversity assessment comprising different lichen habitats for the Kerala state for the development of a lichen database will be very much needful for the further development of lichenology of our state and nation.

REFERENCES

8. References

Abdulla, S.T., Hamid, H., Ali, M., Ansari, S.H. and Alam, M.S., 2007. Two new terpenes from the lichen *Parmelia perlata.* Indian Journal of Chemistry, Section B, Organic and Medicinal Chemistry 46(1), pp. 173-176.

Adamo, P. and Violante, P., 2000. Weathering of rocks and neogenesis of minerals associated with lichen activity. *Applied Clay Science*, *16*(5-6), pp.229-256.

Adenubi, O.T., Famuyide, I.M., McGaw, L.J. and Eloff, J.N., 2022. Lichens: An update on their ethnopharmacological uses and potential as sources of drug leads. *Journal of Ethnopharmacology*, *298*, p.115657.

Ahmadjian, V.A., and Hale, M.E., 1973, "The Lichens," Academic Press, New York and London.

Ahn, C., Moon, K. H. 2016. *Parmotrema clavuliferum* and *P. reticulatum* are independent species. *Journal of Species Research*.5: 254–260.

Airaksinen, M.M., Peura, P. and Antere, S., 1986. Toxicity of Iceland lichen and reindeer lichen. In Toxic Interfaces of Neurones, Smoke and Genes: Proceeding of the European Society of Toxicology Meeting Held in Kuopio, June 16–19, 1985 (pp. 406-409). Springer Berlin Heidelberg.

Andersen, A., 2006. Final report on the safety assessment of benzaldehyde. *International journal of toxicology*, *25*, pp.11-27.

Anilkumar, A., Sequeira, S., Christy, A. and Arsha, S.M., 2022. Macrolichens of Mathikettan Shola National Park, Western Ghats: a preliminary investigation with some new records. *Journal of Threatened Taxa*, *14*(1), pp.20400-20405.

Aparna, V., Dileep, K.V., Mandal, P.K., Karthe, P., Sadasivan, C. and Haridas, M., 2012. Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. *Chemical biology & drug design*, *80*(3), pp.434-439.

Aslan, A., Çiçek, A., Yazici, K., Karagöz, Y., Turan, M., Akkus, F. and Yildirim, O.S., 2011. The assessment of lichens as bioindicator of heavy metal pollution from motor vehicles activites. *African Journal of Agricultural Research*, *6*(7), pp.1698-1706.

Awasthi, D. D. 1976. Lichen genus *Parmelia* in India I – Subgenera *Parmelia* and *Amphigymnia*. *Biological Memoirs*, *Lichenology Series* 1: 155–229.

Awasthi, D.D., 1991. A key to the microlichens of India, Nepal and Sri Lanka. *Bibliotheca Lichenologica* 40: 1-337.

Awasthi, D.D., 2000. A Hand Book of Lichens. Shiva Offset Press, Lucknow, India: 87-92

Awasthi, D.D., 2007. A compendium of the macrolichens from India, Nepal and Sri Lanka. Bishen Singh and Mahendera Pal Singh, Dehradun, India.

Baba, S.A. and Malik, S.A., 2015. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. *Journal of Taibah university for science*, *9*(4), pp.449-454.

Bačkorová, M., Bačkor, M., Mikeš, J., Jendželovský, R. and Fedoročko, P., 2011. Variable responses of different human cancer cells to the lichen compounds parietin, atranorin, usnic acid and gyrophoric acid. *Toxicology in vitro*, *25*(1), pp.37-44.

Balaji P., Hariharan G.N., 2004. Lichen diversity and its distribution pattern in Tropical Dry Evergreen Forest of Guindy National Park (GNP), Chennai. – Indian Forester 130: 1155–1168.

Balaji, P. and Hariharan, G.N., 2007. In vitro antimicrobial Activity of *Parmotrema praesorediosum* thallus extracts. Research Journal of Botany 2(1): 54-59, 2007.

Balaji, P. and Hariharan, G.N., 2013. Checklist of microlichens in Bolampatti II forest range (Siruvani Hills), Western Ghats, Tamil Nadu, India. *Czech Mycology*, *65*, pp.219-232.

Balaji, P., Prithiviraj, B., Ramanathan, V.,i Mohapatra, T., and Hariharan, G.N., 2006. Lichen Bioprospecting - Future Prospects. Journal of Swamy Botanical Club. 23. pp. 81-90.

Baral, B., 2015. Enumeration of lichen diversity in Manaslu conservation area and Sagarmatha National Park of Nepal. *International Journal of Biodiversity and Conservation*, *7*(3), pp.140-147.

Bawingan, P.A., Lardizaval, M.P., Rosuman, P.F., Fajardo, W.T., Azuelo, A., Elix, J.A. and HUR, J., 2017. Philippine species of Parmotrema (Ascomycota, Parmeliaceae). *Philipp J Sci*, *146*(2), pp.145-158.

Beattie, A.J., Hay, M., Magnusson, B., de Nys, R., Smeathers, J. and Vincent, J.F., 2011. Ecology and bioprospecting. *Austral ecology*, *36*(3), pp.341-356.

Bhat, M.., Shukla, V. Upreti, D.K., Verma, S., Sharma, G. and Anand, R., 2014. Assessment of air quality of Rajouri town, Jammu & Kashmir, using lichen transplant technique. *Sci Technol*, *2*(1), pp.15-19.

Biju, H., Bagool, R.G. and Nayaka, S., 2010. Additions to the lichen flora of Kerala State I: Parmelioid macro-lichens. *Journal of Economic and Taxonomic Botany*, *34*(4), pp.890-897.

Biju, H., Bagool, R.G., and Nayaka, S., 2014. Diversity of lichens in Idukki district with new records to flora of Kerala. 37(3):333–340

Blanco, O., Crespo, A., Elix, J.A., Hawksworth, D.L. and Thorsten Lumbsch, H., 2004. A molecular phylogeny and a new classification of parmelioid lichens containing Xanthoparmeliatype lichenan (Ascomycota: Lecanorales). *Taxon*, *53*(4), pp.959-975.

Blanco, O., Crespo, A., Ree, R.H. and Lumbsch, H.T., 2006. Major clades of parmelioid lichens (Parmeliaceae, Ascomycota) and the evolution of their morphological and chemical diversity. *Molecular Phylogenetics and Evolution*, *39*(1), pp.52-69.

Bolton, E.M., 1991. *Lichens for vegetable dyeing*. Julia Bolton Holloway.

Boruah, T., Devi, H. and Sarkar, S., 2024. Lichen as Bio Indicators. *Chemistry, Biology and Pharmacology of Lichen*, pp.289-304.

Boustie, J. and Grube, M., 2005. Lichens—a promising source of bioactive secondary metabolites. *Plant Genetic Resources*, *3*(2), pp.273-287.

Brand-Williams, W., Cuvelier, M.E. and Berset, C.L.W.T., 1995. Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology*, *28*(1), pp.25-30.

Brenna, E.; Fronza, G.; Fuganti, C.; Gatti, F.C., 2016, Serra, S. Biotechnological Tools to Produce Natural Flavours and Methods to Authenticate Their Origin. In *Innovation in Food Engineering*: New Techniques and *Products*; Passos, M.L., Ribeiro, C.P., Eds.; CRC Press, Taylor & Francis, 81–105.

BrijLal and Upreti 1995. Ethnobotanical notes on three Indian lichens. Lichenologist 27(1): 77- 79.

Bungartz, F. and Spielmann, A.A., 2019. The genus *Parmotrema* (Parmeliaceae, Lecanoromycetes) in the Galapagos Islands. *Plant & Fungal Systematics*, *64*(2).

Candan, M., Yılmaz, M., Tay, T., Erdem, M. and Türk, A.Ö., 2007. Antimicrobial activity of extracts of the lichen *Parmelia sulcata* and its salazinic acid constituent. *Zeitschrift für Naturforschung C*, *62*(7-8), pp.619-621.

Cetin, H., Tufan-Cetin, O., Turk, A.O., Tay, T., Candan, M., Yanikoglu, A. and Sumbul, H., 2008. Insecticidal activity of major lichen compounds,(−)-and (+)-usnic acid, against the larvae of house mosquito, *Culex pipiens* L. *Parasitology research*, *102*, pp.1277-1279.

Cetin, H.., Tufan-Cetin, O., Turk, A.O., Tay, T., Candan, M., Yanikoglu, A. and Sumbul, H., 2012. Larvicidal activity of some secondary lichen metabolites against the mosquito *Culiseta longiareolata* Macquart (Diptera: Culicidae). *Natural product research*, *26*(4), pp.350-355.

Chandra, S. and Singh, A., 1971. A lichen crude drug (chharila) from India. *J Res Indian Med*, *6*, pp.209-15.

Chen, H. and Ji, H., 2014. Effect of substitution degree of 2-hydroxypropyl-β-cyclodextrin on the alkaline hydrolysis of cinnamaldehyde to benzaldehyde. *Supramolecular Chemistry*, *26*(10-12), pp.796-803.

Chin, K.W., Tiong, H.L.M., Luang-In, V. and Ma, N.L., 2023. An overview of antibiotic and antibiotic resistance. *Environmental Advances*, *11*, p.100331.

Christy, A., Mohan, A.S., Anilkumar, A. and Sequeira, S., 2022. Diversity of Macrolichens of Wayanad district, Kerala, India with some New Records. *Cryptogam Biodiversity and Assessment*, *6*(01), pp.17-26.

Cole G.M., Lim G.P., Yang F., Teter B., Begum A., Ma Q., Harris-White M.E., Frautschy S.A.,2005, Prevention of Alzheimer's disease: Omega-3 fatty acid and phenolic anti-oxidant interventions. *Neurobiol. Aging.* 1:133–136.

Condò, C., Anfelli, I., Forti, L., Sabia, C., Messi, P. and Iseppi, R., 2023. Lichens as a Natural Source of Compounds Active on Microorganisms of Human Health Interest. *Applied Sciences*, *13*(3), p.1976.

Crawford, M.J. and Gabriel, J.M., 2019. *Drugs on the Page:* Pharmacopoeias and Healing Knowledge in the Early Modern Atlantic World. University of Pittsburgh Press.

Crespo, A., Gavilán, R., Elixt, J.A. and Gutiérrez, G., 1999. A comparison of morphological, chemical and molecular characters in some parmelioid genera. *The Lichenologist*, *31*(5), pp.451-460.

Crespo, A., Kauff, F., Divakar, P.K., Del Prado, R., Pérez-Ortega, S., de Paz, G.A., Ferencova, Z., Blanco, O., Roca-Valiente, B., Nunez-Zapata, J. and Cubas, P., 2010. Phylogenetic generic classification of parmelioid lichens (Parmeliaceae, Ascomycota) based on molecular, morphological and chemical evidence. *Taxon*, *59*(6), pp.1735-1753.

Crespo, A., Lumbsch, H. T., Mattsson, J.-E., Blanco, O., Divakar, P. K., Articus, K., Wiklund, E., Bawingan, P. A. & Wedin, M., 2007. Testing morphology-based hypotheses of phylogenetic relationships in Parmeliaceae (Ascomycota) using three ribosomal markers and the nuclear RPB1 gene. Molecular Phylogenetics and Evolution 44: 812–824.

Curtis, J. T. , McIntosh, R. P.,1950.The interactions of certain analytic and synthetic phytosociological characters.Ecology 31:434-455.

Dai, J. and Mumper, R.J., 2010. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, *15*(10), pp.7313-7352.

Del Olmo, A., Calzada, J. and Nuñez, M., 2017. Benzoic acid and its derivatives as naturally occurring compounds in foods and as additives: Uses, exposure, and controversy. *Critical reviews in food science and nutrition*, *57*(14), pp.3084-3103

Din, L.B., Zakaria, Z., Samsudin, M.W. and Elix, J.A., 2010. Chemical profile of compounds from lichens of Bukit Larut, Peninsular Malaysia. *Sains Malaysiana*, *39*(6), pp.901-908.

Divakar, P.K. and Upreti, D.K., 2003. New species and new records of Parmotrema (Parmeliaceae) from India. *The Lichenologist*, *35*(1), pp.21-26.

Divakar, P.K. and Upreti, D.K., 2005. Parmelioid Lichens in India (A revisionary study). Bishen Singh and Mahendera Pal Singh, Dehradun, India.

Divakar, P.K., Blanco, O., Hawksworth, D.L. and Crespo, A., 2005. Molecular phylogenetic studies on the Parmotrema reticulatum (syn. Rimelia reticulata) complex, including the confirmation of P. pseudoreticulatum as a distinct species. *The Lichenologist*, *37*(1), pp.55-65.

Dodge, C. W., 1959. Some lichens of Tropical Africa, III. *Parmeliaceae*. *Annals of the Missouri Botanical Garden* 46: 39–193..

Dodge, C.W., 1959. Some lichens of tropical Africa. III. Parmeliaceae. *Annals of the Missouri Botanical Garden*, *46*(1/2), pp.39-193.

Easa, P.S., 2003. Biodiversity documentation of Kerala Part 3: Lichens. Kerala Forest Research Institute, Peechi, Kerala, 61 pp.

Elix JA., 1993. Progress in the generic delimitation of Parmelia sensu lato lichens (Ascomycotina: Parmeliaceae) and a synoptic key to the Parmeliaceae. Bryologist 96: 359–383.

Elix, J.A. and Streimann, H., 1989. lichens of Norfolk Island. 1. Introduction and the family Parmeliaceae. In *Proceedings of the Linnean Society of New South Wales*.

Eschweiler, F. G., 1824. Systema lichenum, genera exhibens rite distincta, pluribus novisadaucta. Norimbergae.

Fernández-Moriano, C., Divakar, P.K., Crespo, A. and Cuadrado, M., 2015, July. Antioxidant and cytoprotective potentials of Parmeliaceae lichens and identification of active compounds. In *Anales de la Real Academia Nacional de Farmacia* (Vol. 81, No. 2).

Francolini I, Norris P, Piozzi A, Donelli G, Stoodley P. Usnic acid, a natural antimicrobial agent able to inhibit bacterial biofilm formation on polymer surfaces. Antimicrob Agents Chemother. 2004 Nov;48(11):4360-5. doi: 10.1128/AAC.48.11.4360-4365.2004. PMID: 15504865; PMCID: PMC525405.

Fries, T. M., 1861. Genera Heterolichenum Europaea Recognita. Uppsala: Edquist.

Furmanek, Ł., Czarnota, P. and Seaward, M.R., 2022. A review of the potential of lichen substances as antifungal agents: the effects of extracts and lichen secondary metabolites on Fusarium fungi. *Archives of Microbiology*, *204*(8), p.523.

Galanty, A., Popiół, J., Paczkowska-Walendowska, M., Studzińska-Sroka, E., Paśko, P., Cielecka-Piontek, J., Podolak, I., 2021. (+)-Usnic acid as a promising candidate for a safe and stable topical photoprotective agent. *Molecules*. *26*(17), 5224.

Galla R, Ferrari S, Ruga S, Mantuano B, Rosso G, Tonello S, Rosa L, Valenti P, Uberti F.,2023. Effects of usnic acid to prevent infections by creating a protective barrier in an in vitro study. International Journal of Molecular Sciences;24(4):3695.

Gandhi, A.D., Sathiyaraj, S., Suriyakala, G., Saranya, S., Baskaran, T.N., Ravindran, B. and Babujanarthanam, R., 2020. Lichens in Genus Parmelia: An overview and their application. *Current Pharmaceutical Biotechnology*, *21*(13), pp.1289-1297.

Ganesan, A., Thangapandian, M., Ponnusamy, P. and Sundaraj, J., 2017. Antibacterial and antioxidant activity of *Parmotrema reticulatum* obtained from Eastern Ghats, Southern India. *Biomed Res*, *28*, pp.1593-1597.

Ginwala R., Bhavsar R., Chigbu D.G.I., Jain P., Khan Z.K., 2019. Potential role of flavonoids in treating chronic inflammatory diseases with a special focus on the anti-inflammatory activity of apigenin. *Antioxidants.*;8:35. doi: 10.3390/antiox8020035.

Goel M, Dureja P, Rani A, Uniyal P L, Laatsch H., 2011. "Isolation, characterization and antifungal activity of major constituents of the Himalayan lichen Parmelia reticulata Tayl. Journal of Agriculture and Food Chemistry; 59:2299-2307.

Goga, M., Elečko, J., Marcinčinová, M., Ručová, D., Bačkorová, M. and Bačkor, M., 2020. Lichen metabolites: an overview of some secondary metabolites and their biological potential. *Co-evolution of secondary metabolites*, pp.175-209.

Gomes, A.T., Honda, N.K., Roese, F.M., Muzzi, R.M. and Sauer, L., 2006. Cytotoxic activity of orsellinates. *Zeitschrift für Naturforschung C*, *61*(9-10), pp.653-657.

Goni, R., Raina, A.K., Magotra, R. and Sharma, N., 2015. Lichen flora of Jammu and Kashmir State, India: an updated checklist. *Tropical Plant Research*, *2*(1), pp.64-71.

González-Tejero, M.R., Molero-Mesa, J., Casares-Porcel, M. and Lirola, M.M., 1995. New contributions to the ethnopharmacology of Spain. *Journal of Ethnopharmacology*, *45*(3), pp.157-165.

Gupta, V.K., Darokar, M.P., Saikia, D., Pal, A., Fatima, A. and Khanuja, S.P.S. 2007. Antimycobacterial activity of lichens. Pharmaceutical Biology 45(3): 200–204.

Haffenden, L.J.W.; Yaylayan, V.A.; Fortin, J., 2001. Investigation of Vibrational Theory of Olfaction with Variously Labelled Benzaldehydes. Food Chemistry, 73(*1*), 67–72.

Hale, M. E., 1965. A monograph of *Parmelia* subgenus *Amphigymnia*. *Contributions from the United States National Herbarium* 36:193–358.

Hale, M. E., 1973. Smithsonian Contributions to Botany. 10:9.

Hale, M. E., 1974. New combinations in the lichen genus *Parmotrema* Massalongo. *Phytologia*28: 334–339.

Hale, M. E., 1987. A monograph of the lichen genus *Parmelia* Acharius *sensu stricto* (Ascomycotina: Parmeliaceae). Smithsonian Contributions to Botany 66: 1–55.

Hale, M.E., 1979. How to know the lichens. WC Brown Co..

Hale, M.E., 1983. The biology of lichens. 3 rd Edition. Edward Arnold Ltd. London.

Hale, M.E., 1987. A Monograph of the lichen genus *Parmelia* Acharirius sensu stricto (Ascomycotina, Parmeliaceae). Smithson. Contr. Bot.66: pp.1-55

Halici M, Odabasoglu F, Suleyman H, Cakir A, Aslan A, Bayir Y., 2005. Effects of water extract of Usnea longissima on antioxidant enzyme activity and mucosal damage caused by indomethacin in rats. Phytomedicine. 12:656–662. doi:10.1016/j.phymed.2004.06.021.

Harikrishnan, A., Veena, V., Lakshmi, B., Shanmugavalli, R., Theres, S., Prashantha, C.N., Shah, T., Oshin, K., Togam, R. and Nandi, S., 2021. Atranorin, an antimicrobial metabolite from lichen *Parmotrema* rampoddense exhibited in vitro anti-breast cancer activity through interaction with Akt activity. *Journal of Biomolecular Structure and Dynamics*, *39*(4), pp.1248-1258.

Hawksworth, D.L. and D.J. Hill, 1984. The lichen-forming fungi. Blackie and son Ltd.

Hekking, W. and Sipman, H.J.M., 2018. Checklist of lichenized and lichenicolous fungi from Colombia.

Hertog M.G., Feskens E. J., Hollman P.C., Katan M.B., Kromhout D.,1994. Dietary flavonoids and cancer risk in the Zutphen Elderly Study. *Nutr. Cancer.* 22:175–184. doi: 10.1080/01635589409514342.

Honegger, R., 1991. Functional aspects of the lichen symbiosis. Annual Review of Plant Physiology and Plant Molecular Biology 42, pp. 553–578.

Huang Z.R., Lin ,Y.K., Fang J.Y., 2009. Biological and pharmacological activities of squalene and related compounds: potential uses in cosmetic dermatology. Molecules. 14: pp.540-554.

Huneck, S, Yoshimura Y., 1996. Identification of lichen substances. Springer, Berlin Heidelberg New York

Huneck, S., Hoefle, G ., 1978. Strukturn und C-NMR Spektroskopie von chlorhaltigenFlechtenxanthonen. Tetrahedron 34:2491–2502

Ismed, F., Dévéhat, F.L.L., Rouaud, I., Ferron, S., Bakhtiar, A. and Boustie, J., 2017. NMR reassignment of stictic acid isolated from a Sumatran lichen *Stereocaulon montagneanum* (Stereocaulaceae) with superoxide anion scavenging activities. *Zeitschrift für Naturforschung C*, *72*(1-2), pp.55-62.

Jayalal, U., Divakar, P.K., Joshi, S., Oh, S.O., Koh, Y.J. and Hur, J.S., 2013. The Lichen Genus *Parmotrema* in South Korea. *Mycobiology*, *41*(1), pp.25-36.

Jayapraksha, G.K. and Rao, L.J. 2000. Phenolic constituents from the lichen *Parmotrema stuppeum* (Nyl.) Hale and their antioxidant activity. Zeitschrift für Naturforschung 55(11- 12): 1018-22.

John, E.A., 1992. Distribution patterns and inter thalline interactions of epiphytic foliose lichens. *Canadian Journal of Botany* 70: 818–823

Jones, D., 2019. Lichens and pedogenesis. *Handbook of lichenology*, pp.109-124.

Juan, B., 2017. Bioprospecting and drug development, parameters for a rational search and validation of biodiversity. *J MicrobBiochem Technol*, *9*, p.e128.

Kaur, C. and Kapoor, H.C., 2002. Anti-oxidant activity and total phenolic content of some Asian vegetables. *International Journal of Food Science & Technology*, *37*(2), pp.153-161.

Kekuda, T.P., Ranjitha, M.C., Ghazala, F., Vidya, P. and Vinayaka, K.S., 2015. Antimicrobial activity of selected corticolous macrolichens. *Science, Technology and Arts Research Journal*, *4*(3), pp.169-174.

Kelly GS. Squalene and its potential clinical uses. Altern Med Rev. 1999; 4: 29-36.

Kerboua, M., Monia, A.A., Samba, N., Silva, L., Raposo, C., Díez, D. and Rodilla, J.M., 2022. Phytochemical composition of lichen Parmotremahypoleucinum (J. Steiner) hale from Algeria. *Molecules*, *27*(16), p.5229.

Khosla, C., 2009. Structures and mechanisms of polyketide synthases. *The Journal of organic chemistry*, *74*(17), pp.6416-6420.

Killari, K.N., Polimati, H., Prasanth, D.S., Singh, G., Panda, S.P., Vedula, G.S. and Tatipamula, V.B., 2023. Salazinic acid attenuates male sexual dysfunction and testicular oxidative damage in streptozotocin-induced diabetic albino rats. *RSC advances*, *13*(19), pp.12991-13005.

Kirk, P.M., Cannon, P.F., Minter, D.W. and Stalpers, J.A., 2008. Ainsworth & Bisby's Dictionary of the fungi. CAB International. *Wallingford, United kingdom*.

Kluge, H., Broz, J. and Eder, K., 2006. Effect of benzoic acid on growth performance, nutrient digestibility, nitrogen balance, gastrointestinal microflora and parameters of microbial metabolism in piglets. *Journal of Animal Physiology and Animal Nutrition*, *90*(7-8), pp.316-324.

Koch, N.M., Lucheta, F., Käffer, M.I., Martins, S., De Azevedo, Maria., Vargas, V. and Ferrão, M., 2018. Air quality assessment in different urban areas from Rio Grande do Sul state, Brazil, using lichen transplants. *Anais da Academia Brasileira de Ciências*, *90*, pp.2233-2248.

Kostyuk, V., Potapovich, A., Stancato, A., De Luca, C., Lulli, D., Pastore, S. and Korkina, L., 2012. Photo-oxidation products of skin surface squalene mediate metabolic and inflammatory responses to solar UV in human keratinocytes.

Kukwa, M., Bach, K. Sipman, H.J. and Flakus, A., 2012. Thirty-six species of the lichen genus Parmotrema (Lecanorales, Ascomycota) new to Bolivia. *Polish Botanical Journal*, *57*(1), pp.243-257.

Kuldeep, S. and Prodyut, B., 2015. Lichen as a bio-indicator tool for assessment of climate and air pollution vulnerability. *Int. Res. J. Environ. Sci*, *4*, pp.107-117.

Kumar, K. and Upreti, D.K., 2001. Parmelia spp. (Lichens) in ancient medicinal plantlore of India. Economic Botany 55(3): pp. 458-459.

Kumar, K.C. and Mueller, K., 1999b. Lichen metabolites 2: Antiproliferative and cytotoxic acitivity of Gyrophoric, usnic and diffractaic acid on human keratinocyte growth. J. Nat. Prod. 62(6), pp. 821-823.

Kumar, K.C. and Muller, K. 1999a. Lichen metabolites 1: Inhibitory action against leukotriene B4 biosynthesis by a non-redox mechanism. J. Nat. Prod. 62(6).pp. 817-820.

Kumar, M., 2000. Lichen (macrolichen) flora of Kerala part of Western Ghats. *Kerala Forest Research Institute, Peechi, Thrissur*.

Larsen, R.S., Bell, J.N.B., James, P.W., Chimonides, P.J., Rumsey, F.J., Tremper, A. and Purvis, O.W., 2007. Lichen and bryophyte distribution on oak in London in relation to air pollution and bark acidity. *Environmental pollution*, *146*(2), pp.332-340.

Lawrey, J.D., 1980. Sexual and asexual reproductive patterns in Parmotrema (Parmeliaceae) that correlate with latitude. *Bryologist*, pp.344-350.

Lawrey, J.D., 1986. Biological role of lichen substances. *Bryologist*, pp.111-122.

Lee, H.H., Ahn, J.H., Kwon, A.R., Lee, E.S., Kwak, J.H. and Min, Y.H., 2014. Chemical composition and antimicrobial activity of the essential oil of apricot seed. *Phytotherapy research*, *28*(12), pp.1867-1872.

Lee, K.A. and Kim, M.S., 2000. Glucosidase inhibitor from *Umbelicaria esculenta*. Can. J. Microbiol. 46(11), pp.1077-81.

Llano, G.A., 1948. Economic uses of lichens. *Economic Botany*, *2*(1), pp.15-45.

Lück, E., Jager, M., 1997. Benzoic acid. *Antimicrobial Food Additives: Characteristics· Uses· Effects*, pp.174-182.

Lücking, R., Hodkinson, B.P. and Leavitt, S.D., 2017. The 2016 classification of lichenized fungi in the Ascomycota and Basidiomycota–Approaching one thousand genera. *The Bryologist*, *119*(4), pp.361-416.

Luo, H., Yamamoto, Y., Liu, Y., Jung, J.S., Kahng, H.Y., Koh, Y.J. and Hur, J.S., 2010. The in vitro antioxidant properties of Chinese highland lichens. *Journal of Microbiology and Biotechnology*, *20*(11), pp.1524-1528.

MacEwen, E.G., 1986. Anti-tumor evaluation of benzaldehyde in the dog and cat. *American journal of veterinary research*, *47*(2), pp.451-452.

Maciąg-Dorszyńska, M., Węgrzyn, G. and Guzow-Krzemińska, B., 2014. Antibacterial activity of lichen secondary metabolite usnic acid is primarily caused by inhibition of RNA and DNA synthesis. *FEMS microbiology letters*, *353*(1), pp.57-62.

Mackay, J. T., 1836. Flora Hibernica, comprising the Flowering Plants, Characeae, Musci, Hepaticae, Lichenes and Algae of Ireland. Dublin, London & Edinburgh.

MacKenzie, S., 1952. Ultraviolet absorption spectra of derivatives of usnic acid. *Journal of the American Chemical Society*, *74*(16), pp.4067-4069.

Magaldi, S., Mata-Essayag, S., De Capriles, C.H., Pérez, C., Colella, M.T., Olaizola, C. and Ontiveros, Y., 2004. Well diffusion for antifungal susceptibility testing. *International journal of infectious diseases*, *8*(1), pp.39-45.

Maleki, S.J., Crespo, J.F. and Cabanillas, B., 2019. Anti-inflammatory effects of flavonoids. *Food chemistry*, *299*, p.125124.

Manach, C., Scalbert, A., Morand, C., Rémésy, C. and Jiménez, L., 2004. Polyphenols: food sources and bioavailability. *The American journal of clinical nutrition*, *79*(5), pp.727-747.

Manojlovic, N.T., Vasiljevic, P.J., Maskovic, P.Z., Juskovic, M. and Bogdanovic-Dusanovic, G., 2012. Chemical composition, antioxidant, and antimicrobial activities of lichen Umbilicaria cylindrica (L.) Delise (Umbilicariaceae). *Evidence-Based Complementary and Alternative Medicine*, *2012*(1), p.452431.

Massalongo, A., 1860. Esamecomparativo di alcunigeneri di licheni. *Atti dell'Istituto Veneto di scienze, lettereed arti* 5(3): 247–267, pp.313–337.

Mayer, M., O'Neill, M.A., Murray, K.E., Santos-Magalhães, N.S., Carneiro-Leão, A.M.A., Thompson, A.M. and Appleyard, V.C., 2005. Usnic acid: a non-genotoxic compound with anti-cancer properties. *Anti-cancer drugs*, *16*(8), pp.805-809.

Mehta, V. and Rai, V., 2023. Stochastic modelling and forecasting of relative humidity and wind speed for different zones of Kerala. *MAUSAM*, *74*(4), pp.1053-1064.

Melo, M.J., Nabais, P., Guimarães, M., Araújo, R., Castro, R., Oliveira, M.C. and Whitworth, I., 2016. Organic dyes in illuminated manuscripts: a unique cultural and historic record. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, *374*(2082), pp.20160050.

Mishra, G. and Upreti, D.K., 2017. The lichen genus Parmotrema A. Massal.(Lecanorales, Ascomycota) from India with addition distributional records. *Cryptogam Biodiversity and Assessment*, *2*(02), pp.18-40.

Misra, R., 1968. Ecology work book. Oxford and IBH Publications Co., New Delhi, 244.

Mohammadi, M., Bagheri, L., Badreldin, A., Fatehi, P., Pakzad, L., Suntres, Z. and van Wijnen, A.J., 2022. Biological effects of gyrophoric acid and other lichen derived metabolites, on cell proliferation, apoptosis and cell signaling pathways. *Chemico-biological interactions*, *351*, p.109768.

Monaghan, M.D., 2001. Coats of many colours: dyeing and dye works in classical and Hellenistic Greece. University of Leicester (United Kingdom).

Moon, K.H., Kurokawa, S., Kashiwadani, H., 2001. The genus *Rimelia*(Lichens) from Hawaiian Island. The Journal of Japanese Botany 76: pp.321-328.

Nadkarni, K.M., 1976. Indian materia medica. South Asia Books, India.

Nash, T. H., 1996. Lichen biology: Second Edition, Cambridge University Press, pp 303

Nash, T. H., 2008. Lichen Biology Second Edition. Cambridge, UK: Cambridge University Press 1996. ISBN-13 978-0-511-41407-7/.

Nayaka, S. and Asthana, S., 2014. Diversity and distribution of lichens in India vis a vis its Lichenogeographic Regions. In *Conference: Biodiversity Conservation, Status, Future and Way Forward. National Academy of Biological Science, Chennai* (pp. 79-96).

Nayaka, S. and Haridas, B., 2024. Lichen-Forming and Lichenicolous Fungi of the Western Ghats, India. In *Biodiversity Hotspot of the Western Ghats and Sri Lanka* (pp. 89-107). Apple Academic Press.

Nayaka, S. and Upreti, D.K., 2005. Status of lichen diversity in Western Ghats, India. *Sahyadri E-News, Western Ghats Biodiversity Information System*, *16*, pp.1-28.

Nayaka, S. and Upreti, D.K., 2021. History and Development of Lichen Research in India. *Progress in Mycology: An Indian Perspective*, pp.163-197.

Nayaka, S., 2014. Methods and techniques in collection, preservation and identification of lichens. *Plant Toxonomy and Biosystematics: Classical and Modern Methods*, pp.101-105.

Nayaka. S., Upreti, D.K. and Khare, R. 2010. Medicinal lichens of India. In Drugs from Plants (Ed. P.C. Trivedi). Avishkar Publishers, Distributors, Jaipur, India.

Nayar, T.S., Sibi, M., Rasiya Beegam, A., Mohanan, N. & Rajkumar, G., 2008. Flowering Plants of Kerala: Status and Statistics. Rheedea 18, pp. 95–106.

Neamati, N., Hong, H., Mazumder, A., Wang, S., Sunder, S., Nicklaus, M.C., Milne, G.W., Proksa, B. and Pommier, Y., 1997. Depsides and depsidones as inhibitors of HIV-1 integrase: discovery of novel inhibitors through 3D database searching. *Journal of medicinal chemistry*, *40*(6), pp.942-951.

Odabasoglu, F., Cakir, A., Suleyman, H., Aslan, A., Bayir, Y., Halici, M. and Kazaz, C., 2006. Gastroprotective and antioxidant effects of usnic acid on indomethacin-induced gastric ulcer in rats. *Journal of ethnopharmacology*, *103*(1), pp.59-65.

Ohmura, Y., Kawachi, M., Kasai, F., Sugiura, H., Ohtara, K., Kon, Y. and Hamada, N., 2009. Morphology and chemistry of Parmotrema tinctorum (Parmeliaceae, lichenized Ascomycota) transplanted into sites with different air pollution levels. *Bulletin of the National Museum of Nature and Science, Series B*, *35*, pp.91-98.

Omotoso, D.R., Olubowale, V.O., Aina, F.M. and Daramola, O.O., 2024. Phytochemical Profiling of Basella alba Using Gas chromatography-Mass Spectrometry. *Tropical Journal of Natural Product Research*, *8*(6).

Orange, A., James, P.W. and White, F.J., 2001. *Microchemical methods for the identification of lichens*. Twayne Publishers.

Patwardhan, P.G., 1983. Rare and endemic lichens in the Western Ghats, south western India. In *Assessment of threatened plants of India: proceedings of the seminar held at Dehra Dun, 14-17 September 1981/edited by SK Jain and RR Rao*. Howrath: Botanical Survey of India, Department of Environment, c1983.

Pennington, C.W. ,1969. The Tepehuan of Chihuahua. Univ. of Utah Press.

Peveling, E., 1973. Fine structure. In *The lichens* (pp. 147-182). Academic Press.

Phillips, E.A., 1959. Methods of vegetation study,NewYork:Henry Holt and Co. Inc. pp. 107 pp.

Pinokiyo, A., Singh, K.P. and Singh, J.S., 2008. Diversity and distribution of lichens in relation to altitude within a protected biodiversity hot spot, north-east India. *The Lichenologist*, *40*(1), pp.47-62.

Pires, R.H., Lucarini, R. and Mendes-Giannini, M.J.S., 2012. Effect of usnic acid on Candida orthopsilosis and C. parapsilosis. *Antimicrobial agents and chemotherapy*, *56*(1), pp.595-597.

Pizarro, D., Divakar, P.K., Grewe, F., Crespo, A., Dal Grande, F. and Lumbsch, H.T., 2020. Genome-wide analysis of biosynthetic gene cluster reveals correlated gene loss with absence of usnic acid in lichen-forming fungi. *Genome biology and evolution*, *12*(10), pp.1858-1868.

Plitt, C.C., 1919. A short history of lichenology. *The Bryologist*, *22*(6), pp.77-xii.

Pulliainen, E., 1971, January. Nutritive values of some lichens used as food by reindeer in northeastern Lapland. In *Annales Zoologici Fennici* (pp. 385-389). Societas Biologica Fennica Vanamo.

Purushothaman, D., Nayaka, S. and Thomas, J., 2021. Some new records of lichens from the Goodrical Reserve Forest, Pathanamthitta district of Kerala. Kavaka. 56: pp.65-68.

Quilhot, W., Fernández, E., Rubio, C., Cavieres, M.F., Hidalgo, M.E., Goddard, M. and Galloway, D., 1996. Preliminary data on the accumulation of usnic acid related to ozone depletion in two Antarctic lichens.

Rajan, V.P., Gunasekaran, S., Ramanathan, S., Murugaiyah, V., Samsudin, M.W. and Din, L.B., 2016. Biological activities of four Parmotrema species of Malaysian origin and their chemical constituents. *Journal of Applied Pharmaceutical Science*, *6*(8), pp.036-043.

Ravi, L. and Krishnan, K., 2017. Research article cytotoxic potential of N-hexadecanoic acid extracted from Kigelia pinnata leaves. *Asian J. Cell Biol*, *12*, pp.20-27.

Reddy, L.H. and Couvreur, P., 2009. Squalene: A natural triterpene for use in disease management and therapy. *Advanced drug delivery reviews*, *61*(15), pp.1412-1426.

Reddy, R.G., Veeraval, L., Maitra, S., Chollet-Krugler, M., Tomasi, S., Lohezic-Le Devehat, F., Boustie, J. and Chakravarty, S., 2016. Lichen-derived compounds show potential for central nervous system therapeutics. *Phytomedicine*, *23*(12), pp.1527-1534.

Richardson, D.H., 1988. Medicinal and other economic aspects of lichens. In: Galun M, ed. Handbook of Lichenology. Boca Raton, Florida, USA: CRC Press, pp. 93–108

Rodríguez De Luna, S.L., Ramírez-Garza, R.E. and Serna Saldívar, S.O., 2020. Environmentally friendly methods for flavonoid extraction from plant material: Impact of their operating conditions on yield and antioxidant properties. *The Scientific World Journal*, *2020*(1), p.6792069.

Rodríguez De Luna, S.L., Ramírez-Garza, R.E. and Serna Saldívar, S.O., 2020. Environmentally friendly methods for flavonoid extraction from plant material: Impact of their operating conditions on yield and antioxidant properties. *The Scientific World Journal*, *2020*(1), p.6792069.

Saha, S., Pal, A. and Paul, S., 2021. A review on pharmacological, anti-oxidant activities and phytochemical constituents of a novel Lichen Parmotrema species. *Journal of Biologically Active Products from Nature*, *11*(3), pp.190-203.

Santos, L.C., Honda, N.K., Carlos, I.Z. and Vilegas, W., 2004. Intermediate reactive oxygen and nitrogen from macrophages induced by Brazilian lichens. Fitoterapia. 75, pp. 473-479.

Sasidharan, N., 2012. Flowering Plants of Kerala – Version 2.0. DVD No. 14. Kerala Forest Research Institute, Peechi.

Sati, S.C. and Joshi, S., 2011. Antibacterial activity of the Himalayan lichen *Parmotrema nilgherrense* extracts. *British Microbiology Research Journal*, *1*(2), pp.26-32.

Satvalekar, S.P.D., 1958. Atharvaveda. In Hindi, Printed at Mehra Offset Press, New Delhi.

Satvalekar, S.P.D.,1985. Rigveda. In Hindi, printed at Mehra Offset Press, New Delhi.

Scheidegger, C., Groner, U., Keller, C. and Stofer, S., 2002. Biodiversity assessment tools—Lichens. *Monitoring with lichens—Monitoring lichens*, pp.359-365.

Scheidegger, C., Nimis, P. L., and Wolseley, P., 2002. .Monitoring with Lichens-Monitoring Lichens, Kluwer, Dordrecht.

Schwendener, S., 1868. Ueber die beziehungenzwischenalgen und flechtengonidien. *Botanische Zeitung*, *26*(18), pp.289-292.

Schwendener, S., 1869. *Die algentypen der Flechtengonidien*. C. Schultze.

Sequeira, S., Christy, A., Anilkumar, A. and Arsha, S.M., 2022. Parmotremasahyadrica (Parmeliaceae): A new species of parmelioid lichen from Wayanad, Southern Western Ghats, India. *Phytotaxa*, *539*(3), pp.287-292.

Shah, A.A., Badshah, L., Muhammad, M., Basit, A., Ullah, I., Mohamed, H.I. and Khan, A., 2024. Secondary metabolites of lichens and their application. In *Fungal Secondary Metabolites* (pp. 91-115). Elsevier.

Shannon, C.E., 1949. *The mathematical theory of communication, by CE Shannon (and recent contributions to the mathematical theory of communication), W. Weaver*. Champaign, IL, USA: University of illinois Press.

Sharma, B.C. and Kalikotay, S., 2012. Screening of antioxidant activity of lichens Parmotremareticulatum and Usnea sp. from Darjeeling hills, India. *IOSR Journal of Pharmacy*, *2*(6), pp.54-60.

Sharma, M. and Mohammad, A., 2020. Lichens and lichenology: Historical and economic prospects. *Lichen-Derived Products: Extraction and Applications*, pp.101-118.

Sharma, P. V., 1984. Dravyaguna Vigyan. Chaukhmbha Bharti Academy, Varanasi, India. Bhumika IV, pp.27-28.

Sharma, P. V., 1998. Dravyaguna Vigyan. Chaukhmbha Bharti Academy, Varanasi, India VII, pp. 724-726.

Shrestha, G. and St. Clair, L.L., 2013. Lichens: a promising source of antibiotic and anticancer drugs. *Phytochemistry reviews*, *12*, pp.229-244.

Shukla, A.K., Kumar, M., Sharma, M., Dev, S.K. and Maurya, R., 2022. Usnic acid: A promising bioactive agent for wound healing. *Asian Journal of Pharmacy and Pharmacology*, *8*(6), pp.188-193.

Shukla, V., Joshi, G.P. and Rawat, M.S.M., 2010. Lichens as a potential natural source of bioactive compounds: a review. *Phytochemistry reviews*, *9*, pp.303-314.

Shukla, V., Patel, D.K., Bajpai, R., Semwal, M. and Upreti, D.K., 2016. Ecological implication of variation in the secondary metabolites in Parmelioid lichens with respect to altitude. *Environmental Science and Pollution Research*, *23*, pp.1391-1397.

Simpson, E.H., 1949. Measurement of diversity.Nature. pp.163:688.

Singh, K.P. and Sinha, G.P., 2010. Indian lichens: an annotated checklist. Botanical Survey of India. *Shiva Offset Press, DehraDun, Uttarakhand*.

Singh, T. B., and K. C. Chunekar.,1972. Glossary of vegetable drugs in Brhattrayi. The Chaukhambha Sanskrit Series, Varanasi.

Sinha, G.P., 2021. Documentation of lichen diversity in India. ILS eLetter 1, pp.5-7.

Smith, T.J.,2000. Squalene: potential chemopreventive agent. Expert Opin Invest. Drugs. 9,pp. 1841-1848.

Spielmann, A.A. and Marcelli, M.P., 2009. *Parmotrema* sl (Parmeliaceae, lichenized Ascomycota) from Serra Geral slopes in central Rio Grande do Sul State, Brazil. *Hoehnea*, *36*, pp.551-595.

Spielmann, A.A. and Marcelli, M.P., 2020. Type studies on Parmotrema (Parmeliaceae, Ascomycota) with salazinic acid. *Plant and Fungal Systematics*, *65*(2), pp.403-508.

Sreekumar, V.B., Hussain, K.H. and Renuka, C., 2017. Virtual herbarium of Kerala Forest Research Institute, Peechi, Kerala, India. *Current Science*, pp.466-470.

Studzinska-Sroka, E., Galanty, A. and Bylka, W., 2017. Atranorin-an interesting lichen secondary metabolite. *Mini reviews in medicinal chemistry*, *17*(17), pp.1633-1645.

Svanberg, I. and Egisson, S., 2012. Edible wild plant use in the Faroe Islands and Iceland. *Acta Societatis Botanicorum Poloniae*, *81*(4).

Swathi, D., Suchitha, Y., Kekuda, P.T.R., Venugopal, T.M., Vinayaka, K.S., Mallikarjun, N. and Raghavendra, H.L., 2010. Antimicrobial, anthelmintic and insecticidal activity of a macrolichen Everniastrum cirrhatum (Fr.) Hale. *International Journal of Drug Development and Research*, *2*(4), pp.780-789.

Terahara, N., 2015. Flavonoids in foods: A Review. *Nat. Prod. Com.*10,pp.521–528. doi: 10.1177/1934578X1501000334.

Thadhani, V.M. and Karunaratne, V., 2017. Potential of lichen compounds as antidiabetic agents with antioxidative properties: A review. *Oxidative medicine and cellular longevity*, *2017*(1), p.2079697.

Thajuddin, S., Moorthy, I.G., Dhanasekaran, D., Kumar, R.S. and Thajuddin, N., 2019. Processed Lichens Could be a Potential Functional Food with Special Reference to Traditional Dishes. In *Fermented Food Products* (pp. 67-76). CRC Press.

Thell, A., Kärnefelt, I., Seaward, M. and Westberg, M., 2013. In the footsteps of Erik Acharius. 20th biennial meeting of the Nordic Lichen Society. Vadstena 11-15 August 2013. Programme and Abstracts.

Tiwari, P., Rai, H., Upreti, D.K., Trivedi, S. and Shukla, P., 2011. Antifungal activity of a common Himalayan foliose lichen Parmotrema tinctorum (Despr. ex Nyl.) Hale. *Nat Sci*, *9*(9), pp.167-171.

Torrallardona, D., Badiola, I. and Broz, J., 2007. Effects of benzoic acid on performance and ecology of gastrointestinal microbiota in weanling piglets. *Livestock Science*, *108*(1-3), pp.210-213.

Torres-Benítez, A., Rivera-Montalvo, M., Sepúlveda, B., Castro, O.N., Nagles, E., Simirgiotis, M.J., García-Beltrán, O. and Areche, C., 2017. Metabolomic analysis of

two Parmotrema lichens: P. robustum (Degel.) Hale and P. andinum (Mull. Arg.) Hale using UHPLC-ESI-OT-MS-MS. *Molecules*, *22*(11), p.1861.

Townsend, G.F., 1963. Benzaldehyde: a new repellent for driving bees. *Bee World*, *44*(4), pp.146-149.

Tripathi, A.H., Negi, N., Gahtori, R., Kumari, A., Joshi, P., Tewari, L.M., Joshi, Y., Bajpai, R., Upreti, D.K. and Upadhyay, S.K., 2022. A review of anti-cancer and related properties of lichen-extracts and metabolites. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)*, *22*(1), pp.115-142..

Tuan, N.T., Dam, N.P., Van Hieu, M., Trang, D.T.X., Danh, L.T., Men, T.T., De, T.Q., Bach, L.T. and Kanaori, K., 2020. Chemical constituents of the lichen Parmotrema tinctorum and their antifungal activity. *Chemistry of Natural Compounds*, *56*, pp.315-317.

Upreti, D.K., Divakar, P.K. and Nayaka, S., 2005. Commercial and ethnic use of lichens in India. *Economic botany*, *59*(3), pp.269-273.

Valgas, C., Souza, S.M.D., Smânia, E.F. and Smânia Jr, A., 2007. Screening methods to determine antibacterial activity of natural products. *Brazilian journal of microbiology*, *38*, pp.369-380.

Vartia, K.O., 1973. Antibiotics in lichens. In *The lichens* (pp. 547-561). Academic Press.

Verma, N., Behera, B.C., Sonone, A. and Makhija, U. 2008b. Cell aggregates derived from natural lichen thallus fragments: Antioxidant activities of lichen metabolites developed in vitro. Natural Product Communications. 3 (11), pp. 1911-1918.

Verma, N., Behera, B.C., Sonone, A. and Makhija, U., 2008 a. Lipid peroxidation and tyrosinase inhibition by lichen symbionts grown in vitro. *Afr J Biochem Res*, *2*(12), pp.225-231.

Wang, Y., Shao, J., Yao, S., Zhang, S., Yan, J., Wang, H. and Chen, Y., 2014. Study on the antithrombotic activity of Umbilicaria esculenta polysaccharide. *Carbohydrate polymers*, *105*, pp.231-236.

Xu, H. and He, X.Q., 2010. Natural products-based insecticidal agents 6. Design, semisynthesis, and insecticidal activity of novel mono methyl phthalate derivatives of podophyllotoxin against Mythimna separata Walker in vivo. *Bioorganic & medicinal chemistry letters*, *20*(15), pp.4503-4506.

Xu, R., Fazio, G.C. and Matsuda, S.P., 2004. On the origins of triterpenoid skeletal diversity. *Phyto- chemistry*, *65*(3), pp.261-291.

Yamamoto, Y., Kinoshita, Y., Matsubara, H., Kinoshita, K., Koyama, K., Takahashi, K., Kurokawa, T. and Yoshimura, I., 1998. Screening of biological activities and isolation of biological-active compounds from lichens.

Yanık, B. and Ateş, F.B., 2023. The Effects Of Orcinol On Proliferation And Apoptosis Of Sw480 Human Colorectal Cancer Cells. *Journal of Faculty of Pharmacy of Ankara University*, *47*(2), pp.375-382.

Zahlbruckner, A., 1926. 'Lichens'. In:Engler and Prantl (eds.) Naturl. Pflanzenfam. 2ed. Bd. 8 Leipizig.

Zambare, V.P. and Christopher, L.P., 2012. Biopharmaceutical potential of lichens. *Pharmaceutical Biology*, *50*(6), pp.778-798.

Zhang, L., Ma, J., Yang, M., Zhao, T., Han, M.Y., Zheng, D., Mapook, A., Lu, Y. and Jayawardena, R.S., 2024. Polyketides from Neohelicosporium griseum: structure assignment and bioactivity investigation. *Medicinal Chemistry Research*, *33*(2), pp.308-313.

Zhou, R., Yang, Y., Park, S.Y., Nguyen, T.T., Seo, Y.W., Lee, K.H., Lee, J.H., Kim, K.K., Hur, J.S. and Kim, H., 2017. The lichen secondary metabolite atranorin suppresses lung cancer cell motility and tumorigenesis. *Scientific reports*, *7*(1), p.8136.

Zorrilla, J.G., D'Addabbo, T., Roscetto, E., Varriale, C., Catania, M.R., Zonno, M.C., Altomare, C., Surico, G., Nimis, P.L. and Evidente, A., 2022. Antibiotic and nematocidal metabolites from two lichen species collected on the Island of Lampedusa (Sicily). *International Journal of Molecular Sciences*, *23*(15), p.8471.