PHARMACOLOGICAL STUDIES OF SELECTED LICHENS OF MIZORAM

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NURPEN MEITEI THANGJAM

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DEPARTMENT OF HORTICULTURE AROMATIC AND MEDICINAL PLANTS,
SCHOOL OF EARTH SCIENCES AND NATURAL RESOURCES MANAGEMENT, MIZORAM UNIVERSITY

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PHARMACOLOGICAL STUDIES OF SELECTED LICHENS OF MIZORAM

BY

NURPEN MEITEI THANGJAM

Department of Horticulture Aromatic and Medicinal Plants

Under the Supervisor

Dr. AWADHESH KUMAR

Submitted
In partial fulfillment of the requirement of the degree of Doctor of Philosophy
in Department of Horticulture Aromatic and Medicinal Plants of
Mizoram University, Aizawl

CERTIFICATE

This is to certify that the thesis entitled **"Pharmacological Studies of Selected Lichens of Mizoram."** submitted by **Mr. Nurpen Meitei Thangjam** (Ph.D. Regn. No.**MZU/Ph.D/911 of 13.04.2016)**, in partial fulfillment of the requirements for the award of degree of Doctor of Philosophy in Horticulture Aromatic and Medicinal Plants of Mizoram University, Aizawl embodies the record of his original investigation under my supervision. He has duly registered and the thesis presented is worthy of being considered for the award of the Doctor of Philosophy (Ph. D.) Degree. The work has not been submitted previously for any degree to this or any other university.

(Dr. D.K.Upreti)

Joint Supervisor

डा० डी० के० उप्रेती
DR. D. K. UPRETI
इमेरिटस वैज्ञानिक / EMERITUS SCIENTIST
लाईकेनोलॉजी लैब / LICHENOLOGY LABORATORY
पादप विविधता वर्गिकी एवं पादपालय विभाग
PLANT DIVERSITY SYSTEMATICS AND HERBARIUM DIVISION
सी०एस०आई०आर० राष्ट्रीय वनस्पति अनुसंधान संस्थान
CSIR NATIONAL BOTANICAL RESEARCH INSTITUTE
लखनऊ-226001 (भारत) / LUCKNOW-226001 (INDIA)

(Dr. Awadhesh Kumar)

Supervisor

Dr. AWADHESH KUMAR *M.Sc., Ph.D.*
Assistant Professor
Department of Horticulture Aromatic
& Medicinal Plants
Mizoram University
Tanhril, Aizawl-796 004, Mizoram

DECLARATION

I, **Nurpen Meitei Thangjam**, hereby declare that the subject matter of this thesis entitled "**Pharmacological Studies of Selected Lichens of Mizoram.**" is the record of the work done by me, that the contents of this thesis did not form basis of the award of any previous degree or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University / Institute.

This thesis is being submitted to the Mizoram University for the degree of **Doctor of Philosophy** in the Department of Horticulture Aromatic and Medicinal Plants.

(Nurpen Meitei Thangjam)

HEAD

विभागाध्यक्ष
Head
बागवानी सुगन्धित एवं औषधीय वनस्पति विभाग
Dept. of Horticulture, Aromatic & Medicinal Plants
मिजोरम विश्वविद्यालय
Mizoram University

Dr. Awadhesh Kumar
Supervisor
Dr. AWADHESH KUMAR *M.Sc., Ph.D.*
Assistant Professor
Department of Horticulture Aromatic
& Medicinal Plants
Mizoram University
Tanhril, Aizawl-796 004, Mizoram

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Place: Aizawl.

Th. Nurpen

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LIST OF ABBREVIATIONS

TSM	Traditional system of Medicine
TCM	Traditional Chinese Medicine
SPP.	Species
MIC	Minimum inhibitory concentration
mg	Microgram
Kg	Kilogram
%	Percentage
ml	Milliliteres
GA	Gallic acid
STZ	Streptozotocin
CRU	cold restraint
MTCC	Microbial Type Culture Collection
PDA	Potato Dextrose Agar
AS	Aspirin
DMSO	Dimethyl Sulfoxide
BOD	Biological Oxygen Demand
AL	Alcohol
PL	pyloric ligation
GERD	Gastroesophageal reflux disease
ANOVA	Analysis of Variance
CCl4	Carbon tetrachloride
LOX	Lipoxygenase
NER	North Eastern Region
BIS	Botanical Survey of India
°C	Degree celcius
Mts	Meters
Mm	Millimeters
GIS	Geographic Information System
MZU	Mizoram University
MNP	Murlen National Park
TLC	Thin Layer Chromatography

+ve	Positive
-ve	Negative
KOH	Potassium hydroxide
PD	paraphenylenediamine
C	Calcium hypochlorite
I	Potassium iodide
UV	Ultra violet
LWG	lichen herbarium
HCL	Hydrochloric acid
$FeCl_3$	Ferric chloride
H_2SO_4	Sulphuric acid
NaOH	Sodium hydroxide
HNO_3	Nitric acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
NM	Nanometer
NBT	Nitrobluetetrazolium
TPC	Total phenolic content
GAE	Gallic acid equivalent
QE	Quercetin equivalent
IMTECH	Institute of Microbial Technology
PDB	Potato dextrose Broth
LBS	Pound
ZIC	Zone of inhibition
CPCSEA	The Committee for the Purpose of Control and Supervision of Experiments on Animals
MZUAEC	Mizoram University Animal Ethics Committee
OECD	Organisation for Economic Co-operation and Development
CMC	Carboxy methyl cellulose

CHAPTER -1

INTRODUCTION

1.1 HISTORY, DEFINITION AND CLASSIFICATION OF LICHENS

The term "Lichen" was given by Theopharastus (The Father of Botany) in 371-286 BC for superficial growth on bark of Oleaeuropea (olive) tree. The word lichens is a Greek origin which is pronounced as “Lie Kens” (Hawksworth and Hill, 1984). Erik Acharius, Swedish botanist who was devoted his scientific work to lichens; he defined many new species and arranged in 40 distinct genera, he studied on the basis of external morphology in his monumental works Methodica Lichenum, Lichenographina Universalis and Synopsis Methodica Lichenum. Since he was a pioneered researcher in lichens taxonomy, henceforth, he known as the “Father of Lichenology”;

In the year 1867; Swiss Botanist Simon Schwendener’s proposed a theory of the dual nature of lichens which is accepted universally, where lichen consists of a fungal and an algal component (Honegger, 2000). However, in the later century, a number of definitions of lichens were provided by many of leading contemporary lichenologist. In the year 1886-89 Scientist Bonnier synthesized lichen by growing the fungal spore with algae. Moreover, Debary (1870), Reinke (1872), Crombie (1885), Chopra (1934), Plessel (1963), Ahmadjian (1960) were the others who worked prominently in the field of lichens in the early stages of 19^{th} century (Web.1).

Lichens are considered as a constant and self-sustaining relationship between fungi as mycobionts and the algal partners as photobionts. As the mycobiont is sole in the symbiotic relationship and it is typically controls the relationship (Ranković and Kosanić, 2015). Lichens is also known as one of the most positive symbiotic relations of a fungus, a green and/ or blue green alga, which can be survive everywhere in this planet (Rai et al., 2015). The field of botany which deals with the study of lichens is called as lichenology and the Scientist or the researcher who works in the field of lichenology are called as lichenologist.

Lichen doesn’t have the proper natural system of classification; they are classified based on the nature and kinds of fruiting bodies of the fungal partner. Thus, they are integrated into the system of fungi. Austrian botanist Zahlbruckner (1926)

classified lichens into 2 main groups based on the structure of fruiting bodies of fungal partners as ascolichens (belongs to the fungal member of lichen acomycotina) and basidiolichen (belongs to the fungal member of lichen basidiomycotina) (Büdel and Henssen, 1983). Later, Alexopoulos and Mims (1979) again classified into 3 different main groups as basidiolichen (basidiomycetes), deuterolichen (deuteromycetes) and ascolichen (ascomycetes) (Web.2).

Lichens are the perennial plants which are very slow in growth specially the mycobiont. The supply of the nutrients and the food produced through photosynthesis by the photobiont at the growing sites is utilized by the fungal partner (mycobiont). The growth form of lichens is centrifungal, apical and marginal (Shukla et al., 2014). Lichens as a composite the mycobiont dominates the thallus (body) with 90% of the thallus volume and provides shape, structure and colour to the lichen with 10% contribution from photobiont. Since, the visible part of lichen thallus from outer surface is fungal part which upholds the algal cell inside. Therefore, the lichens are placed in the kingdom – mycota (Fungi). The fungi present in lichens are called as lichenized fungi. Among the 20,000 lichen species known in the world 95% belongs to the ascomycetes group of fungi while bacidiomycetes and deuteriomycetes groups are represented by only 3% and 2% of species respectively.

1.2 HABIT AND HABITAT OF LICHENS

Lichen can grow everywhere; they are highly diverse in distribution, capable of living from hot deserts to high-altitude Antarctic. They can tolerate extremes of climate (cold, heat and drought); lichens are grown on any types of substratum like rocks, soil and tree bark and at any non-living objects too. On basis of their occurrence, lichens are categorized by different names; as, the lichens that are growing on tree trunk and bark (corticolous), twig inhabiting (ramicolous), on decaying or dead wood (legnicolous), on rocks (saxicolous), on moss (muscicolous), on soil (terricolous), on evergreen leaves (foliicolous). The lichen which is growing commonly on other plant is called as epiphytic lichens. The lichens can also grow on some insects and animals as well. The lichens are widely distributed in almost all the phytogeographical regions of the world

(Jørgensens, 1983). The growth and abundance of the lichens is also depends on the moisture, elevation, free from pollution and favorable substratum.

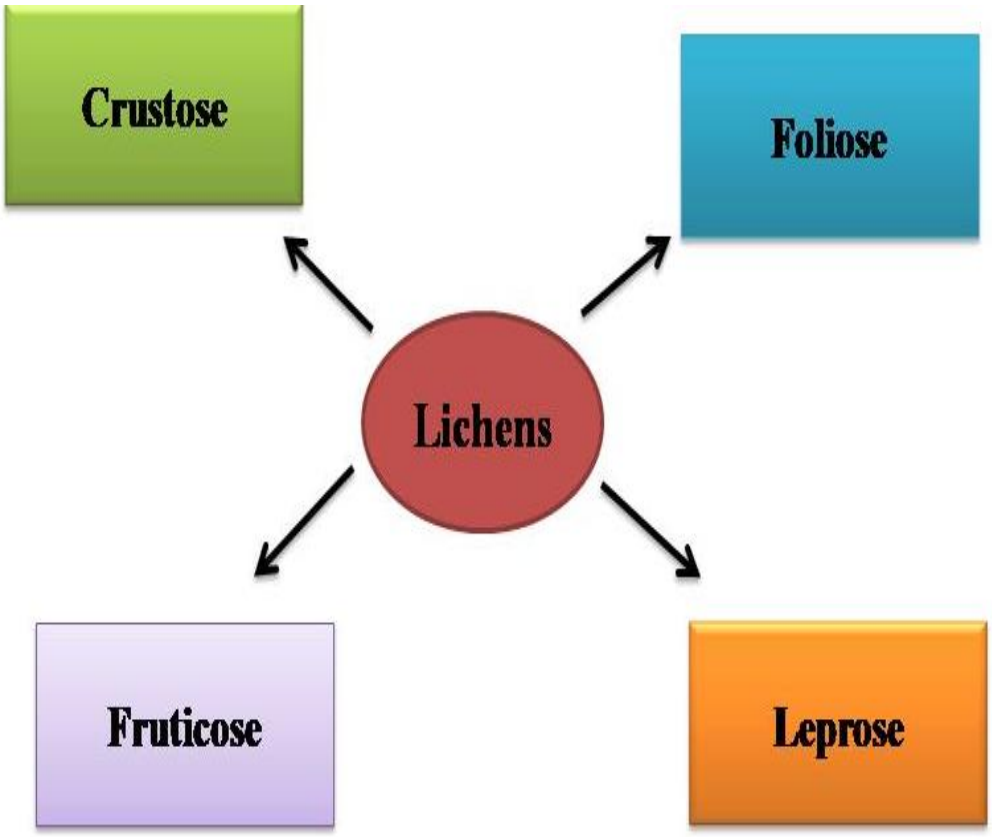


Figure 1.1 Types of Lichens

Considering the morphology of lichen thallus, it is found that lichens are growing in different growth forms such as crustose (used to grow attached to the substratum) followed by foliose (used to grow like leafy and slackly attached to the substratum) and lastly fruticose (used to grow like bushes which is hanging or upright growing on substratum) (Gaurav and Upreti, 2016). In addition to this, several other growth form of lichens like leprose (powdery or granular), Placodioid (thallus is closely attached to the substratum at centre and lobate or free at the margin, but lack rhizines), squamulose (thallus is in the form of minute lobes, having dorsiventral differentiation), dimorphic (single thallus has the characters of both foliose/squamulose and fruticose lichens

(Figure 1). The squamules are the primary thallus, which bears erect body of fruticose lichen *ie.* secondary thallus). These leprose, crustose including few placodioid and squamulose lichens are generally called as microlichens, because they are smaller in size and require microscopic studies to identify. On the other hand, foliose, dimorphic and fruticose lichens are called as macrolichens. The macrolichens have comparatively larger thallus and a hand-lens, dissection or stereozoom microscope is sufficient to identify them.

1.3 DIVERSITY AND DISTRIBUTION OF LICHENS

Lichens are widely distributed in all the geographical region of the world. The total amount of lichens species and lichenized fungi is estimated about 17,000 to 20,000 species around the world (Galloway, 1992). However; Aptroot (1997) reported that the biodiversity of fungi is very high but the number projected of the species is varied and it was estimated 30,000 as a recognized species with known ascomycetes. Later; the latest update of lichens species was reported that number of accepted species is 19,387 in 995 genera, 115 families. Parmeliaceae family is the highest number of species with 2,765 spp. and 77 genera followed by graphidaceae with 2,161 spp. and 79 genera (Lücking et al., 2017).

India was count as the fifth nation around the world for its biodiversity and overall, 2303 lichen species were projected under 305 genera and 74 families was recorded in India. Foliose lichens of family Parmeliaceae dominants the diversity with 345 spp. widely distributed in subtropical, temperate and alpine regions; crustose of family Graphidaceae with 278 spp. distributed in tropical and subtropical regions of India; followed by the families Thelotremataceae, Pyrenulaceae, Caliciaceae, Lecanoraceae, Physciaceae, Tryptheliaceae, Teloschistaceae and Collemataceae with their descending order as 130, 116, 103, 99, 97, 77, 75, 67 spp. respectively. Likewise; genus *Graphis* dominate high diversity with 111 spp.; followed by genera *Pyrenula* and *Lecanora* with 84 spp.; and mostly these lichens are widely distributed in tropical and subtropical regions of India (Rashmi and Rajkumar 2015; Singha et al., 2010). Moreover; Singha et al., (2018) reported the addition of 411 species to the list of

annotated checklist 2010, therefore the total number of lichens recorded from India became 2714 species where the family Graphidaceae with 106 spp. And the genus *Graphis* with 34 spp. followed by order arthoniales and opegrapha with 52 and 17 spp. respectively.

In the context of eastern Himalayas; Singh et al., (2018) reported 1047 species in 592 crustose, 321 foliose, 125 fruticose and 9 squamulose growth forms which are widely distributed in tropical, sub-tropical, temperate, sub alpine and alpine regions comprising of about 41.22 % of the total lichens diversity of India. However, in the eastern Himalayan region including Sikkim and the seven other North-Eastern states of India are still unexplored and most of the researchers related to lichens are attempting to explore for the new accounts from the country. The literature of some recent publication shown that research about lichens exploration was began from last 18 years only (Singh and Pinokiyo, 2004, Pinokiyo et al., 2008, Rout et al., 2010, Daimari et al., 2014, Devi et al., 2015, Upreti et al., 2015, Singh and Singh, 2016). Further, Logesh et al., (2017) reported 159 species of lichens in Mizoram including Thangjam et al., (2019) and Thangjam et al., (2020).

1.4 SECONDARY METABOLITES OF LICHENS

Lichens are known for its unique secondary metabolites, it can synthesize a great variety of metabolites (Richardson, 1988; Lawrey, 1989; Elix, 1996). Lichens compounds are classified into 3 groups *viz.* aliphatic lichen, aromatic lichen substances and carbohydrates; which comprise of aliphatic, cycloaliphatic, aromatic, and terpenic compounds (Huneck, 1999). Looking its biosynthetic pathways, Lichens further classified in 3 pathways to produce its compounds named as polymalonate (polyketide), shikimic acid and mevalonic acid pathways and most of the metabolites are derived from polyketide synthetic pathway; others derived from shikimic acid and mevalonic acid pathways (Boustie and Grube, 2005) (Figure 2). Primary metabolites include amino acids, polyols, carotenoids, polysaccharides and vitamins, whereas; the secondary metabolites of lichens are produced by the mycobiont (fungi) and present in the form of amorphous/ crystal on the outer surface of the hyphae. So far 800 secondary metabolites

of lichens has been updated in the recent scientific publications, lichens and its bioactive secondary metabolites have a major role in pharmaceutical field for its active phytochemical constituent and application (Ranković B and Kosanić M, 2019).

Basically, Lichen acid are derived from above mentioned biosynthetic pathways such as shikimic acid pathways which is the source of pulvinic acid derivatives (yellow pigments), mevalonic acid pathways a source of terpenes and acetate-polymalonate pathway which is considered as the biggest pathway, because majority of the lichen compounds are produced by this pathway such as depside, depsidones, usnic acid, anthraquinones, xanthine, aliphatic acids. Mevalonic acid pathway produce steroids and triterpenes like zeorin. Usnic acid is one of the known medicinally important lichen acids (Ivanova and Ivanov, 2009).

The secondary metabolites of lichens are not importance only for their existence and growth; they used to make good relationship with the environment also. They have ability to protect themselves from different factors such as herbivores, pathogens, UV radiation etc. Since, many lichens are known for its edible properties and most of their metabolites are also explored for biological activities (KatalinMolnára and Edit Farkas, 2010). Usnic acid is the most common acid which has wider ranges of properties; moreover, there are some other metabolites which have a high potent medicinal value such as atranorin, diffractaic acid, lecanoric acid, lobaric acid, stictic acid, salazinic acid, fumarprotocetraric acid, physodic acid and orsellinates. All of these secondary metabolites of lichens have the properties of antioxidant and antimicrobial activities (White et al., 2014).

Figure 1.2 Classification of lichen substances according to their biosynthesis (Huneck, 1999).

1.5 ECOLOGICAL IMPORTANCE OF LICHENS

Lichens are known for its ability for their adaptation in diverse climatic conditions; they can be able to with stand from high temperature to the cold Arctic and Antarctica regions and are widely distributed in all the geographical regions around the world. Hence, it is true that most of the diversity of the lichens is dependent on the climatic conditions. As per the recent researches, the monitoring and measurement by using natural systems as an indicator for checking the ecosystems now become a trend. In the same line, lichens are also used as an indicator for monitoring the ecological

conditions and its helps to provide the warning at the earliest time. Lichens are highly sensitive to environment and pollution because they don't possess vascular system and it used to synthesize their food by captivating their requirements like water and nutrients from the surrounding environment. Therefore, lichens are important tools to check out the details of environmental changes such as climate, pollution and other biological process (Kuldeep and Prodyut, 2015). The studies of forest ecosystem by using lichens communities as an indicator also provides the information which is concerned about the biodiversity, the air quality as well as the sustainability of timber production (McCune, 1998).

Biodiversity of lichens and its conservation have assumed an important goal for the researchers for its exploration, documentation, conservation and sustainable utilization. It's also an important factor for all socio-economic development of the society. Lichens are one the most reasonable indicators in ecological aspect for its known sensitiveness to different types of environmental stressors like pollution and climate change. Due to this, lichens are also used as terrestrial bio-monitors of environmental contamination in industrial area, urban and rural habitats.There are other several methods have alsobeen proposed for lichen as monitors of heavy metals pollution and climate change in different parts of the world (Upreti, 1995; Upreti and Nayaka, 2008).

1.6 ECONOMICAL IMPORTANCE OF LICHENS

Lichens are used as a household since the time immemorial with their vast properties. Lichens were believed that it is widely used in different traditional system of medicines (TSM) such as Ayurveda, Chinese medicine (TCM), Homeopathic and Unani for the treatment of various ailments (Saklani and Upreti, 1992). They play a very important role in the ancient and traditional system of medicinal practices. Lichens are also used in different other purpose *viz.* food, fodder, spices, dyes, cosmetics, perfumery. Kumar and Upreti (2001) indicate the early used of lichens in various cultural or rituals functions for the first time in India. Shipal in atharveda (1500 B.C.) is the first record of the use of lichen as medicine.Very popular vernacular name Charila is widely used in

ayurveda, an ancient system of medicine in India.The Sanskrit synonyms for lichens are Shailaya and Shila Pushp (Shila, rock; Pushp, flower). Many ayurvedic and unani products prepared from different species of lichens are marketed in India with the trade name as charila and ushna. Lichens are also used as holy lichens; and burned in holy sacrificial fires known as “Hawan or Homa” often mixed with other aromatic herbs.

The major problem where people face while eating lichens are that, their secondary lichen compounds are mostly acid and gives acrid flavors, and it is also a complex carbohydrate which are very difficult to digest and irritating to the digestive tracts. Some lichens such as usnic and vulpunic acid are also found to be poison to the human being if those compounds ingested in large amounts. People have traditionally used lichens in various preparations for consuming by removing the secondary compounds and hydrolyzing the polysaccharides. The technique which is commonly used for preparation is by boiling and steaming; and it is mostly practices in Europe, India and North America. The lichen polysaccharides can be hydrolyzing by boiling into digestible formed (Ivanova and Ivanov, 2009).

Lichen powder or the whole parts are used as an important ingredients as a spices in the food. Many species of lichens are used as food or as flavourant in the foods. Parmelioid lichens (Lichens belong to family parmeliaceae) are available in the market as condiment. *Parmotrema tinctorum* is used as a spice and flavouring agent for meat and vegetables by ethnic groups in India and Nepal (Upreti et al 2005). It is called Al-Sheba in Arabic and used as a spice in food, in Arab countries. (Abo-Khatwa et al. 1997; Mohaptra, 2011; Anupama et al., 2017).

In addition to this, it also reported that, the perfume produced from the some of the lichen was popular since last 800 years in a town called Kannauj of Uttar Pradesh, now also perfume preparation is carried out named as ’Otto’ (the Hina Attar) in the present generation (Upreti et al. 2005). *Parmotrema nilgherrense, Everniastrum nepalense, Ramalina subcomplanata* and *Usnea lucea* are more popular in the regions of Ramanagar and Tanakpur of the Uttar Pradesh hills for commercial use.

1.7 PHARMACOLOGICAL IMPORTANCE OF LICHENS

Lichens are highly importance from time immemorial forits various medicinal activities including the role of medicinal practitioners. Lichens are listed in various pharmacopoeias particularly for their antimicrobial and antioxidant properties. The study of lichens on its antibiotic properties was initiated by Burkholder in 1944 and later Vartia (1950); where they tested with 9 crystalline lichenic acids against 11 species of bacteria and it was proved that lichens are having antimicrobial properties against the gram positive bacteria. Vartia (1973) again studied with secondary metabolites such asusnic acids, the lichesterinic acid group, orcinol-type depsides and depsidones to check out the ability to inhibit the growths of bacteria. Similarly, many prominent scientist starts working on different lichens to investigate the potent of lichens against the bacteria. Further, lichens are also known for its antifungal properties, Burzlaff, (1950) investigated that the ethanolic and aqueous extract of *Parmelia molliuscula* can inhibit the growth of *Penicillium* and *Rhizopus*.

Lichens are also considered as one of the most important sources for its bioactive molecules in various pharmaceutical activities as secondary metabolites have been well known for its inhibition of different microbes.Lichens are known for their unique chemical constituents such as atranorin, fumarprotocetraric acid, gyrophoric acid lecanoric acid, physodic acid, protocetraric acid, stictic acid and usnic acid. They possess high therapeutic and pharmacological properties. Usnic acid is one of the most commonly used compound uses in the different ailments like treatment of dermatitis and infected burns as a traditional medicine (Rowe et al 1999). Moreover, Lichens as whole and its identified metabolites are also having numerous biological activities as antimicrobial, antiprotozoal, antiviral, antiproliferative, anti-inflammatory, analgesic, antipyretic, antitermite, antioxidant, cytotoxic, enzyme inhibitory, insecticidal, wound healing, antitumor as well as enzyme inhibitory (Yılmaz et al., 2004, Kosanić et al., 2013, Rajan et al., 2016).

Antioxidants are the molecules which protect the cellular damages caused by oxidation of other molecules. Oxidation is a biochemical reaction that transfers electrons

from one molecule to other oxidizing agent.And these Oxidation reactions are the one produce free radicals as oxygen, which is highly reactive atom capable of destroying the unwanted molecules known as free radicals. Free radicals are able to kill the healthy cells of the body which can make the changes of their structure and function. Antioxidants are also consider as one of the first line protection from the damage caused by the free radicals and helps to maintain the human health (Percival, 1997; Misra et al. 2014). Further, many previous researches also discovered the ability of natural antioxidants against deadly disease such as acquired immuno deficiency syndrome, alzheimer, diabetes and other cardio vascular related diseases (Millot et al., 2017; (Ingelfinger et al., 2020).

Fungal infections are becoming a very serious kind of disease in the new generation around the world. The fungal kingdom includes yeasts, molds, rusts and mushrooms. The infections are causes by the microorganism that attack on the epithelial tissues. Filamentous keratinophilic fungi causing cutaneous mycoses are called dermatophytes (Pathak et al., 2016); *Trichophyton*, *Epidermophyton* and *Aspergillu*s genera are some dermatophytic agents which infect on particular sites of infection (Web.3). Moreover, Gastric ulcer is one of the most common diseases of gastrointestinal system; characterized by bleeding, perforation, and obstruction of the mucosal wall due to excess acid secretion (Saranya et al., 2011). Hence, antifungal and gastroprotective agents obtained from the natural resources are attractive vision for most of the developing countries. Lichens are one most important natural resource which is made from the symbiosis relationship of fungi and algae and have potency to inhibit the growth of such dermatophytes and other stomach related problems.

SCOPE OF THE PRESENT WORK

In India, more than 2714 lichen species have been reported so far, out of this, hardly 1% of the species are screened out for their biological activities. The use of lichens and their products in medicine still hold a considerable interest as alternative medicine to cure various diseases in different parts of the world. More than 50% lichen species known from the world exhibited presence of peculiar antibiotic substances.

Thus, there is a lot of scope for carrying out such studies in India and particularly in the state of Mizoram where lichen grows luxuriantly.

Many lichen species of the Himalayan region are said to effectively cure dyspepsia, bleeding piles, bronchitis, scabies, stomach disorders and many disorders of blood and heart (Saklani and Upreti 1992; Negi and Kareem 1996; Sochting 1999). Bioactive Secondary metabolites have been isolated from lichens and they are used in pharmaceutical purposes. Lichen(s) compounds are also reported to have antimicrobial activities *viz.*, antifungal, antiviral, anti-tumor, anti-inflammatory, analgesic, antipyretic, anti-proliferative, anti-protozoal (Lawrey, 1986; Huneck 1999; Shahi et al., 2001; Shukla et al., 2003; Patrice and Haluwin 2004; Shukla et al., 2011; Pianga et al., 2014; Shahi et al., 2014; Pianga et al., 2016). Many species are used for human nutrition, animal nutrition, for getting colors, perfumes, alcohol and in the medicinal industry (Süheyla, et al., 2003). Several lichens species have been used in folk medicine (Ethnolichenology) for treatment of stomach diseases, diabetes, whooping cough, pulmonary tuberculosis, cancer treatment, and skin diseases.

It is well known that long term use of synthetic drugs often causes numerous side effects and sometimes its develop the resistance against pathogenic strain as well as diseases too (Şahin et al., 2003). Since, many traditional plants reported to possess pharmacological properties as they are known to have various secondary metabolites like alkaloids, flavonoids, glycosides, saponins, steroids, tannins and terpenes, therefore, it should be utilized to combat the disease causing pathogens (Hussain et al., 2011).

The main target of this research purpose is to find out the antifungal effects to reinforce bioprospecting of lichens as herbal medicine. The first phase of the experiment was involve the collection of the lichens species and their identification, preparation of the extracts and its phytochemical properties followed by determination of antifungal activity against the common pathogenic fungi; using broth micro dilution method (NCCLS, 2002). In addition to this, extract was also evaluated for its antioxidants and gastroprotective activity.

AIMS & OBJECTIVES

Following are the important objectives, considering under the proposed research work:

i) Collection and characterization of lichens spp;
ii) Extraction of the secondary metabolites, and antifungal screening;
iii) Phytochemical characterization of potential lichen samples;
iv) Detail *in vitro* antifungal investigation of selected lichens, against common pathogenic fungi viz., *Epidermophyton floccosum* Hartz (MTCC-7880); *Trichophyton mentagrophytes* (Robin) Blanchard (MTCC-8476); *Aspergillus flavus* Link (MTCC 9064) and *Aspergillus fumigatus* Fresenius (MTCC 1811).

CHAPTER -2

Review of Literature

2.1 INTERNATIONAL SCENARIO

Lichen is a word from Greek origin which referred to the superficial growth of fungus like organism on the bark of the olive trees; it was introduced during 300 BC into the groups of plants to this scientific world. The comprehensive history of lichens is incredible one; and as separate field of the plant system known as Lichenology was published by Krempelhuber in 1867. Later on, many authors were briefly explained about lichens to the science into 6 different periods; for example, Theophrastus to tournefort (1694), Tourneft to Micheli (1729), Micheli to Weber (1779), Weber to Acharius (1810) Acharius to De Notaris (1845), De Notaris to1867. But, Schneider (1898) found some limitations and further, hemodified in the fourth period from Weber to Wallroth and Meyer (1825), Wallroth and Meyer to Schwendener (1868) and on the six periods from Schwendener to Reinke (1894) with addition of 7 periods.

There was a confusion raised in the seventeen century about the classification of lichens as a group of plants; they neither considered asmosses and fungi nor algae, sponges and liverworts. And it was happened due to lack of knowledge of this lower plants. Even, Linnaeus never interested to the cryptograms and selected as “rusticipauperrimi," which has been translated as "the poor trash” of vegetation. Later, at the end of the eighteen century 500 species or forms of lichens were known out by the efforts of some lichenographers (Schneider 1898).

Lichens have a very long and diverse evolutionary history with very unique secondary metabolites and their adaptability is highly diverse in all the geographical features around the world. Lichens have a wide range of primary and secondary metabolites which gives an important hint in their taxonomic and evolutionary associations (Galloway, 1991). Genus like *Peltigerales* illustrate special chemical diversity; explaining the active constituent from the entire major secondary metabolites pathway such as acetate polymalonate pathway, shikimic acid pathway and mevalonic acid pathway whereas some families Nephromataceae, Peltigeraceae and Solorinaceae

compounds are produced from acetate-polymalonate and mevalonic acid pathways (Galloway, 1994).

In the olden time; Some lichens had resemblance with certain parts of our human body; *Usnea barbata* was consider as old Man's beard were used to promote the growth of hair, whereas, *Xanthoria parietina* which has yellow wall lichen is used for the treatment of Jaundice. *Peltigera canina* was included into the London Pharmacopoeia for its unique activity as anti-hydrophobia powder (dried and finely powder mixed with red pepper). *Ceiraria islandica* contains high percentage of lichen starch, which used as a food. Even France is the country which is manufacturing the dyes from the lichen (Marshall 1910). About the lichens, there are very famous lines indicating its specific nature are as follows:

LICHENS

"Little lichen, fondly clinging

In the wild wood to the tree,

Covering all unseemly places,

Hiding all thy tender graces,

Ever dwelling in the shade,

Never seeing sunny glade."

R. M. E... Lichens.

Lichens were not given much importance for a certain period of time but it was believed that 10% of the earth terrestrial habitats were covered by this flora. It was also estimated that, about 13500 to 20000 species belonging to 650 genera of lichens from all overthe world were reported (Hawksworth and Hill, 1984). Further with the development and the discovery in the interest of lichens increased the number of lichens in the world (Sipman and Aptroot, 2001). The first taxonomic work was reported by the

name of lichens with 80 species and published under 24thclass of cryptogamic-algae in book Plantarum by Linnaeus (1753). Lichens were well documented and working out in various aspects in many countries of Europe, North America, South America,Asia, Africa and even in Australia and New Zealand also.

The first checklist of the lichens of the smaller Pacific islands was published in 1998, a total of 291 genera were listed with 2422 accepted species including intraspecific taxa and 1710 synonyms. Among them 749 of the accepted taxa were based on type specimens from the region (Elix, and McCarthy 1998).The beginning of the lichen checklist about North American (north of Mexico) was produced by Mason Hale and Bill Culberson in 1956 with a large number of 2,280 species with 193 genera; after a long gap of years, Esslinger & Egan (1995) increased the total number as 3,580 of lichen species, which was published in the sixth version. Esslinger (2018) again reported a total of 5,561 species with 755 genera as the latest checklist for lichen-forming, Lichenicolous and allied Fungi of the Continental United States and Canada. Moreover, Breuss and John (2004) reported about new records of 46 species of Turkish lichen flora with *Caloplaca floridana*, a record for the first time to the scientific world. Savic and Tibell, (2006) listed 586 species of lichenized fungi from Serbia as an annotated checklist of the country. In addition to these, the first report from the Alps was published with distribution records in eight countries and 42 operational geographic units. The reported lichens species of eight countries were Austria (2,337), Italy (2,169), France (2,028), Switzerland (1,835), Germany (1,168), Slovenia (890) and Lichtenstein (152) respectively; but, there was no report of lichen taxa from Monaco (Nimis et al. 2018).

In respect to India, the diversity and distribution are also very vast in the neighbouring countries and other south East Asian countries. *Architrypethelium murisporum* Luangsuphabool, Lumbsch & Sangvichiena crustose lichen from genus *Architrypethelium* was reported for the first time in Southeast Asia found in the dry evergreen forest of Thailand (Luangsuphabool et al. 2018). Joshi et al. (2011) explained briefly about 14 subtropical to temperate lichen species as new record to South Korea. *Bacidia, Cresponea, Diploschistes, Fissurina, Fuscidea, Micarea, Mycoblastus,*

Phyllopsora, *Sarcogyne*, *Scoliciosporum,* and *Toninia* were the genera reported for the first time from South Korea. *Fuscidea recensa* var. *arcuatula, Micarea elachista, Sarcogyne privigna,* and *Toninia cinereovirens* also represented as new records for East Asia including China and Japan. In a similar way, Firdous et al. (2017) was also reported 34 species belongs to 24 genera under 15 families from the Pakistan showing the diversity and distribution of lichens at the altitude of 1000-2200 m. Aptroot and Feijen (2002) presented an annotated checklist of 287 lichens and lichenicolous fungi from Bhutan with 225 species as a new report for the country and *Pyrrhospora bhutanensis* was introduced as new to Lichens biota. Many previous studies shown that the distributions of lichens in the elevation between 200–7400m; so with this link, there were 525 Nepalese lichen species reported from the country Nepal, which proves the richness of the species at the altitude between 3100–3400 and 4000–4100m, respectively (Baniya, et al. 2010). As already mentioned above that, lichen has very unique secondary metabolites with wide ranges of biological activity, the knowledge of isolation of lichens secondary metabolites were developed recently with the improvement of culture techniques, because it makes easy for the extraction of metabolites (Oksanen 2006). Müller (2001) did a review about the pharmacologically relevant secondary metabolites of lichens and it was found that the presence of secondary metabolites like aliphatic acids, pulvinic acid derivatives, depsides anddepsidones, dibenzofuans, anthraquinones, naphthoquinones and epidithiopiperazinediones which has a high effectiveness as antibiotic, antimycobacterial, antiviral, antiinflammatory, analgesic, antipyretic, antiproliferative and cytotoxic effects. But so far the potential of lichens are not yet fully explored and not reach upto the level of pharmaceutical companies.

Lichen was used as a traditional medicine in various part of the world, temperate and arctic region in particular; it was believed that these European start using lichens from the fifteenth and sixteenth centuries. 52 lichen taxa were listed as a traditional medicine practiced by different culture and genus *Usnea* is amongst the widely used in the world (Crawford 2019). The lichens like *Parmeliopsis ambigua, Parmelia pertusa*

and *Hypogymnia physodes* extract were prepared with acetone solvents and tested for its antioxidant by DPPH superoxide anion scavenging activity, reducing power, antimicrobial by minimal inhibitory concentration against various pathogen and anticancer activities by microculture tetrazolium test on FemX (human melanoma) and LS174 (human colon carcinoma) cell lines. Out of these, *Parmeliopsis ambigua* was found to be effective in free radical scavenging properties. In addition to this, the contents of phenolic and flavanoids compounds were also determined as pyrocatechol and rutin equivalent. In case of anticancer activity, it was resulted with IC_{50} values ranging from 6.84 to 43.45 μg/ml (Kosanić et al., 2013). Recently, the Tomovic et al. (2019) reported briefly about the lichen extract of *Physcia semipinnata* as a new source of pharmacologically active compounds by evaluating the chemical characterization and biological activity and it was found that it contains lecanoric acid, methyl-β-orcinol carboxylate, ethyl haematommate, evernic acid, obtusic acid and atranorin, and they proved for their antioxidant activity and cytotoxic/cytostatic activity.

According to previous lab studies, it was found that, lichens have very unique antimicrobial properties, lichens extract with different solvents such as acetone, aqueous and methanol were used to treat against different bacteria and fungi species such as *Lecanora atra, L. muralis, Parmelia saxatilis, P. sulcate* and *Parmeliopsis ambigua* were tested against 6 different bacteria and 10 fungi species by using disc-diffusion technique to determine minimum inhibitory concentration (MIC) (Kosanić and Ranković, 2010). Acetone, diethyl ether and ethanol of *Cetraria aculeate* have also been tested against 12 bacteria and 8 fungi and it was found active that is due to the presence of active constituents of protolichesterinic acid (Türk et al., 2003). Moreover, the lichens extract of *Cladonia furcata, Parmelia caperata, Parmelia pertusa, Hypogymnia physodes* and *Umbilicaria polyphylla* were also reported by using disc diffusion methods against Gram-positive and Gram-negative bacteria and fungal organisms (Ranković et al., 2007).

Another study made by Odabasoglu et al., (2006) also reported on gastroprotective effect of usnic acid in rat model; which was based on traditional use of

Usnea longissima in Turkey for the treatment of gastric ulcer. The isolated usnic acid from *Usnea longissima* was conducted the test in indomethacin-induced gastric ulcers in rats at doses of 25, 50, 100 and 200 mg/kg body weight and found effective with the reduced of gastric lesion. Further, Sepulvedaet al., (2013) reported that lichens might be considered as a source of gastroprotective molecules by investigation of secondary metabolites of lichens depsidone and depsidewere preventing the gastric ulcer affected model at the doses of 30 mg/kg. The other metabolites like lobaric acid, atranorin and psoromic acid were able to reduced the gastric lesions by 76%, 63% and 65%, whereas variolaric acid, diffractaic acidand perlatolic acid with 32%, 14% and 45%, respectively.

2.2 NATIONAL SCENARIO

The knowledge of lichen in Indian subcontinent was from the time of Linnaeus, who reported two taxa from India (Linnaeus 1753, 1767). More taxa were added and published by Acharius (1810, 1814), Belanger (1838), Montagne (1842), Taylor (1847) and Babington (1852). A substantial increase in the knowledge of lichen taxa of Indian subcontinent took place in the second half of the 19th century based on collections by Hooker & Thomson, S. Kurz, Thwaites, Gray, Almquist and Watt. They were worked out by Nylander (1860, 1867, 1869, 1873, 1900), Leighton (1869), Mulier Argoviensis (1874-1891, 1892, 1895), Stirtoil (1875-77, 1879, 1881a, b, 1882, 1883), and Hue (J 900b). There were few additions in the earlier part of 20th century, as evidenced by the publications by Jatta (1902, 1905, 1911), Zahlbruckner (1911, 1932), A.L. Smith (J 931), Chopra (1934) and Motyka (1936-38). The descriptions of the 80 taxa of lichens by Chopra (1934), the first Indian to take up the study of lichens, were essentially based on the determinations by A.L. Smith (1931) and Zahlbruckner (1932). Lichenology had been a neglected field of study in the Indian Universities for teaching and research, and thereby basic facilities of requisite literature and authentic specimens for comparison, essential for taxonomic work, were not available.

There was no particular lichens reported from India at the early period, in the middle of 20th century, Awasthi (1965) reported 1310 species under 150 genera, then Singh (1980) made an addition of 685 species. Awasthi (1973) reported a new species of

Heterodermia himalayensis from the Pithoragarh area of Kumaun zone and so on the importance of lichens were rooted up in India. Further; Upreti (1998) revised the lichen genus *Pyrenula* from India. Upreti and Aptroot (1996) described a new species of *Lithothelium himalayense* from the Uttrakhand. According to checklist of lichens Singha et al. (2010) compiled 2303 lichen species under 305 genera and 74 families from India; Singha et al. (2018) reported the addition of 411 species to the list of annotated checklist 2010, therefore the total number of lichens recorded from India is 2714 species.

Asahina and Shibata (1954) reported that the acid present in the lichens were having efficacy to treat against antibacterial or antiviral and these acids were mostly phenolic-carboxylic acids such as atranorin, lobaric, salazinic acids, fatty acids like protolichesterenic acid and triterpenes derivatives zeorin. The preliminary phytochemical screening of some lichens such as *Flavoparmelia caperata, Roccella montagnei, Teloschistes flavicans, Physcia aipolia, Parmotrema austrosinensis, P. grayanum, P. tinctorum Nyl., P. reticulatum* and *Usnea subflorida* collected from Kodagu district, Karnataka were investigated by making lichens extract using with different solvents. And it was resulted that most of the lichens extracts were shown the presence of carbohydrates, proteins, steroids, tannins (Rashmi andRajkumar, 2014).

Saklani and Upreti (1992) have initiated the studies of ethnomedicinal properties on lichen species by collecting the important information of their traditional uses from the various parts of India (tribal and non-tribal communities). Further, Upreti et al. (2005) reported 15 lichen species commonly used in India by different tribes in day to day life for its medicinal as well as asthetics values. The studies also highlighted that western Himalayas are the main location for the collection of lichens and same lichens either raw form or after processing distributed to other parts of the country. Moreover, Upreti and Chatterjee (2007) had reviewed ethnomedicinal values of lichens and they found that more than 50 lichen taxa were used in different forms throughout the globe. Identically, Nayaka et al., (2010) reported 137 lichens possess medicinal properties from India from which 36 species were traditionally used; 55 species for antimicrobial activity, 57 for antioxidant property and 37 for anti-cancer activity. His finding also

showed that, *Cetraria islandica, Cladonia rangiferina, Evernia prunastri, Everniastrum cirrhatum, Hypogymnia physodes, Parmotrema chinense, Peltigera canina* and *Usnea longissima* were the common lichen species explored more for their uses in traditional medicine as well as some other biological purposes. But, due to over collection now these species are at their alarming stage.

Jayakumar et al., (2016) reported that endolichenic fungi known as *Talaromyces tratensis* isolated from the *Lecanora* species which was collected from the Andhra Pradesh. These endolichenic fungi used to identified on the basis of ITS4 and ITS5 ribosomal gene sequences and it was found active against bacteria and fungi; it also posses antioxidant properties. Devi et al., (2015) also evaluated the different solvent extract of *Parmotrema andinum* (Müll. Arg.) Hale andfound good antimicrobial properties against human pathogenic bacteria and fungiby using the Kirby-Bauer disc diffusion technique. Radika (2013) from Coimbatore, Tamil Nadu also reported that the extract of *Xanthoparmelia caperata* were tested against 2 bacteria and 3 fungi and found effective against *Escherichia coli*and *Staphylococcus aureus* only. In addition to this, the same extract was investigated for wound healing property on the rat model by coating the lichen extracts on the inner surface of bandage and the results was found good as compared with the normal bandage. Pavithra et al. (2013) investigated a macrolichen (*Usnea pictoides* G. Awasthi) collected from Mullayanagiri, Western Ghats of Chikmagalur, Karnataka, for its antioxidants, antimicrobial and the total phenolic contents. TLC results revealed that the presence of usnic acid and the phenolic contents was higher in methanolic extract; it was also reported for high susceptibility against both *S.aureus* and *C. neoformans* strain. Moreover, the dose dependent scavenging activities were observed in DPPH and ferric reducing assay.

Kambar et al. (2014) reported four lichens (*Leptogium burnetiae* C.W. Dodge, *Ramalina hossei* H. Magn and G. Awasthi, *Roccella montagnei* Bel. Em. D.D. Awasthi and *Heterodermia diademata* (Taylor) D.D. Awasthi) from Karnataka. Here also, the different extracts were tested for clinical isolates of burn, dental caries and urinary tract infections; where, extracts were more susceptibleto gram positive bacteria isolated from

urinary tract infection. Further, Tiwari et al. (2011) carried out the studies on the extracts of four lichen species namely *Bulbothirx setschwanensis, Everniastrum nepalense, Heterodermia diademata, Parmelaria thomsonii*) collected from Pithoragarh district, Uttarakhand for its antifungal activity against seven plant pathogenic fungi such as *Aspergillus flavus, A. fumigatus, Alternaria alternata, Fusarium oxysporum, F. solani, F. roseum* and *Penicillium citrinum*). And, acetone and methanol extracts of lichens were comparatively more effective against some broad spectrum plant pathogenic fungi. Additionally, Sati and Joshi (2011) reported the investigation of *Parmotrema nilgherrense* species collected from Nainital (Kumaun), Himalaya for its antibacterial properties by making out extract with 4 different solvents (methanol, ethanol, chloroform and aqueous) *Bacillus subtilis, Erwiniachrysanthemi, Escherichia coli, Agrobacterium tumefaciens* and *Xanthomonas phaseoli* by following agar well assay. From all extracts, chloroform extract was reported for higher zone of inhibition (23-38mm) followed by others; but there was no any zone of inhibition shown by aqueous extract.

Moreover, Bisht et al. (2014) studied the aqueous and methanol extract of *Peltigera* sp.and *Cladomia* sp. for total phenol contents and its antimicrobial activities against bacterial and fungal pathogens. The finding resulted that, the MIC of both sample have much variation as 0.7-27mg/ml. whereas, total phenolic contents were higher in *Peltigera* sp. both extract and values were recorded as 15.6mg GA/g and 14.3 respectively. Likewise, Pathak et al. (2016) were carried out the screening of lichens for antidermatophytic activity with the extracts of five lichens as *Bulbothrix setschwanensis, Myelochroa aurulenta, Parmotrema nilgherrense, Parmotrema reticulatum, and Ramalina conduplicans*) collected from Chakrata district, Uttarakhand. The broth microdilution technique was used for evaluation of their fungistatic and fungicidal doses against three dermatophytic fungi (*Microsporum canis, Trichophyton mentagrophytes* and *Trichophyton rubrum*). In comparison to all lichens species, *Myelochroa aurulenta* was found more active against the tested fungi. In addition to this, the study also had shown the report of two secondary metabolites (secalonic acid and leucotylic acid).

Further, Sharmaet al. (2012) reported that, among the 5 different lichens such as *Cladonia* sp., *Everniastrum* sp., *Parmelia* sp., *Stereocaulon*s p. and *Usnea* sp. collected from Darjeeling hills were evaluated against the gram positive and gram negative bacteria. Except *Everniastrum* sp. all above species were shown activity against the tested bacterial strain.

Lakshmi et al. (2013) reported the antidiabetic properties of *Parmelia perlata* extract in streptozotocin induced diabetic rats. The ethanol extract showed good activity of 21.5% at 500 mg/kg dose of extract in STZ-s animal model. Similarly, Lakshmi et al. (2013) investigated the ethanolic extract of lichen *Parmelia perlata* (Huds.) Ach.for its gastroprotective activity against the cold restraint (CRU), aspirin (AS), alcohol (AL) and pyloric ligation (PL) induced gastric ulcer models in rats. The significantly effective result of the extracts was found against CRU (50.0%), AS (37.5%), AL (65.41%) and PL (50.00%) induced ulcer models. It's also reduced free acidity (19.04%), total acidity (14.43%) and upregulated mucin secretion by 14.17% respectively. Whereas; Sharma et al., (2014) reported about the effectiveness of *usnic* acid; one of the most important secondary metabolites of lichen and the extract of *Cladonia furcata* on gastroesophageal reflux disease (GERD) in animal model. The study was carried out at different dose *C. furcata* extract, and the result was significantly reduced oesophageal index from 2.35±0.11 to 0.92±0.04. Further another study on *Parmelia perlata* (Huds.) Ach.which is also known as Dagadphool or stone flower in India from the family parmeliaceae was tested was tested in CCl4 intoxicated Albino Wistar rat as hepatoprotective agent with comparison to Silymarin as a standard drug.The results were found at 0.7 g / kg and1.0 g / kg dose (Shailajan et al., 2014). Behera et al. (2016) evaluated the ethyl aetate extract of 4 *Heterodermia* genus viz. *H. diademata, H. flabellata, H. antillarum* and *H. incana* for antilipoxygenase, radical scavenging and antimicrobial activities. The extracts were shown a significant lipoxygenase (LOX) inhibition at the concentration with 0.123, 0.153, 0.160, 0.150 mg/ml respectively. Further, the extracts were also shown radical scavenging properties as well as effective antimicrobial activity against the tested pathogens at the concentration of 0.232 mg/ml to 0.591 mg/ml.

2.3 NORTH EASTERN REGION

The north eastern region (NER) comprises of Arunachal Pradesh, Assam, Manipur, Meghalaya, Nagaland, Mizoram, Sikkim and Tripura considered as the hotspot region of biodiversity in the Indo-Burma region. NER is very interesting in their geographical features and favorable climatic conditions which support the growth of different varieties of flora and faunas (Myers et al., 2000). Lichens are also one of the most important resources from plant kingdom due to its luxuriant growth and wide distribution in whole NER. The first records of collection of lichens were made by Simons from Khasi hills and G. Watt from Manipur, later foliose and fruticose lichens was collected by Rolla Seshagiri Rao and Dr. G. Panigrahi of the Botanical Survey of India from Assam and North-East Frontier Agency during the years 1956-58. The collected samples were sent for identification to Dr. D.D. Awasthi, where 35 lichen specimens were reported from the state Assam and other 110 from the North-East Frontier Agency under 72 species under 18genera and 9 families (Awasthi, 1961).But, the exploration of lichens in various remote areas of north eastern region were exactly started with establishment of Botanical Survey of India (BSI) in Shillongfor the eastern circle in the year 1981. So far, prominent scientist and taxonomist were initiated the identification of various species of lichens and added many more new records for India (Sinha and Singh, 1986, 1987; Singh, 1981). In connection to this, Singh et al. (2018) have reported that the totaldata identified lichens were 2540 of India, where 1047 species comprised from the eastern region of Himalayas; which is about 41.22% of the total lichen biota of India.

In Assam, Routet al. (2010) identified 55 species under 26 genera and 15 families of lichens from Reserve forest of southern Assam. The study reflected the different growth forms, where crustose forms dominated with 89% of species followed by foliose with 11 % of total species including dominance of family Graphidaceae and Pyrenulaceae in this diversity hotspot region. Further, Daimari et al. (2014) documented the distribution of epiphytic lichens from 3 different districts like Baksa, Kamrup and Sonitpur of Assam and reported 67 species under 12 families and 24 genera have been

recorded along with 41 lichen species as new records for the state Assam. It also showed that, family Physciaceae dominated with 20 species followed by Graphidaceae with 16 species. Recently, Gogoi et al., (2019) added 25 new records of lichens under 19 genera and 11 families for the state of Assam, India. From, Arunachal Pradesh, Pinokiyo et al. (2008) reported 177 species under 71 genera and 35 families from the Mehao wildlife sanctuary in Arunachal Pradesh. Corticolous lichens was dominated the distribution followed by saxicolous and terricolous with 158, 17, 2 species respectively. Further, Singh et al. (2013) reported *Rhabdodiscus indicus* as a new record of species in lichen biota from Arunachal Pradesh including two other species *Ocellularia neopertusariiformis* Hale and *Ocellularia subgranulosa* (Homchantara & Coppins) Lumbsch & Papong as a new record for the India also. Moreover, Singh and Singh (2014) reported *Cyphelium inquinansa* calicioid lichens again from Arunachal Pradesh as a new distributional record from India. Recently, Debnath et al. (2018) added 17 new species collected West Kameng district and Tawang district which is located in the Eastern Himalayas of Arunachal Pradesh. In case of Manipur state, Devi et al. (2015) reported a total of 39 lichen species as new records for the state of Manipur, Northeast Indiaand 14 species as an addition for the north east lichen biota from the total of 140 species under 50 genera and 23 families. Crustose growth form dominated with 49% followed by foliose, dimorphic, fruticose, leprose and squamulose with 43%, 4%, 2%, 1% and 1% respectively. The occurrence of lichens have shown that the high diversity of this flora in the region.

Singh and Singh (2015) reported 38 species of graphidoid lichens as a new distribution from Manipur, Meghalaya and Nagaland in North-East India with *Graphis crassilabra* and *G. pavoniana* were also new records for India. Further, Singh and Singh (2016) have shown the first report from Meghalaya on distribution of lichens with 39 species including *Porina eminentior* as a new record for India. Further, Sinha and Ram (2011) have reported about rich diversityof lichens in Sikkim state. The study was initiated at lichen section of Sikkim Himalayan Regional Centre, Gangtok which was established in 1994, hence, till today 506 lichen species was reported from the region.

On the another hand, Sinha (2011) evaluated the acetone, methanol and aqueous extract of *Usnea baileyi* (Stirt) Zahlbr., *Parmotrema reticulatum* (Taylor) M.choisy, *Everniastrum nepalense* (Tayler) Hale ex sipman and *Cladonia mitis* (Sandst) Hustich collected from Ravangla, Sikkim for antibacterial activity against *Staphylococcus aureus, Escherichia coli, Vibrio cholerae, Shigella dysenteriae* and *Shigella flexneri*. And it was found that the methanol extracts showed active against all the bacteria strain while aqueous extracts didn't shows effect against all tested bacteria.

Likewise, in Mizoram, the natural resources are very rich due to the diverse species of flora and fauna. The state is situated in between the border of Myanmar and Bangladesh, where hilly region coveredwith 90% evergreen forest. Somehow, Chinlampianga et al. (2013) referred 2 species viz. *Cladonia fruticulosa* Kremp. And *Cladonia submultiformis* Asahina as first report of lichen collection from Mizoram in his paper. Moreover, in same finding 10 more species of lichen from different parts of the state were reported by him. Further; Logesh et al. (2015) have reported 159 species from different localities of Murlen National Park, Champhai district as well as different other places of Aizawl and Mamit district of the state. Where, 14 new species were recorded as the new for India these were named as *Buellia aeruginascens, Chaenotheca chrysocephala, Diorygma reniforme, Gassicurtia acidobaeomyceta, Graphis granulosa, Hafellia demutans, Phyllopsora soralifera, Ramboldia sorediata, R. subnexa, Relicina sublanea, Stigmatochroma adaucta, S. gerontoides, S. kryptoviolascens* and *S. metaleptodes.* Recently, Lalremruata et al. (2018) also investigated about the distribution of lichens in 2 different reserve forests like Ṭawi Wildlife Sanctuary, Aizawl District and Thorangtlang Wildlife Sanctuary, Lunglei District; and reported 118 species under 37 genus and 17 families with 10 species as new record for the state of Mizoram such as *Bacidia connexula, Buellia curtisii, Caloplaca citrina, Herpothallon philippinum, Leptogium delavayi, Pertusaria pertusa, Pertusaria pseudococcodes, Pyrenula andina, Trichothelium epiphyllum* and *Usnea nipparensis*. Additionally, Thangjam et al. (2019) evaluated the diversity and distribution of lichens in Central Part of Murlen National Park (MNP), Mizoram; and enumerated 20 species under 15 genera and 10 families. The

family parmeliaceae showed the highest number of species. Moreover, the growth form was dominated by crustose compared to foliose and fruticose. The richness of lichen diversity in this protected area specifies the diversity of lichen in Mizoram.

Moreover, in relation to lichen antimicrobial studies, there was only one literature is available so far; where, Shukla et al. (2011) studied the antifungal property of aqueous and acetone extracts of two selected lichens *Ramalina* sp. and *Stereocaulon* sp. against plant pathogenic fungi such as *Alternaria alternata*, *Aspergillus flavus* and *Penicillium italicum*. And it was found that acetone extracts showed more potent activity than aqueous extracts.

CHAPTER -3

Materials and Methods

3.1 PROFILE OF MIZORAM

Mizoram is the states belong in the North Eastern Region of India. The name Mizoram is taken from **"Mi"** that indicates (people); "**Zo"** that indicates the (hill) and Ram (land), therefore, Mizoram is known as the "Land of the hill people". The state Mizoram lies between21^{o}58' north to 24^{o}35' north latitude and 92^{o}15' east to 93^{o}29' east longitude. The total covering area of the state is 21,087sq.km. Mizoram shared the border with Cachar district of Assam and Manipur state in the northern part, the ranges of Arakan hills of Myanmar on the southern side, on the eastern part of the side Chin Hills of Myanmar and Tripura and Chittagong hills of Bangladesh on the western region respectively. Aizawl district is the capital of the state. The state is covered maximum part by forest according to their forest report of 2015 (GB Pant, 2017)

The topography of the region consists of parallel hill ranges running in north-south direction, in the altitude vary from 40m to 2157m. The state has a total forest area of 18576sq km. The terrain is highly dissected with streams and rivers. Forest cover is primary and secondary type comprising tropical wet evergreen, tropical semi-evergreen and sub-tropical type forests. The climatic condition and the forest types both provide a suitable condition for growth of many plant groups from lower to higher including lichens. The climate of Mizoram is moderate, a warm climatic condition in the summer and cold winter with the rainy season from april to october. Temperature varies for around 11°C in the cold winter to 30°C in the hot summer approximately. The state has receives the total annual rainfall between 2000 - 3600 mm from both north-east and south-west monsoons respectively.

3.2 THE STUDY AREA

There were three area have been selected for study of lichens biota shown in (Figure. 3.1)

3.2.1 MURLEN NATIONAL PARK (MNP)

The Murlen National Park is located in Champhai district of Mizoram, India; and its lies between N 23º32'42" to 23º41'36" (Latitude); E 92º13'12" to 92º27'24" (Longitude) and having an altitude of 400 to 1897 meters (Figure 3.1 (green). The total coverage area of the park is 200km^2. The park is also located around 245 km east of Aizawl district (State Capital). The Park is close to the Indo-Myanmar border and is significant for its proximity to the Chin Hills. This Park is also known to be one of the dense forests in the country, which allows the sunlight to penetrate at minimal level. MNP is situated at Murlen Village, once it was ruled by the chief of Hnahlan. It was declared as National Park in 1991; vide Government of Mizoram No. B.11011/13/84-FST dated 8.7.91. (Chaudhary, 2018). Recently, it has been categorized as one of the National Parks under Category II of United Nations list of protected area (PA). It is the last home of Himalayan Black Bear in the Eastern corner of the country. Moreover, it is homely to a variety of floral and faunal species that are endemic to Mizoram and the NER. The forest is a type of Sub-tropical semi evergreen and Semi-montane type and the region fall within the geographical sub- tropic and enjoys sub- tropical climate; the climate of MNP is warm and moist in the summer and cool temperature and dry condition in the winter. The Annual rainfall ranges between 1700mm to 3900mm respectively (MoEF 2018). The park is also rich in biodiversity, shelter and protects rare, endangered & threatened species.

Location	Champhai District, Mizoram
Latitude	23º 32' 42" to 23º 41' 36" N
Longitude	92º 13' 12" to 92º 27' 24" E
Altitude	400 mts to 1897 mts
Area	200 Sq.km
Average Rainfall	2300 mm

Climate	Pleasant and equable warm climate throughout the year with moderate to chilly winter during November - January at higher altitudes
Temperature	8°C to 30°C

3.2.2 REIEK HILL

Reiek hill is located in the Mamit districts of Mizoram just 29 km from the main capital city of Aizawl. It lies between 20°45' and 22°28' N latitude and 92°37' and 93°46' E longitude with the altitude of 1465 metres (Figure 3.1 (Blue).Since 1980s the Sailo chief was declared Reiek forest was declared as a protected area and it was following a very strict prohibition rules and regulation against killing of wild life animals and the plants that was found in the protected area to maintain the conservation of biodiversity. The forest has a pleasant climatic condition with mild winters and hot summer and the temperature ranges from 8-22℃ and 20-28℃ in winter and summer respectively. The forest is covered with rocky-sandy soil in the top layer, hard stone plates in the peak and the rest parts with sandy-loam to black humus. The vegetation of the forest is tropical semi-evergreen forest or subtropical evergreen forest (Lalzarzovi ST and Lalnuntluanga, 2013, Lalzarzovi ST and Lalnuntluanga, 2018).

Reiek is a mountain and it is one of the most breathtaking tourist spot of Mizoram, Reiek is a typical type of Mizo village where the ancient traditional huts of the different mizo's sub- tribes is preserved and maintain by the Tourism Department, Govt of Mizoram. Reiek hill was surrounded by the thick lush green temperate trees and bushes and the plain region of the Bangladesh can be seen clearly from the hill top on the fine climate day. This area is also the venue for the annually conducted festival called "Anthurium" (Web.4).

Location	Mamit District, Mizoram
Latitude	20°45' and 22°28' N
Longitude	92°37' and 93°46' E
Altitude	1465 metres
Average Rainfall	2500mm
Climate	Pleasant climate with mild winters and summers temperature
Temperature	8℃-22℃ in winter and 20℃-28℃ in summer.

3.2.3 MIZORAM UNIVERSITY CAMPUS

The present studies were carried out in Mizoram University (MZU) as well (Figure 3(orange). MZU is located nearby Tanhril village which is located in the western part of the Aizawlat a distance of 15 km from the Aizawl city. It lies between 23°45’25" and 23°43’37" N latitude and 92°38’39" and 92°40’23" E and cover the areas of 978.1988 acres with altitude ranging from 300 m to 880 m. The climatic condition is humid with winter (November – February), summer (March – October) and heavy rainfall. The temperature ranges from 13-36℃ throughout the year (Lalrinawmi et al., 2018).

The campus comes under the Tanhril village which was established in the year 1881. The name of the village was given Tanhril taken from the first local chief of the village ‘‘Tanhrila’’. The area of the region is a dense forest and fully vegetation till 1960 later it was used for agriculture as it was the main occupation of the people during those days. The vegetation of the campus falls under tropical semi-evergreen forests. Plant diversity in this area is quite rich particularly in the western sides representing the forests with less biotic disturbance. Mizoram university campus is highly diverse in its natural habitat and ecosystem (Web 5).

Location	Aizawl District, Mizoram
Latitude	23°45’25" and 23°43’37" N
Longitude	92°38’39’’ and 92°40’23" E
Altitude	300 to 880 meters
Area	978.1988 acres
Average Rainfall	182 mm.
Climate	Pleasant climate throughout the year with short winter and long summer, Heavy rainfall.
Temperature	8°C to 32°C

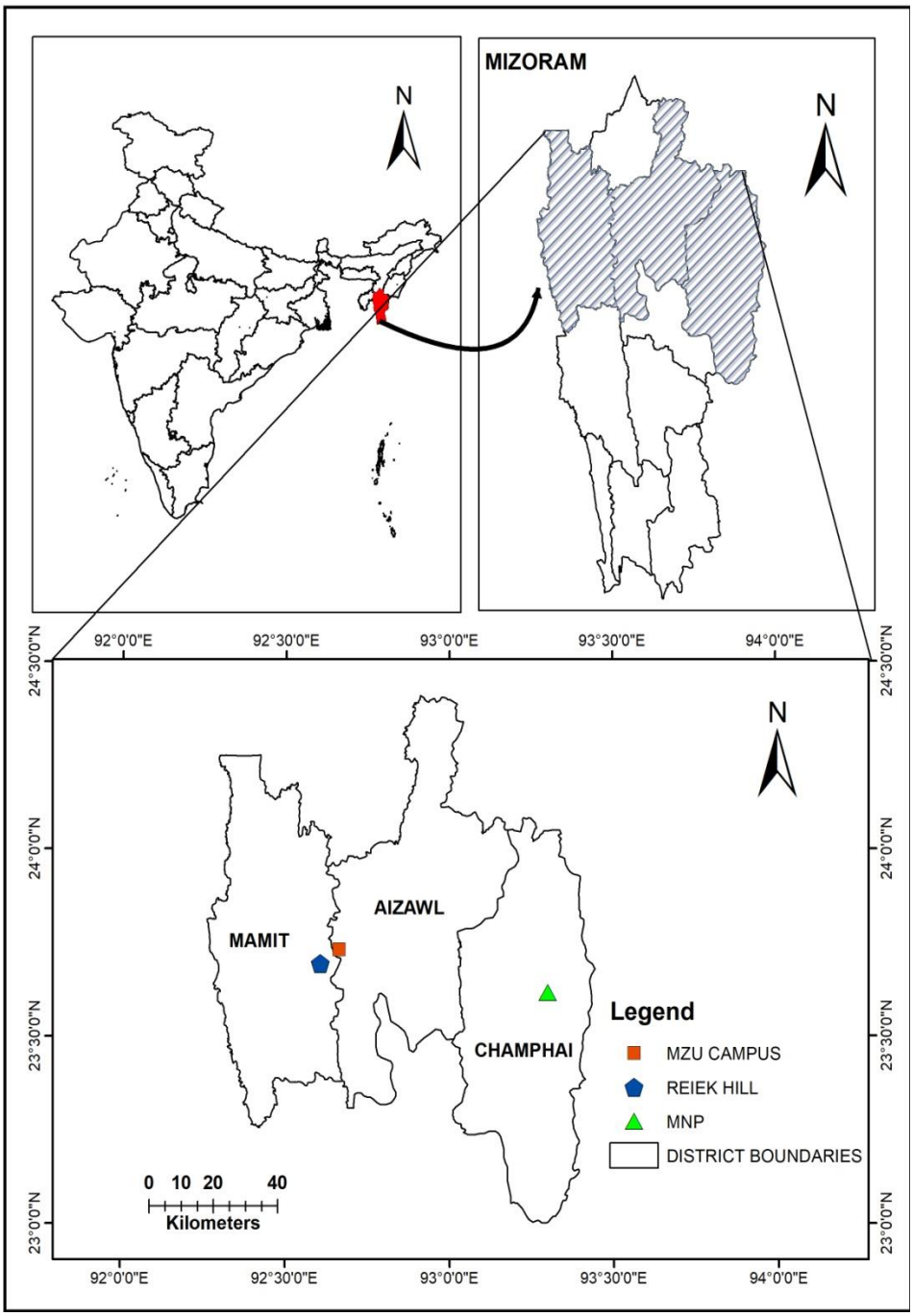


Figure 3.1 GIS Mapping of 3 study sites (A) MZU Campus (B) Reiek Hill (C) Murlen National Parks (MNP).

3.3 METHODS

3.3.1 COLLECTION AND CATEGORIZATION OF LICHENS

A frequent field visit was conducted at different seasons in all the selected sites for collection of lichen specimens as per thestandard procedures (Nayaka, 2014; Devi et al. 2015). Further, the processing of specimens like removal of unwanted materials (Bryophytes and substratum) as well as drying by using hot air oven was carried out in Department of Horticulture, Aromatic and Medicinal Plants (HAMP), Mizoram University, Aizawl; then the collected specimens were preserved for herbarium. Based on growth forms of lichen (crustose, foliose and fruticose) the categorization process was made. The lichens have their ethnomedicinal importance along with other commonly used lichen species were emphasized for detail studies during the research work.

MORPHOLOGICAL IDENTIFICATION OF LICHENS

The lichens specimens' wereidentified through comparative studies based on their anatomical, morphological and biochemical observations and it was done at Lichenology Laboratory, CSIR–National Botanical Research Institute (NBRI), Lucknow. The morphological variations of each specimen were studied by using Leica S8APO stereo-zoom microscope, whereas anatomical studies were made by Leica DM500 compound microscope. The microscopic details of each specimen were performed by cutting the transverse section of fruiting bodies, where the cotton blue was used as a staining media and lactophenol as mounting media (Thangjam et al., 2019). Moreover, the specimens were identified and authenticated with previous literatures on lichens by (Awasthi 1993, 2007; Nayaka 2004, Divakar and Upreti 2005, Singh and Sinha 2010, Shukla et al., 2014)

The external morphology was examined, generally in dry condition but dark brown to bluish specimens of some cyanophycean lichens was also be examined in wet condition. The anatomical structures were studied after cutting the sections of dry material by microtome or with the help of safety razor blade. The thin dry sections of the thallus or apothecia was immersed in 90% ethyl alcohol to drive off the intercellular or

inter-hyphal air bubbles and the sections was mounted in water or in cotton blue in lactophenol. The colour of medulla, epithecium, hypothecium and ascus was observed. The asci and ascospores were taken from the sections when sections were mounted in water and shapes, sizes are recorded. The measurements of the thallus, medulla, epithecium, and hymenium were generally taken in the sections mounted in cotton blue. The thallus sizes was measure in centimetre, lobe and apothecia size in millimetre and thallus medulla, epithecium, hymenium thickness, asci and ascospores size in millimicron.

CHEMICAL IDENTIFICATION OF LICHENS

Chemical identification of the lichen specimens were carried out with colour tests and thin layer chromatography (TLC).

(a) Colour tests

Colour test were performed by applying the chemicals reagents on thallus and medulla and observed with the colour change.

A positive change denoted by a positive symbol (+ve), followed by the colour produced and no change in colour denoted by a negative symbol (-ve).

The chemical reagents required for the test are summarizing as follows:

***K* test:**

10-25% aqueous solutions of potassium hydroxide (10g KOH pellets + 100 ml distilled water) were applied to cortex, medulla and part of apothecia. It can be used as a clearing agent for sections of fruiting bodies and thalli, as it often dissolves the crystalline lichen substances and removes some mucilage that may obscure detail in sections.

Pd test:

Solution of paraphenylenediamine was prepared in ethanol or alcohol in a small quantity for the use of a day, because it is unstable and cannot be used for the next day. A more stable solution called Steiner's Pd was prepared by dissolving 1.0gm of paraphenylene diamine and 10gm of sodium sulphite in 100ml of distilled water with 1.0ml of a liquid detergent. This reagent can keeps well for about a month.

C test:

A freshly prepared aqueous solution of calcium hypochlorite or bleaching powder or modern commercial bleaching fluid containing active chlorine was used. This can be prepared by dissolving calcium hypochlorite in the distilled water in 2% ratio.

KC test:

At a particular spot of thallus, K was applied first and immediately followed by C.

I test:

2-5gm of iodine was dissolved in water with 0.5 gm of potassium iodide. It does not react with lichen secondary metabolites but rather with starch like polysaccharides in the thallus or fruiting body. The reagent keeps well for several days and is to be renewed when colour fades.

(b)Micro-imaging of the Crystals

The method does not need elaborate equipment. A small fragment of lichen to be investigated were placed on the middle part of a microscopic glass slide and one-two drops of acetone or any other organic solvent was dripped on to the fragment by means of dropper pipette. Lichen substances if present were dissolved in the solvent and extracted on the slide as residue in a ring formed around the fragment as soon as the solvent evaporates. The thallus fragment were blow off. A micro-cover glass was placed over the residue and a drop of one of the crystallizing fluids (detailed below) and placed at the edge of the cover glass. The fluid gradually seeps in. The slide will be heated gently over a sprit lamp. The residue dissolves in the fluid and lichen substances gradually crystallize into their characteristic of shapes on cooling. These crystals may be observed under low power of microscope and identified by comparison with the photographs or line diagram. Identification of depsides, depsidones and dibenzo-furans can usually beconfirmed by this method.

The crystallizing fluids will be as follows:

- G.E. Glycerol: acetic acid, 1:3

- G.A.W- Glycerol: ethanol: water, 1:1:1
- G.A.Ot- Glycerol: ethanol: ortho-toluidine, 2:2:1
- G.A.An- Glycerol: ethanol: aniline, 2:2:1
- G.A.Q- Glycerol: ethanol: quinoline, 2:2:1.

(c) Chromatographic separation

It was mainly performed in solvent system A (Toluene: 1, 4-dioxane: acetic acid: 180: 60: 8ml) but sometimes in solvent system B (Hexane: Diethyl ether: Formic acid: 130: 100: 20ml). The chemical substances were extracted in acetone and loaded on silica gel pre-coated aluminium plates. After running in solvent system A, the TLC plates were sprayed with distilled water for checking the presence of fatty acids. Later the plates was sprayed with 10% H_2SO_4 solution and heated in hot air oven at 110°-120°C till the colour spots develops, due to charring. *Parmelinella wallichiana* (Taylor) Elix and Hale, having Salazinic acid (Rf class 2), Norstictic acid (Rf class 4) and Atranorin (Rf class 7), were used as reference material. The TLC was observed under UV radiations at 350nm wavelength before and after charring.

Table 3.1 Identification technique of Rf classes of lichen by TLC, colour spot, colour test and substances.

Rf class	**Colour of spot**	**Identification of lichen substances**	**Colour test**
1 - 2	Dark grey	Fumarprotocetraric acid, Protocetraric acid	PD+ yellow- red
1 - 2	Grey - Orange	Thamnolic acid	K+ yellow – orange, PD+ orange
2	Yellow - Orange	Salazinic acid	K+ yellow – orange, PD+ red
2	Pale violet grey	Pannaric acid	C+green
3	Orange	Stitic acid	K+yellow, PD+orange

3	Pale	Physodic acid	KC+ orange - red
3	Yellow or grey	Gyrophoric acid	C+ red
3	Yellow - grey	Lecanoric acid	C+ red
3	Pale green – grey	Lobaric acid	KC+ red
3	Dull yellow - brown	Psoromic acid	PD- yellow – red
3 – 4	Pale straw	Olivetoric acid	C+red
4	Bright yellow	Norstictic acid	K+ red, PD+ orange
4	Orange	Sekakaic acid	-
4	Yellow	Barbatic acid	-
4 - 5	Yellow - orange	Perlatalic acid	-
5	Violet	Zeorin	-
6-7	Pale	Lichexanthone	UV+, yellow- orange
7	Greenish grey	Usnic acid	UV+ quench
7	Dark green	Pannarin	PD Orange
7	Yellow - orange	Atranorin	K+ yellow

(d) Other colour tests

A dilute aqueous solution of nitric acid and an aqueous solution of ferric chloride are sometime used for identification of some crustose species. The spot tests can be done on any part of the thallus but younger parts give better results. Colour test was done at small fragment of the thallus part of desired lichen or thallus or ascocarp. A definite colour comes showing the presence of any lichen.

UV test

A number of secondary metabolites in lichens exhibit a characteristic fluorescence under UV light. The response of (+ve or -ve) these metabolites plays a vital role in the lichen identification.

DOCUMENTATION OF LICHENS (HERBARIUM DATABASE)

Based on the above morphological and chemical characterizations; the identified lichens were confirmed with the help of Lichenologist in the Laboratory of Lichenology,

National Botanical Research Institute (CSIR Lab), Lucknow and the identified lichens specimens were properly packed inside a herbarium packet which is specifically made for lichens. The herbarium sheets were categorized with area of collection, date of collection, name of lichens and its family, altitude and name of collector. Lastly, all the specimens were deposited in lichen herbarium (LWG) of CSIR-NBRI, Lucknow as well as Department of HAMP, Mizoram University, Aizawl.

EXTRACTION OF THE SECONDARY METABOLITESAND ITS ANTIFUNGAL SCREENING

Lichen samples were sort, clean of substratum and dry for extraction. Three different solvent systems *i.e*. aqueous, acetone and methanol were used for extraction of the secondary metabolites/ constituents. The selected lichens thus extracted were investigated for preliminary phytochemical screening, antioxidant, antifungal screening and gastro-protective activity.

3.3.2 EXTRACTION OF THE SECONDARY METABOLITES FROM SELECTED LICHENS

The selected lichen specimens were done with the shade dry at room temperature. The dry thalli of the particular lichen were grounded into powdery form with the help of the grinder. 100g of the powder was soaked at 300 ml of acetone, aqueous and methanol solvent respectively for 48 hours by cold maceration process. The solution was filtered using Whatman filter paper No.1 then the filtered were concentrated and the solvents were evaporated using rotary vacuum evaporator. Further the extract was again concentrated by keeping in the water bath at 20- 40°C. The complete dry extracts obtained were stored at the refrigerator at 4°C for further experiments.

3.3.3 PHYTOCHEMICAL SCREENING

The phytochemical characterizations of the 6 potential lichen samples were carried out by following the methods of (Rashmi and Rajkumar, 2014).

(a) Test for Tannins:

Ferric chloride Test- 2 ml crude extract mix with few drops of 5% ferric chloride solution; formation of blue color indicates presence of hydrolysable tannins.

(b)Test for Alkaloids:

Dragondroff's test- 2 ml of crude extract adds to 1% HCl, steam for 10 minutes. Further, add 6 drops of Dragondroff's reagent; reddish brown precipitate indicates the presence of alkaloids.

(c) Test for Saponins:

Frothing test- 2 ml of crude extract mix with 5 ml of distilled water in a test tube and shake vigorously; formation of stable foam indicates the presence of saponins.

(d) Test for glycosides:

Keller-kilani test- 2 ml of crude extracts mix with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. Poured the mixture into another test tube, containing 2 ml of concentrated H_2SO_4; brown ring at the interphase indicates the presence of cardiac glycosides.

(e)Test for Flavonoids:

NaOH solution test- 2 ml of crude extract adds into 2 ml of 10% NaOH solution; yellow to orange colour indicates the presence of flavanoids.

(f)Test for Proteins:

Xanthoproteic test- 2 ml of crude extract adds into 2 ml of HNO_3, boil in a water bath; orange colour indicates the presence of proteins.

(g)Test for Triterpenoids:

Salkowski Test- 2 ml of crude extract shake with 1 ml of chloroform and add few drops of concentrated sulphuric acid (H_2SO_4) along with the side of the test tube. Red brown colour formed at the interface; indicates the presence of triterpenoids.

(h) Test for carbohydrates:

Benedict's test- 2 ml of crude extract mix with 2 ml of Benedict's reagent and boiled; reddish-brown precipitate indicates the presence of carbohydrates

(i) Test for Steroids:

Liebermann-Burchard reaction- 2 ml of crude extract adds into 2 ml acetic anhydride and adds few drops of conc. H_2SO_4; blue-green ring indicates the presence of steroids.

3.3.4 ANTIOXIDANTS PROPERTIES OF SELECTED LICHENS

DPPH Free Radical Scavenging Activity:

Radical scavenging activities of the selected lichens extract were determined by calorimetric assay using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a source of free radical according as a source of free radical following the method of Blois (1958) with a slight modification. Different concentrations of extract and standard were prepared in the solvent methanol and mixed with to the clean test tube containing 2ml of 0.1 mM methanol DPPH solution separately.The mixture was then shaken vigorously and left for 30 minutes at room temperature in the dark. The absorbance was measured at 517nm in UV visible spectrophometer. The absorbance of the DPPH control was also noted.

This experiment was called as percent DPPH scavenging activity and is calculated as per the formula given below-

$$\% \text{ DPPH Scavenging} = \frac{Control\ absorbance - Sample\ absorbance}{Control\ absorbance} \times 100$$

The IC_{50} value was also calculated**.**

Reducing Power:

The reducing power of the methanolic extract of different selected lichens extract was evaluated by using the method of Oyaizu (1986) with slight modification. In this activity, 10mg of the extract into 1 ml of distilled water was mixed in the mixture of 2.5 ml of 0.2M phosphate buffer (pH 6.6) and 2.5 ml of 1% Potassium ferricyanide and incubated for 20 minutes at 50^{o}C. After incubation, 2.5 ml of 10% tri chloro acetic acid was added to the mixture and then centrifuged at 3000 rpm for 10 min. Later on, the supernatant layer of the solution (2.5ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1 %); further absorbance was recorded at 517nm. The increased value of absorbance of the reaction mixture indicated the increased reducing power or high reducing power.

Superoxide radical scavenging activity by alkaline DMSO method:

The Superoxide radical scavenging activity of the different selected lichens extract was evaluated by following the method of Kunchandy and Rao (1990). In this method basically, the superoxide radical is produced with the addition of Sodium hydroxide to air saturated DMSO. The generated superoxide remains stable in solution and reduces nitrobluetetrazolium (NBT) into formazan dye at room temperature which can be measured at 560 nm. The weighted 0.1 mL of NBT (1 mg/mL) was added to the reaction mixture containing 1 mL of alkaline DMSO (1 mL DMSO containing 5 mMNaOH in 0.1 mL water) and 0.3 mL of the extract in freshly distilled DMSO at various concentrations and made a final volume of 1.4 ml. The absorbance was measured at 560 nm.

Determination of total phenolic contents:

The total phenolic content (TPC) of the different selected lichens extract was determined by the Folin-Ciocalteu colorimetric method given by Slinkard and Singleton (1977). Here, different concentrationsof extracts were mixed with 1.0 ml of 10 fold diluted Folin-Ciocalteu reagent and 1 ml of saturated sodiumcarbonate solution. Further the solution was kept for 30 min at 30°C without any disturbances and then, absorbance was recorded at 725 nm with a UV-Visible spectrophotometer. Following the same procedures the standard curve of gallic acid solution was prepared and used for measurement of total phenolic content where results were expressed as mg gallic acid equivalent (GAE)/100 g of extract. The standard curve of gallic acid was used for comparative study with extracts. All the experiments were carried out in triplicate.

Determination of total Flavonoids contents:

The total Flavonoids contents of different selected lichens extract was evaluated by using the aluminum chloride colorimetric method. Quercetin was used to make the standard calibration curve. The stock solution of quercetin was prepared by dissolving 1mg of quercetin in 1ml of methanol. 2ml of 2% aluminum chloride was mixed with the same volume of extract solution or standard quercetin solutions. After mixing, the solution was incubated for 10 min at room temperature. The absorbance of the reaction

mixtures was measured against blank at 415 nm with a UV-Vis spectrophotometer. Further, total flavonoids content were calculated using a standard quercetin curve and results were expressed as mg quercetin equivalent (QE)/g of extract. All the experiments were carried out in triplicate.

3.3.5 ANTI-FUNGAL PROPERTIES OF SELECTED LICHENS

Procurement and multiplication of test pathogens

The authentic cultures (Microbial type culture collection) of the targeted pathogenic fungi were procured from the Institute of Microbial Technology (IMTECH), Chandigarh, India. The cultures thus procured was revived and multiplied on the potato dextrose agar (PDA) for further investigations, and the routine sub-culturing was done for maintaining the pure culture throughout the study.

Names of tested pathogens with their MTCC number

1. *Epidermophyton floccosum* Hartz (MTCC-7880)
2. *Trichophyton mentagrophytes* (Robin) Blanchard (MTCC-8476)
3. *Aspergillus flavus* Link (MTCC 9064)
4. *Aspergillus fumigatus* Fresenius (MTCC 1811).

PREPARATION OF MEDIA

Potato Dextrose Agar medium

The commercially available (HiMedia) potato dextrose agar medium (39g) was suspended in 1000ml of distilled water. The medium was dissolved completely by boiling and was then autoclaved at 15 lbs pressure (121ºC) for 15 minutes. Further, the pure plates were prepared by addition of antibiotics and performed the antifungal experiments afterwards.

Potato Dextrose Broth medium

The commercially available (HiMedia) potato dextrose broth medium (24g) was suspended in 1000ml of distilled water. The medium was dissolved completely by boiling and was then autoclaved at 15 lbs pressure (121ºC) for 15 minutes. Further, the pure culture tubes were prepared by addition of antibiotics and performed the antifungal experiments afterwards.

IN-VITRO STUDY OF SELECTED LICHENS EXTRACT AGAINST TEST PATHOGENS

The method was used to determine the antifungal activity of selected lichens extract and it was performed by the agar well diffusion method against fungal pathogens in order to evaluate their virtues required for an ideal antifungal agent. Further, the minimum inhibitory concentration (MIC) of each extract was calculated separately with help of zone of inhibition.

Agar well diffusion assay:

The experiment was carried out by agar well diffusion method to investigate the Minimum inhibitory concentration (MIC) of lichen extracts against the 4 selected fungi. The test fungi were inoculated into sterile Potato dextrose broth in the test tubes and incubated at 25±2^{o}C for 72 hours. The broth culture was spread onto the media plate by using the L shaped glass/plastic spreader in a sterile condition (LAF). The inoculated plate was punched for making a well of 6mm with help of sterile cork borer. 100µl of lichen extracts (200mg/ml of DMSO (Dimethylsulfoxide), 2 standard drugs meconazole and ketoconazole and negative control DMSO was transferred into their respective wells (as marked behind the plates before experiments). The plates were incubated in the BOD at the set temperature of 25±2^{o}C for 72 hours and zones of inhibition values were recorded using antibiotic measuring scale. Each experiment was repeated in triplicate. The zone of inhibition was calculated by using the following formula:

$$W = \frac{T - D}{2}$$

Where:

W - Diameter of clear zone of inhibition

T - Total diameter of including well and clear zone

D - Diameter of the well.

Comparison with some Synthetic Fungicides:

The efficacy of lichens extract was compared with some synthetic antifungal drugs including determination of MICs values. This activity was carried out by method

given by Shukla et al, (2003).

The above investigation was carried out at Natural Product Research Laboratory, Department of Horticulture, Aromatic and Medicinal Plants, Mizoram University Aizawl and Natural Product Chemistry Laboratory, Institute of Bioresource and Sustainaible Development, Imphal.

3.3.6 *IN VIVO* GASTROPROTECTIVE ACTIVITIES OF SELECTED LICHEN EXTRACTS ON ALBINO RATS

Animals:

Inbred adult male and female albino rats of 150-200 g were used for the experiment. The animals were maintained in a well-ventilated room at a temperature of 25 ± 2^{0}C with 12:12 hour light/dark cycle in polypropylene cages. The standard pellet feed (Hindustan lever, Bangalore) and tap water were provided *ad libitum* throughout the experimentation period. Animals were acclimatized to laboratory conditions of about 10 days prior to initiation of experiments. All the experimental process and protocol used for this study were reviewed by the Institutional Animal Ethical Committee and it was according to guidelines of the CPCSEA (2003).

Ethical statement:

The experiment was performed in compliance with the guidelines for the care and use of laboratory animals in line under Mizoram University Animal Ethics Committee (MZUAEC), Mizoram University, Aizawl, Mizoram, India. Approval no. (**MZU-IAEC/ 2018-2019/11**).

Acute toxicity test

The acute toxicity studies of the methanol extract of *Usnea baileyi* was carried out for the selection of dose according to the OECD guidelines (No, 2001). The extract was administered orally at different doses up to 2000mg/kg and the observation was made for 48 hrs for its behavioral changes, any toxicity, and mortality. No acute toxicity effect was found and accordingly the dose was selected at 50, 100 and 200mg/kg for gastro- protective evaluation (Odabasoglu et al., 2006, thangjam et al., 2019)

EXPERIMENTAL DESIGN OF GASTROPROTECTIVE STUDY

Ethanol-induced Gastric ulcer

Adult Male and female wistar albino rats weighed 150-200g were withdrawn from food 24 hrs and water was also removed prior to the experiment. Six group having 5 animals in each group were maintained, control groups were administered orally with 0.5% CMC vehicle (5 ml/kg); Standard groups were given omeprazole drugs with 20 mg/kg body wt. in 0.5% CMC; Selected lichen extract (*Usnea baileyi*) were administered orally with 50, 100, 200 mg/kg body wt. respectively as pretreatment in the 3 extract groups. After 1 hour of pretreatment Ulcer groups were fed with 0.5% CMC and absolute ethanol (5 mL/kg) along with all the others pretreated groups. After one hour later; all the animals were euthanized through the overdose of chloroform, stomachs were dissected and opened along greater curvature for evaluating the number and the length of gastric lesions (Sagun et al., 2013; Sistani et al., 2019).

Measurement of gastric juice pH and acidity

Gatric juice obtained from the open stomach were drained and collected, then centrifuged at 1000rpm for 10 mins at 4°Cand checked the pH at 580 nm; the gastric contents were observed for the hydrogen ion concentrations using pH meter titration with 0.1 N NaOH. The total acidity was determined and expressed as mEq/L. The gastric mucosa of the stomach was scraped smoothly using a glass slide and weighed using an electronic balance (AL-Wajeeh et al. 2016, Sagun et al. 2017)

Acidity = volume of NaOH × Normality of NaOH × Molecular volume ×1000 mEq/L

Ulcer index and preventive index

Ulcer index was measured according to the scoring of the ulcer by using magnifying lens. The score was made as normal colour, red colouration, spot ulcer, hemorrhagic streak, deep ulcers, perforation count as 0, 1, 1.5, 2, 2.5, 3 respectively (Raju et al. 2009; Sagun et al. 2017) and it was calculated by formula given as follows:

$$\text{Ulcer Score} = \frac{\text{Total number of ulcers}}{\text{numbers of rats}}$$

$$\text{Ulcer Index} = \frac{\text{Total number of ulcers}}{\text{numbers of rats}} \times 100$$

$$\text{Preventive Index} = \frac{Ulcer\ index\ of\ Ulcer\ group - Ulcer\ index\ of\ Treated\ group}{Ulcer\ index\ of\ Ulcer\ group} \times 100$$

Histopathology

The section of each group at 5 µm thickness was cut from pieces of stomach with the help of rotary microtome (Leica, model RM2125 RTS); embedded into paraffin wax and deparaffinized in xylene; stained with hematoxylin-eosin and examined under microscope with standard light microscope (Leica DM 2500) attached with a digital camera (model-DFC 450C) (Leica Microsystems, Wetzlar, Germany) for histopathological alterations and photographed (Sagun et al. 2017; Yau et al. 2017).

3.3.7 STATISTICAL ANALYSIS

The data obtained from the experiment of antioxidant activity and TPC and TFC assays were expressed by a mean and standard deviation. To evaluate statistical differences for the antifungal and gastroprotective, Data were analyzed by using One-way ANOVA followed by Duncan Multiple rangetest ($p<0.05$).

CHAPTER -4

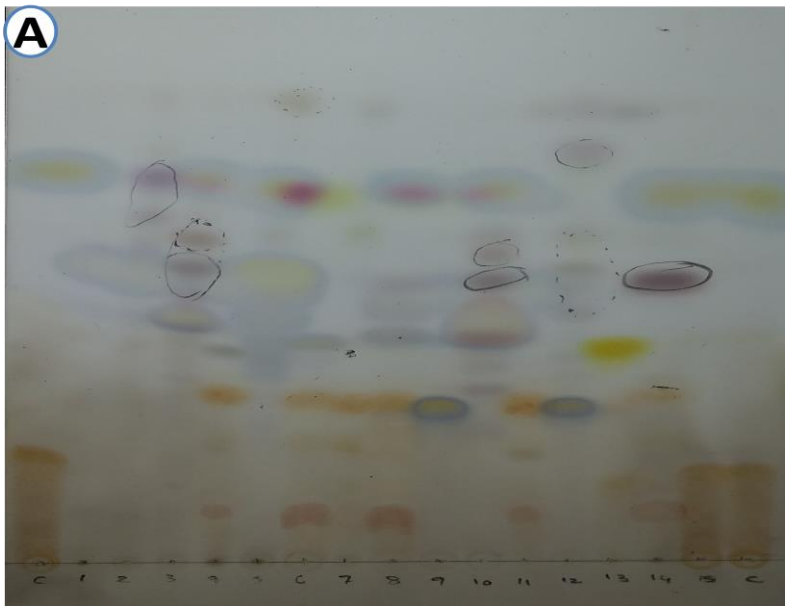
Results

4.1SYSTEMATIC INVESTIGATION

The present studies revealed that 78 lichens species were identified from 3 different selected sites. Out of total identified lichens, 22 new species were reported as the new records for the North East Region (NER) as well as for state of Mizoramincluding 1 new species (*Pyrenula dissimulans*) as the record of India lichen biota. In addition to this, 20 species from central part of the Murlen National Park, Champhai District, Mizoram; 20 species from the Reiek hill which is located in the Mamit District and 16 species from the Mizoram University Campus, Aizawl District were also reported.

4.2CHROMATOGRAPHY OF COLLECTED LICHENS SPECIMENS

Including various techniques, the chromatography (TLC- thin layer chromatography) was the very important to check out the presence of acids in collected lichens samples. The different colors were observed during the TLC, which later on became the main source for identification as shown in following Photo plate 1 (A & B).



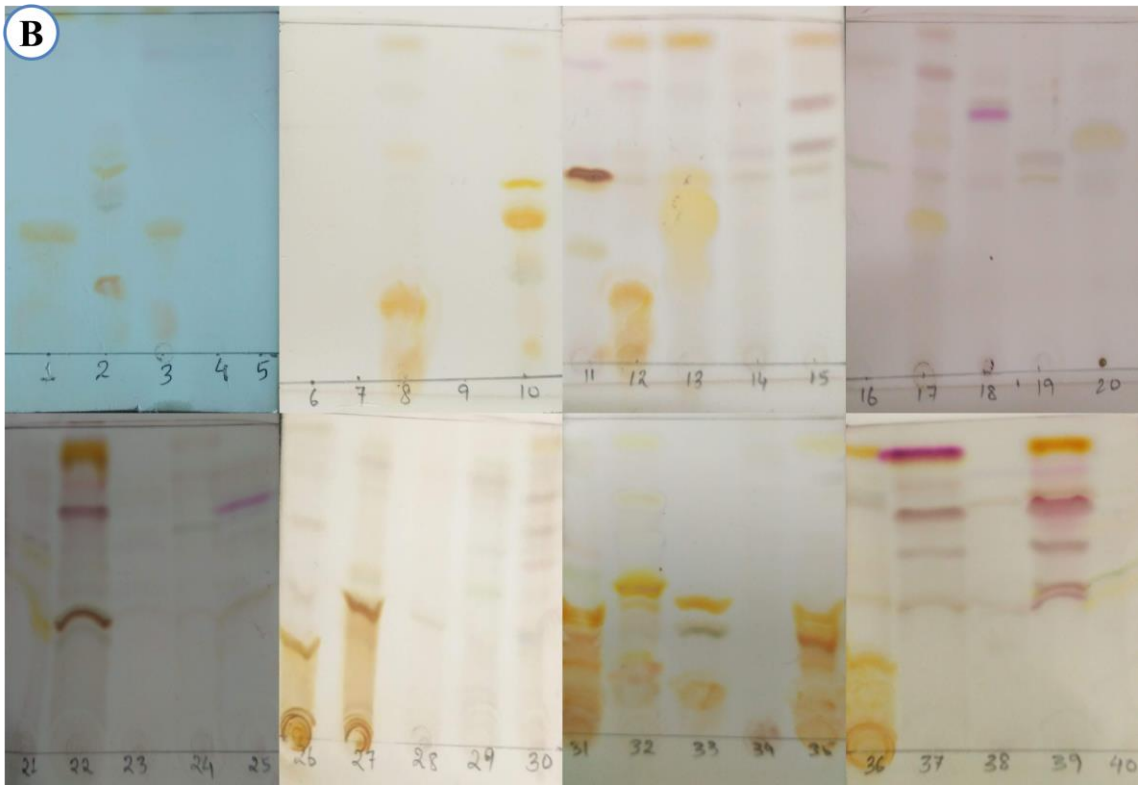


Photo Plate 1 (A&B) Constituents of secondary metabolites of the lichens detected in TLC

4.3.1 TAXONOMIC TREATMENT OF NEW SPECIES

***Pyrenula dissimulans* a new record of India (family Pyrenulaceae)**

Genus *Pyrenula*

Pyrenula dissimulans (Müll. Arg.) R.C. Harris, 1995

Basionym: *Pleurothelium dissimulans* Müll. Arg., Bot. Jb. 6:387 (1885) (Figure 4.1)

Description

Thallus crustose, corticolous, yellowish, brown to buff smooth without pseudocyphellae and pigment. Ascomata-perithecia, 0.5 to 1.5mm in diameter, osteoleapical, immerse in thallus upto the ostioles or upper part naked, peridium carbonized globose, slightly spreading laterally near the ostioles. Hemathecium-filamentous mostly unbranched, without oil, 4 to 8 spored, ascospores-ellipsoid rounded apically, without oil, 20-45 × 14-2 µm, the species has a neotropical. *Pyrenula dissimulans* is close to *Pyrenula welwitschii* (Upreti & Ajay Singh) Aptroot and

Pyrenula thelemorpha in morphology and shape of ascospores. However *Pyrenula welwitschii* differs in having smaller size (25-35 µm) and *Pyrenula thelemorpha* has less wide (11-15 µm) size of spore.

4.3.2 SPECIMEN EXAMINED

Murlen National Park (central), Mizoram, N23º38'22.3" E 93º17' 44.6"and 1745 m altitude on the bark, Nurpen Meitei Thangjam, 16-030757(LWG).

Distribution and Habitat

Earlier the species is recorded from Florida (Harris 1995). This species is a new record for India which is found as a Crustose lichens grown on the bark of trees.

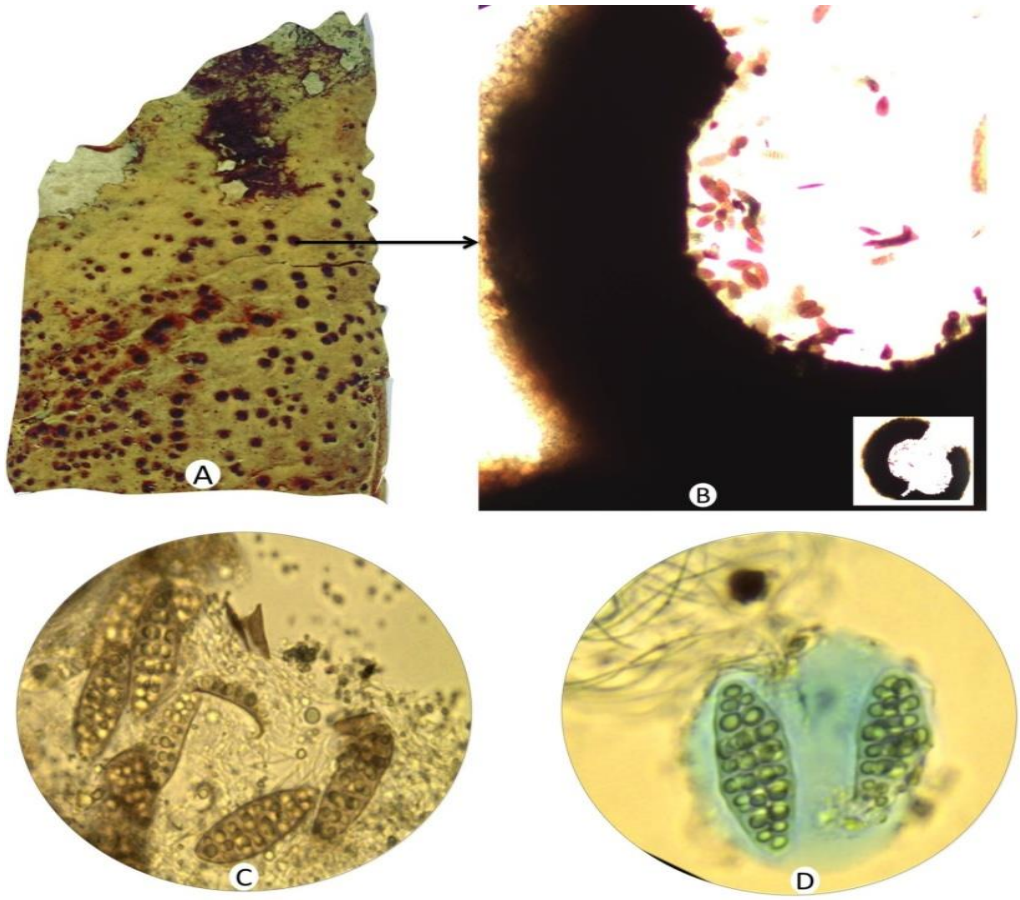


Figure 4.1 *Pyrenula dissimulans* (Mull. Arg.) R.C. Harris, (A) Thallus on the bark, (B)T.S. of apothecia (C) Asci, (D) Asci with ascospores enlarge scale.

4.3.3 ADDITIONS OF LICHENS SPECIES NEW TO THESTATE OF MIZORAM, NORTH EAST INDIA

As it was mentioned above that 22 lichens were reported from the central part of the MNP, Champhai district, Mizoram which are new to state. Out of 22 species recorded in the present study, the lichen family Parmeliaceae showed luxuriant growth form with 7 species and 5 genera followed by Arthoniaceae with 3 species and 2 genera, Pyrenulaceae with 3 species and single genus, Ramalinaceae with 2 species with 2 genera, Collemataceae as well as Porinaceae reported with 2 species and single genus, however, Brigantiaeaceae, Graphidaceae and Physciaceae were represented by single species with single genera (Table 4.1; Figure 4.2).

Moreover, 20 other lichens were reported from the same site, which was already documented from other parts of the MNP in previous studies, and counted as common lichens. These common lichens of different species were belonging to 15 genera and 9 families.Considering the growth forms of lichens, it was observed that crustose lichens were growing luxuriantly and represented by 9 species followed by foliose having 7 species and fruticose with 4 species respectively.On account of family's distribution, it was found that the family Parmeliaceae possessed highest number of species (8 sp.) which is followed by Graphidaceae with 3 sp. Further, family Physciaceae as well as Ramalinaceae with 2 sp. holds the third position and Arthoniaceae, Haematommataceae, Lecideaceae, Pyrenulaceae and Ramboldiaceae were also contributed 1 sp. in each family (Table 4.2; Fig. 4.3).

In addition to this, the present finding was also recorded a new species under genus *Pyrenula* ie., *P. dissimulans* for India as already shown in figure 4.1; with which the contribution of genus *Pyrenula* for lichen biota of India was increased with one and now the number became 78. Further, the details of new species with their distribution including specimens examined were summarized as follows:

Family Arthoniaceae

Cryptothecia dissimilis Makhija & Patw., Biovigyanam 13(2): 44 (1987)

Distribution – *C. dissimilis* is a new report for Mizoram and is previously recorded from Andaman & Nicobar Islands and Manipur of India.

Specimen examined– India, Mizoram, Champai district, Murlen National Park (central), 1584m altitude, on the bark, Nurpen Meitei Thangjam 16-030754 (LWG).

Cryptothecia scriblitella (Nyl.) Makhija & Patw., Biovigyanam 11(1): 8 (1985)

Basionym: *Arthonia scriblitella* Nyl., Acta Soc. Sci. fenn. 7(2): 481 (1863)

Arthothelium scriblitellum (Nyl.) Zahlbr., Catalogus Lichenum Universalis 2:133 (1923)

Distribution– *C. scriblitella* is a new report for North East India including Mizoram and is previously reported from Andaman & Nicobar Islands

Specimen examined– India, Mizoram, Champai district, Murlen National Park (central), 1745 m altitude, on the bark, Nurpen Meitei Thangjam, 16-030758 (LWG).

Herpothallon granulosum Jagad. Ram & G.P. Sinha, Lichenologist 41(6): 610 (2009)

Distribution – *H. granulosum* is a new report for Mizoram and is previously reported from Meghalaya and West Bengal.

Specimen examined– India, Mizoram, Champai district, Murlen National Park (central), 1768m altitude, on the bark, Nurpen Meitei Thangjam 16-030745 (LWG).

Family Brigantiaeaceae

Brigantiaea nigra D.D. Awasthi, in Awasthi & Srivastava, Proc. Indian Acad. Sci., Pl. Sci. 99(3): 172 (1989).

Basionym: *Lopadium nigrum* (D.D. Awasthi) Kr.P. Singh & G.P. Sinha, Indian Lichens, An Annotated Checklist (Kolkata): 13 (2010).

Distribution– *B. nigra* is a new report for North East India including Mizoram and is previously recorded from Kerala.

Specimen examined– India, Mizoram, Champai district, Murlen National Park (central), 1594m altitude, on the bark, Nurpen Meitei Thangjam 16-030756 (LWG).

Family Collemataceae

Leptogium arisanense Asahina, J. Jap. Bot. 12: 250-255 (1936)

Distribution – *L. arisanense* is a new report for Mizoram and is previously known from Arunachal Pradesh, Manipur, Sikkim and West Bengal.

Specimen examined– India, Mizoram, Champai district, Murlen National Park (central), 1723m altitude, on the bark, Nurpen Meitei Thangjam 16-030725 (LWG).

Leptogium moluccanum (Pers.) Vain., Acta Soc. Fauna Flora fenn. 7(no. 1): 223 (1890) Basionym: Collema *moluccanum*Gaudichaud-Beaupré, C., Pers., Botanique (Nagpur) 5:203 (1827)

Distribution – *L.moluccanum*is a new report for Mizoram and is previously recorded from Andaman & Nicobar Islands, Arunachal Pradesh, Maharashtra, Manipur, Nagaland, Tamil Nadu and West Bengal.

Specimen examined– India, Mizoram, Champai district, Murlen National Park (central), 1749m altitude, on the bark, Nurpen Meitei Thangjam 16-030744 (LWG).

Family Graphidaceae

Acanthothecis gracilis Staiger & Kalb, Mycotaxon 73: 99 (1999)

Distribution – *A. gracilis* is a new report for North East India including Mizoram and is previously recorded from Kerala, Tamil Naduand Maharashtra.

Specimen examined– India, Mizoram, Champai district, Murlen National Park (central), 1584m altitude, on the bark, Nurpen Meitei Thangjam 16-030738 (LWG).

Family Parmeliaceae

Bulbothrix isidiza (Nyl.) Hale, Phytologia 28:480 (1974)

Basionym: *Parmelia isidiza* Nyl., Bolm Soc. broteriana, Coimbra, sér. 1 3:130 (1884)

Distribution – *B. isidiza* is a new report for Mizoram and is previously recorded from Arunachal Pradesh, Karnataka, Kerala, Madhya Pradesh, Manipur, Meghalaya, Maharashtra, Nagaland, Sikkim, Tamil Nadu, Uttar Pradesh, Uttarakhand and West Bengal.

Specimen examined– India, Mizoram, Champai district, Murlen National Park (central), 1629m altitude, on the bark, Nurpen Meitei Thangjam 16-030720 (LWG).

Hypotrachyna awasthii Hale & Patw., Bryologist 77(4): 637 (1975) [1974]

Basionym: *Remototrachyna awasthii* (Hale & Patw.) Divakar & A. Crespo, in Divakar, Lumbsch, Ferencová, Prado & Crespo, Am. J. Bot. 97(4): 586 (2010)

Distribution – *H. awasthii* is a new report for Mizoram and is previously known from Karnataka, Kerala, Maharashtra, Manipur, Nagaland and Tamil Nadu.

Specimen examined– India, Mizoram, Champai district, Murlen National Park (central), 1765m altitude, on the bark, Nurpen Meitei Thangjam, 16-030707 (LWG).

Hypotrachyna neosingularis Divakar, Upreti & Elix, Mycotaxon 80: 355 (2001)

Distribution – *H. neosingularis* is a new report for Mizoram and is previously known from Sikkim.

Specimen examined– India, Mizoram, Champai district, Murlen National Park (central), 1743m altitude, on the bark, Nurpen Meitei Thangjam, 16-030733 (LWG).

Myelochroa subaurulenta (Nyl.) Elix & Hale, Mycotaxon 29: 241 (1987)

Distribution – *M. subaurulenta* is a new report for Mizoram and is previously recorded from Arunachal Pradesh, Kerala, Nagaland, Sikkim, Tamil Nadu, Uttarakhand and West Bengal.

Specimen examined– India, Mizoram, Champai district, Murlen National Park (central), 1628m altitude, on the bark, Nurpen Meitei Thangjam, 16-030719 (LWG).

Parmotrema latissimum (Fée) Hale, Phytologia 28: 337 (1974)

Distribution– *P. latissimum* is a new report for North East India including Mizoram and is previously recorded from Himachal Pradesh, Maharastra and West Bengal.

Specimen examined– India, Mizoram, Champai district, Murlen National Park (central), 1734m altitude, on the bark, Nurpen Meitei Thangjam, 16-030724 (LWG).

Usnea eumitrioides Motyka, Lich. Gen. Usnea Monogr. 2(1): 322. (1937)

Distribution – *U. eumitrioides* is a new report for Mizoram and is previously recorded from Himachal Pradesh, Nagaland, Sikkim, Tamil Nadu, Uttarakhand and West Bengal.

Specimen examined– India, Mizoram, Champai district, Murlen National Park (central), 1478m altitude, on the bark, Nurpen Meitei Thangjam 16-030764 (LWG).

Usnea nipparensis Asahina, Lich. Jpn. 3: 91 (1956)

Basionym: *Usnea nipparensis* f. reagens Asahina, J. Jpn. Bot. 47: 257 (1972)

Distribution – *U. nipparensis* is a new report for Mizoram and is previously reported from Sikkim and West Bengal.

Specimen examined– India, Mizoram, Champai district, Murlen National Park (central), 1610m altitude, on the bark, Nurpen Meitei Thangjam 16-030734 (LWG).

Family Physciaceae

Heterodermia hypoleuca (Ach.) Trevis. Atti Soc. Ital. Nat. 11: 615 (1868)

Distribution – *H. hypoleuca* is a new report for Mizoram and is previously recorded from Jammu & Kashmir, Maharashtra, Sikkim and Tamil Nadu.

Specimen examined– India, Mizoram, Champai district, Murlen National Park (central), 1749m altitude, on the bark, Nurpen Meitei Thangjam, 16-030762 (LWG).

Family Porinaceae

Porina internigrans (Nyl.) Müll. Arg., Rep. Australas. Assoc. Advancem. Sci. 1895: 452 (1895)

Distribution – *P. internigrans* is a new report for Mizoram and is previously recorded fromAndaman & Nicobar Islands, Arunachal Pradesh, Assam, Goa, Karnataka, Kerala, Meghalaya, Nagaland, Tamil Nadu and West Bengal.

Specimen examined– India, Mizoram, Champai district, Murlen National Park (central), 1667m altitude, on the bark, Nurpen Meitei Thangjam, 16-030750 (LWG).

Porina interstes (Nyl.) Harm., Bull. Soc. Sci. Nancy, sér. 3, 12: 126 (1911)

Distribution – *P. interstes* is a new report for Mizoram and is previously reported from Andaman & Nicobar Islands, Arunachal Pradesh, Goa, Karnataka, Madhya Pradesh, Nagaland, Orissa, Sikkim, Tamil Nadu and West Bengal.

Specimen examined– India, Mizoram, Champai district, Murlen National Park (central), 1764m altitude, on the bark, Nurpen Meitei Thangjam, 16-030701 (LWG).

Family Pyrenulaceae

Pyrenula platystoma (Müll. Arg.) Aptroot, Lichenologist 44(1): 36 (2011)

Basionym: *Anthracothecium platystomum* Müll. Arg., Revue mycol., Toulouse 10(no. 40): 184 (1888)

Distribution – *P. platystoma* is a new report for Mizoram and is previously reported from Arunachal Pradesh, Himachal Pradesh, Sikkim, Tamil Nadu, Uttarakhand and West Bengal.

Specimen examined– India, Mizoram, Champai district, Murlen National Park (central), 1721m altitude, on the bark, Nurpen Meitei Thangjam, 16-030717 (LWG).

Pyrenula subducta (Nyl.) Müll. Arg., Flora, Regensburg 67:666 (1884)

Distribution – *P. subducta* is a new report for Mizoram and is previously reported from Manipur and Nagaland.

Specimen examined– India, Mizoram, Champai district, Murlen National Park (central), 1745 m altitude, on the bark, Nurpen Meitei Thangjam, 16-030749 (LWG).

Family Ramalinaceae

Biatora vernalis (L.) Fr., K. svenska Vetensk-Akad.Handl., ser. 3: 271 (1822)

Distribution – *B. vernalis* is a new report for Mizoram and is previously recorded from Arunachal Pradesh and Manipur.

Specimen examined– India, Mizoram, Champai district, Murlen National Park (central), 1694m altitude, on the bark, Nurpen Meitei Thangjam, 16-030763 (LWG).

Phyllopsora furfuracea (Pers.) Zahlbr., in Engler & Prantl, Nat. Pflanzenfam. 1(1):138 (1905)

Distributio*n* – *P. furfuracea* is a new report for North East India including Mizoram and is previously known from Karnataka, Kerala and Uttarakhand.

Specimen examined– India, Mizoram, Champai district, Murlen National Park (central), 1584m altitude, on the bark, Nurpen Meitei Thangjam, 16-030742 (LWG).

Table 4.1 New Lichen flora for the state of Mizoram from Murlen National park, Champhai district, Mizoram

S.no	**Family**	**Genera**	**Species**	**Growth form**	**Field number**
1	Arthoniaceae	*Cryptothecia*	*Cryptothecia dissimilis* Makhija & Patw.	Crustose	16-030754
2	Arthoniaceae	*Cryptothecia*	*Cryptothecia scriblitella*	Crustose	16-

			(Nyl.) Makhija & Patw.		030758
3	Arthoniaceae	*Herpothallon*	*Herpothallon granulosum* Jagadeesh & G.P Singha	Crustose	16-030745
4	Brigantiaeaceae	*Brigantiaea*	*Brigantiaea nigra* D.D. Awasthi	Crustose	16-030756
5	Collemataceae	*Leptogium*	*Leptogium arisanense* Asahina	Foliose	16-030725
6	Collemataceae	*Leptogium*	*Leptogium moluccanum* (Pers.) Vain.	Foliose	10-030744
7	Graphidaceae	*Acanthothecis*	*Acanthothecis gracilis* Staiger& Kalb	Crustose	16-030738
8	Parmeliaceae	*Bulbothrix*	*Bulbothrix isidiza* (Nyl.) Hale	Foliose	16-030720
9	Parmeliaceae	*Hypotrachyna*	*Hypotrachyna awasthi* Hale & Patw.	Foliose	16-030707
10	Parmeliaceae	*Hypotrachyna*	*Hypotrachyna neosingularis* Divakar, Upreti & Elix	Foliose	16-030733
11	Parmeliaceae	*Myelochroa*	*Myelochroa subaurulenta* (Nyl.) Elix& Hale	Foliose	16-030719
12	Parmeliaceae	*Parmotrema*	*Parmotrema latissimum* (Fée) Hale	Foliose	16-030724
13	Parmeliaceae	*Usnea*	*Usnea eumitrioides* Motyka	Fruticose	16-030764
14	Parmeliaceae	*Usnea*	*Usnea nipparensis* Asahina	Fruticose	16-030734
15	Physciaceae	*Heterodermia*	*Heterodermia hypoleuca* (Ach.) Trevis.	Foliose	16-030762

16	Porinaceae	*Porina*	*Porina internigrans* (Nyl.) Müll. Arg.	Crustose	16-030750
17	Porinaceae	*Porina*	*Porina interstes* (Nyl.) Harm.	Crustose	16-030701
18	Pyrenulaceae	*Pyrenula*	*Pyrenula dissimulans* (Mull. Arg.) R.C. Harris	Crustose	16-030757
19	Pyrenulaceae	*Pyrenula*	*Pyrenula platystoma* (Müll. Arg.) Aptroot	Crustose	16-030717
20	Pyrenulaceae	*Pyrenula*	*Pyrenula subducta* (Nyl.) Müll. Arg.	Crustose	16-030749
21	Ramalinaceae	*Biatora*	*Biatora vernalis* (L.) Fr.	Crustose	16-030763
22	Ramalinaceae	*Phyllopsora*	*Phyllopsora furfuracea* Zahlbr.	Crustose	16-030742

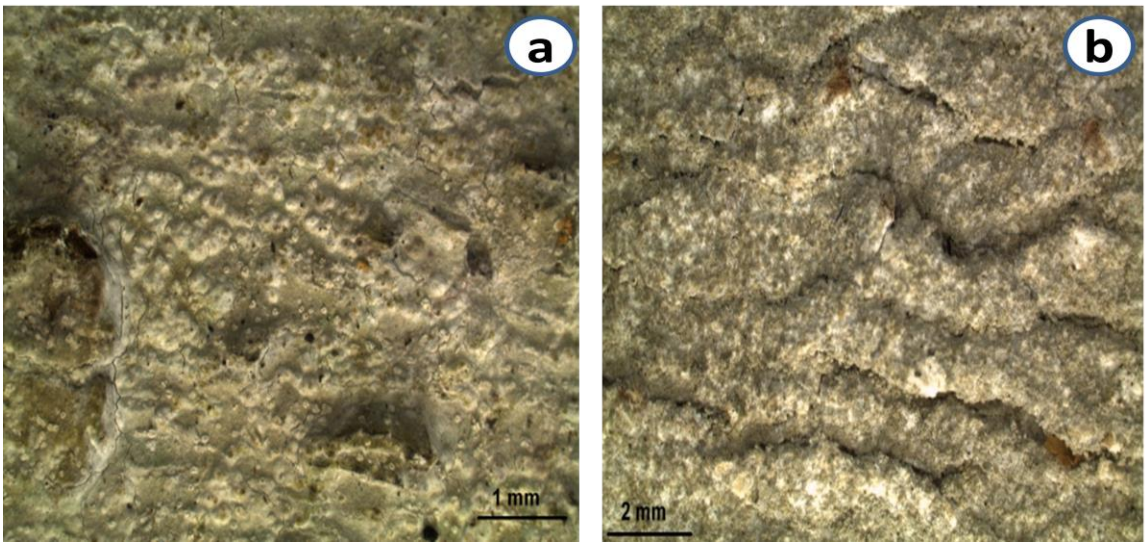


Figure 4.2 New Lichen flora for the state of Mizoram from Murlen National park, Champhai district, Mizoram, Habitus (contd…)

(a) *Cryptothecia dissimilis* Makhija & Patw.

(b) *Cryptothecia scriblitella* (Nyl.) Makhija & Patw.

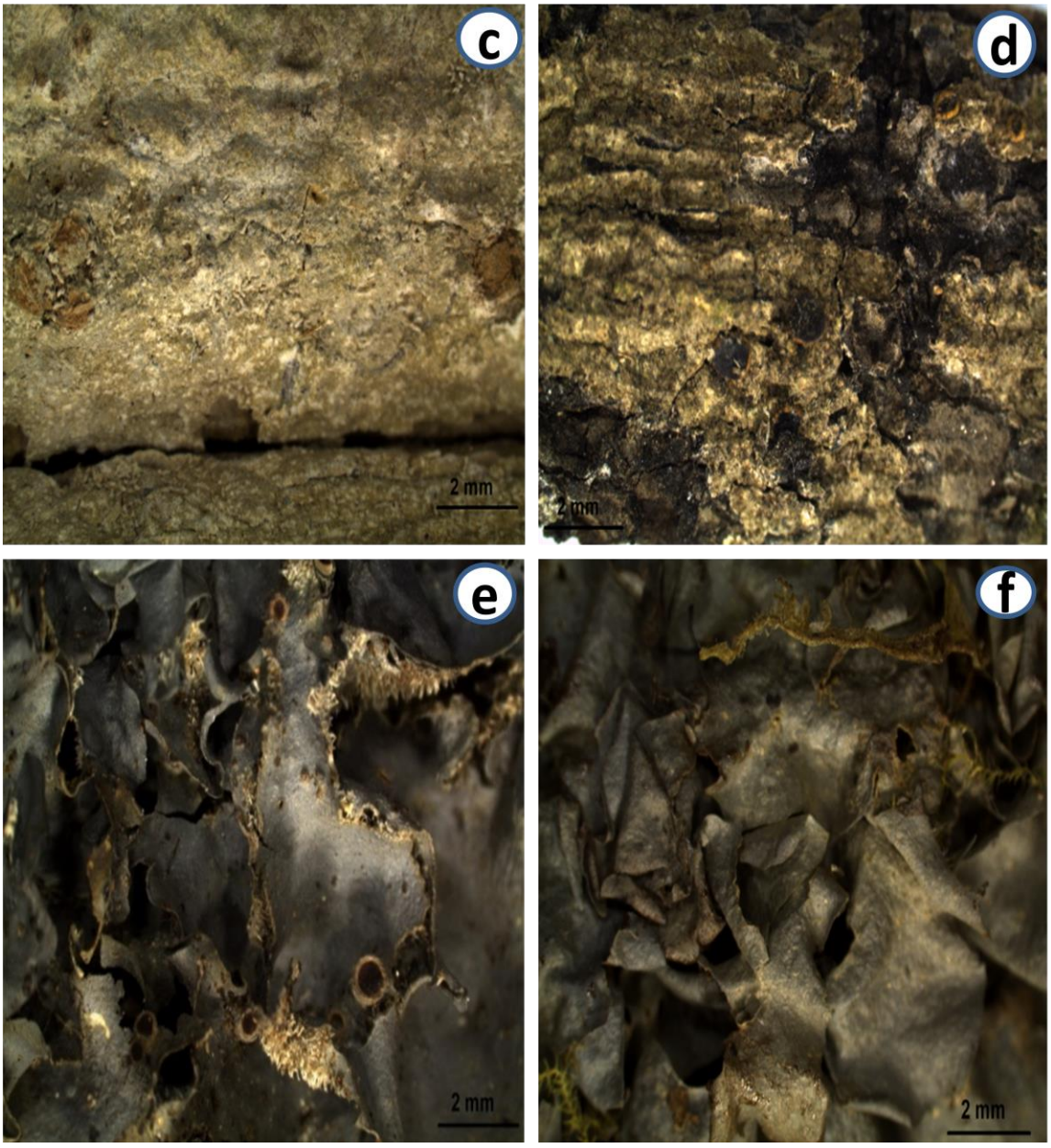


Figure 4.2 New Lichen flora for the state of Mizoram from Murlen National park, Champhai district, Mizoram, Habitus (contd…)

(c) *Herpothallon granulosum* Jagadeesh & G.P Singha
(d) *Brigantiaea nigra* D.D. Awasthi.
(e) *Leptogium arisanense* Asahina
(f) *Leptogium moluccanum* (Pers.) Vain.

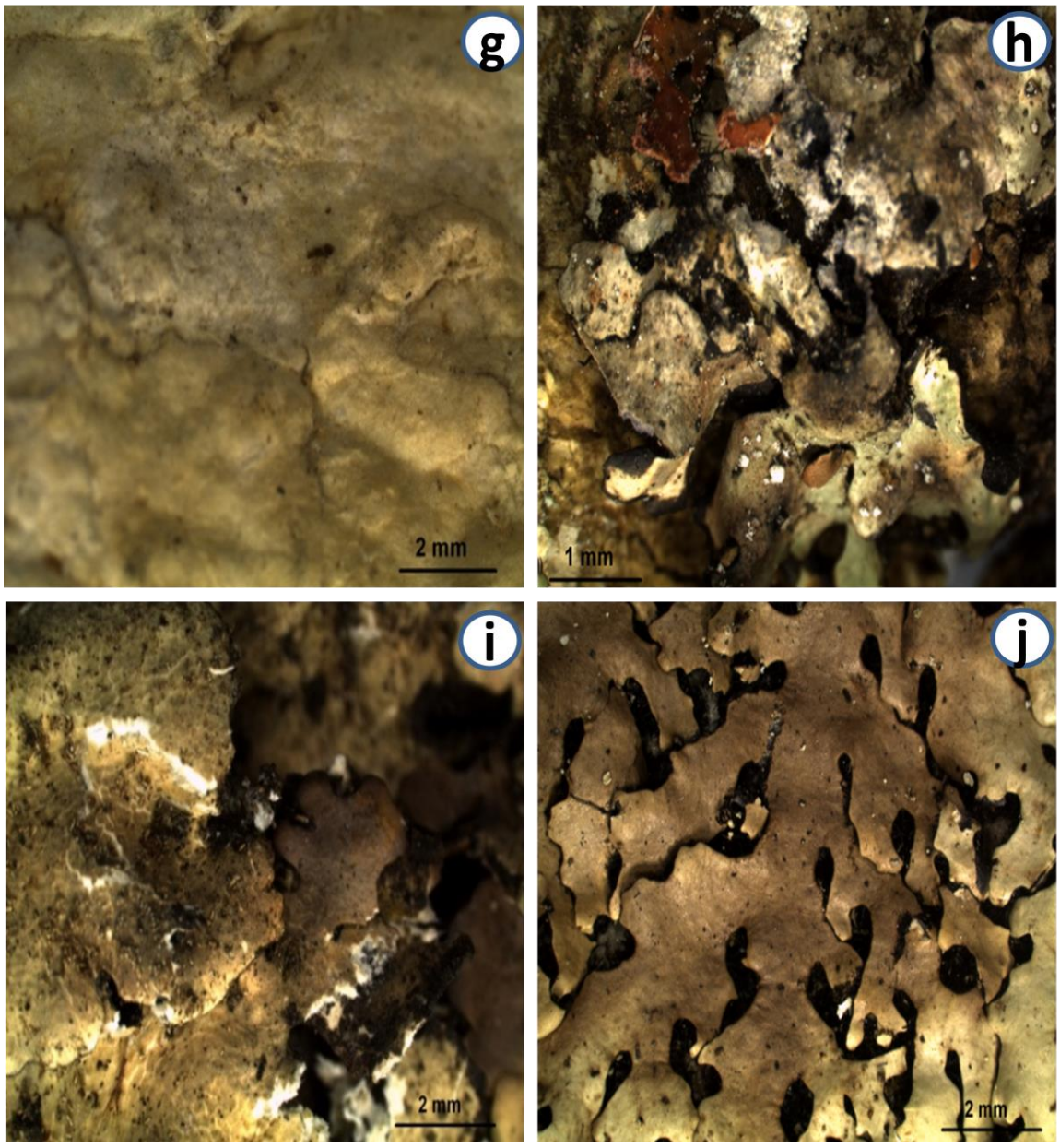


Figure 4.2 New Lichen flora for the state of Mizoram from Murlen National park, Champhai district, Mizoram, Habitus (contd…)

(g) *Acanthothecis gracilis* Staiger & Kalb

(h) *Bulbothrix isidiza* (Nyl.) Hale

(i) *Hypotrachyna awasthi* Hale & Patw.,

(j) *Hypotrachyna neosingularis* Divakar, Upreti & Elix

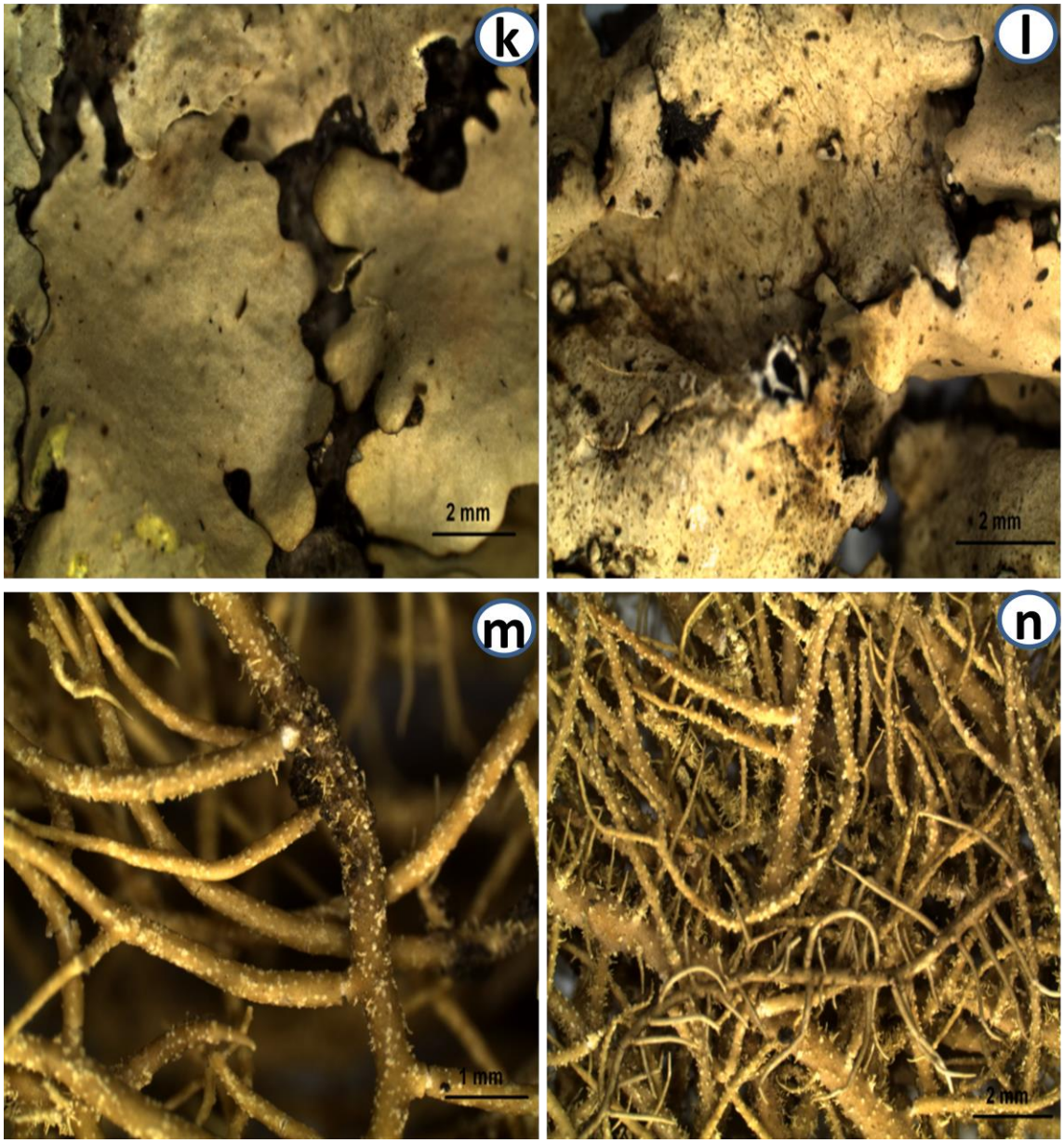


Figure 4.2 New Lichen flora for the state of Mizoram from Murlen National park, Champhai district, Mizoram, Habitus (contd…)

(k) *Myelochroa subaurulenta* (Nyl.) Elix & Hale

(l) *Parmotrema latissimum* (Fée) Hale

(m) *Usnea eumitrioides* Motyka

(n) *Usnea nipparensis* Asahina

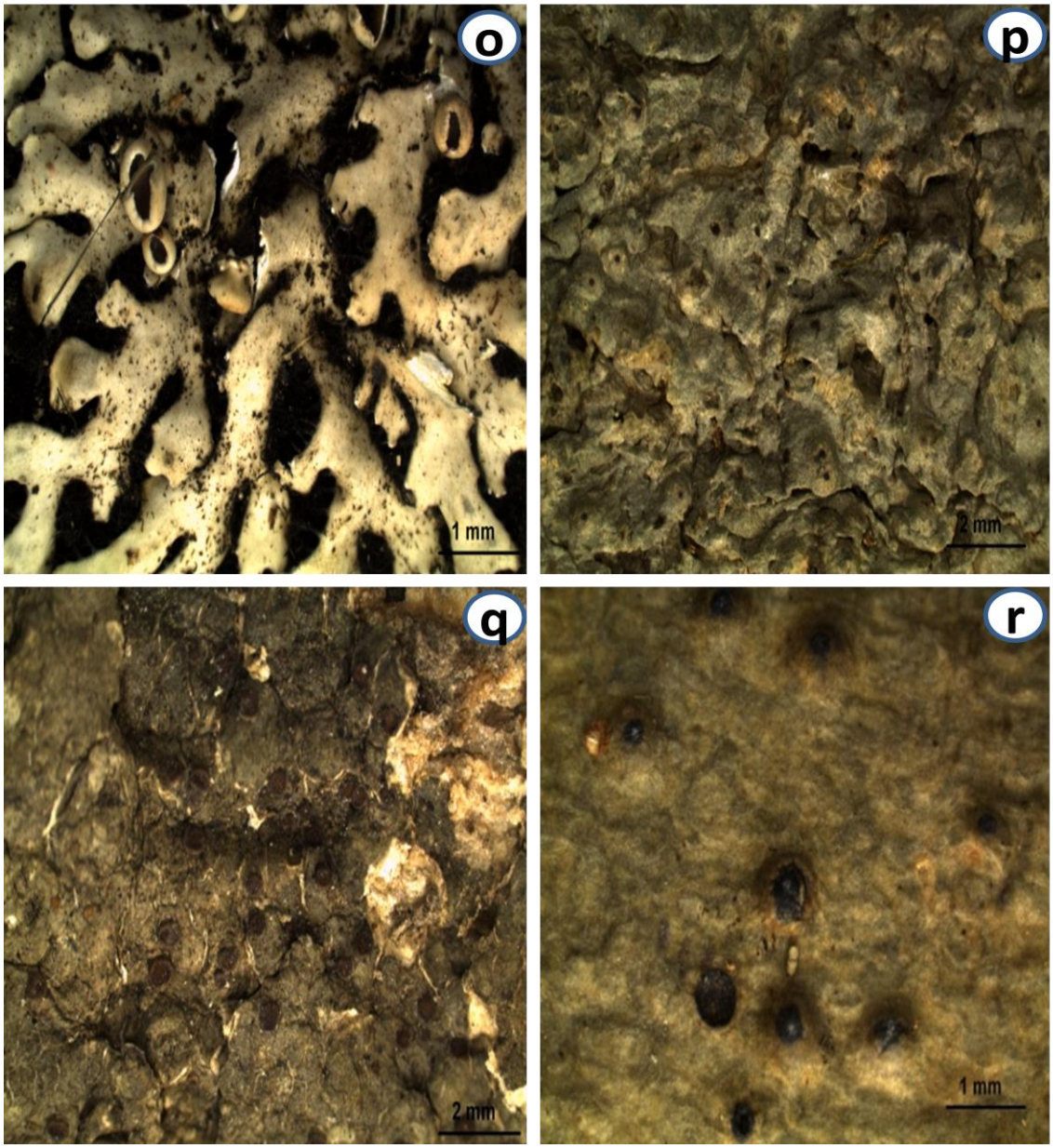


Figure 4.2 New Lichen flora for the state of Mizoram from Murlen National park, Champhai district, Mizoram, Habitus (contd…)

(o) *Heterodermia hypaleuca* (Muhl.)Trevis.

(p) *Porina internigrans* (Nyl.) Müll. Arg

(q) *Porina interstes* (Nyl.) Harm.

(r) *Pyrenula platystoma* (Müll. Arg.) Aptroot

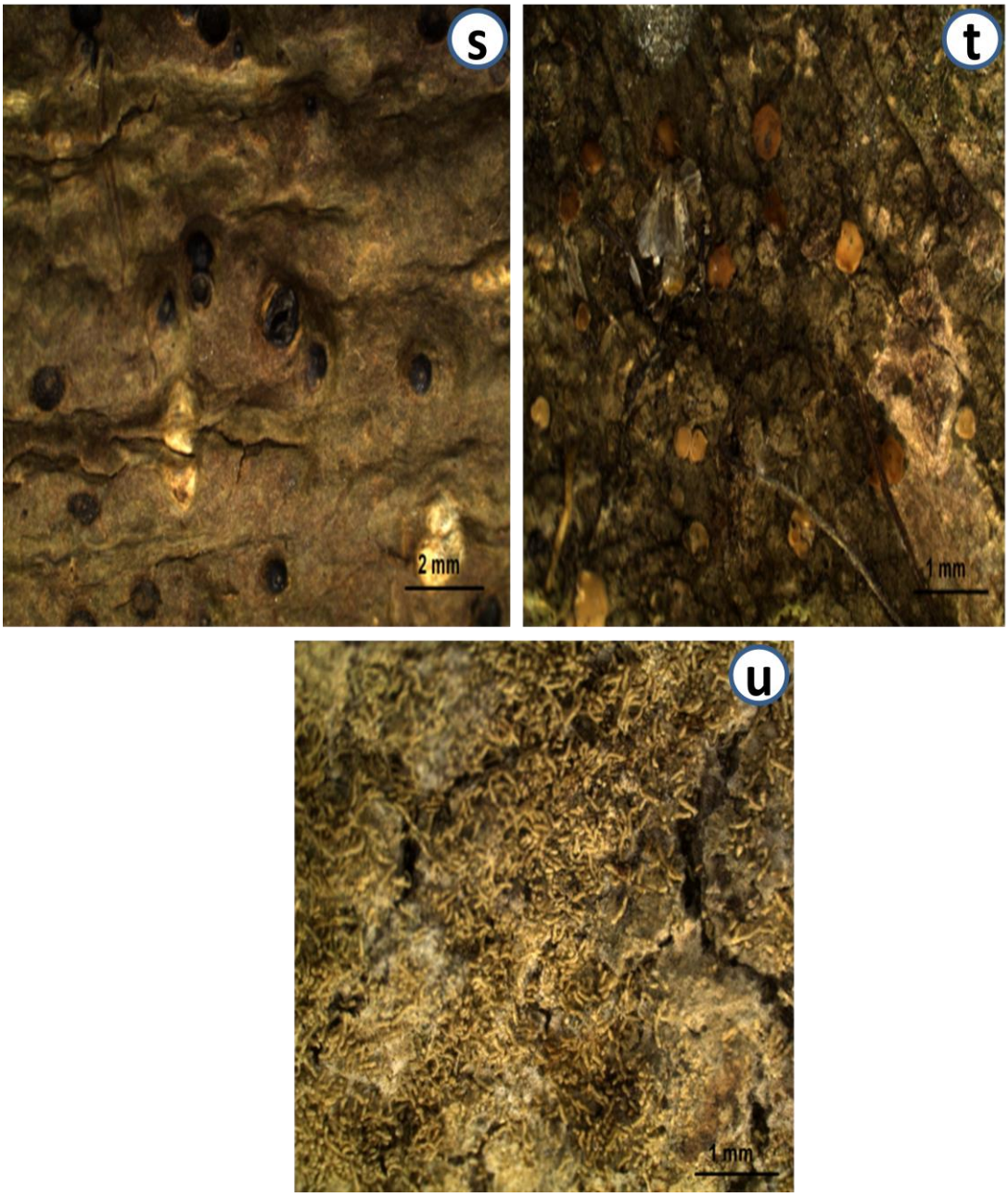


Figure 4.2 New Lichen flora for the state of Mizoram from Murlen National park, Champhai district, Mizoram, Habitus (contd…)

(s) *Pyrenula subducta* (Nyl.) Müll.Arg.

(t) *Biatora vernalis* (L.) Fr.

(u) *Phyllopsora furfuracea* Zahlbr.

Table 4.2 List of common Lichens recorded from Murlen National Park, Champhai district, Mizoram.

S.no	**Family**	**Genera**	**Species**	**Growth form**	**Field number**
1	Arthoniaceae	*Crytothecia*	*Cryptothecia lunulata* (Zahlbr.) Makhija & Patw.	Crustose	16-030740
2	Graphidaceae	*Diorygma*	*Diorygma hieroglyphicum* (Pers.) Staiger & Kalb	Crustose	16-030760
3	Graphidaceae	*Graphis*	*Graphis* Sp.	Crustose	16-030739
4	Graphidaceae	*Myriotrema*	*Myriotrema clandestinum* (Fée) Hale	Crustose	16-030714
5	Haematommataceae	*Haematomma*	*Haematomma puniceum* (Sw.) A. Massal.	Crustose	16-030721
6	Lecideaceae	*Lecidea*	*Lecideagranifera* (Ach.) Vain.	Crustose	16-030748
7	Parmeliaceae	*Everniastrum*	*Everniastrum cirrhatum* (Fr.) Hale ex Sipman	Foliose	16-030726
8	Parmeliaceae	*Myelochroa*	*Myelochroa xantholepis* (Mont. & Bosch) Elix& Hale	Foliose	16-030708
9	Parmeliaceae	*Parmotrema*	*Parmotrema reticulatum* (Taylor) M. Choisy	Foliose	16-030703
10	Parmeliaceae	*Parmotrema*	*Parmotrema saccatilobum* (Taylor) Hale	Foliose	16-030729
11	Parmeliaceae	*Parmotrema*	*Parmotrema tinctorum* (Despr. exNyl.) Hale	Foliose	16-030702
12	Parmeliaceae	*Usnea*	*Usnea baileyi* (Stirt.) Zahlbr.	Fruticose	16-030743

13	Parmeliaceae	*Usnea*	*Usnea orientalis* Motyka	Fruticose	16-030751
14	Parmeliaceae	*Usnea*	*Usnea undulata* Stirt.	Fruticose	16-030747
15	Physciaceae	*Heterodermia*	*Heterodermia diademata*(Taylor) D.D. Awasthi	Foliose	16-030765
16	Physciaceae	*Heterodermia*	*Heterodermia speciosa* (Wulfen) Trevis.	Foliose	16-030704
17	Pyrenulaceae	*Anthracotheciu m*	*Anthracothecium macrosporum* (Hepp) Müll. Arg.	Crustose	16-030713
18	Ramalinaceae	*Phyllopsora*	*Phyllopsora* sp.	Crustose	16-030746
19	Ramalinaceae	*Ramalina*	*Ramalina conduplicans* Vain.	Fruticose	16-030723
20	Ramboldiaceae	*Ramboldia*	*Ramboldia manipurensis* (Kr.P. Singh) Kalb, Lumbsch & Elix	Crustose	16-030752

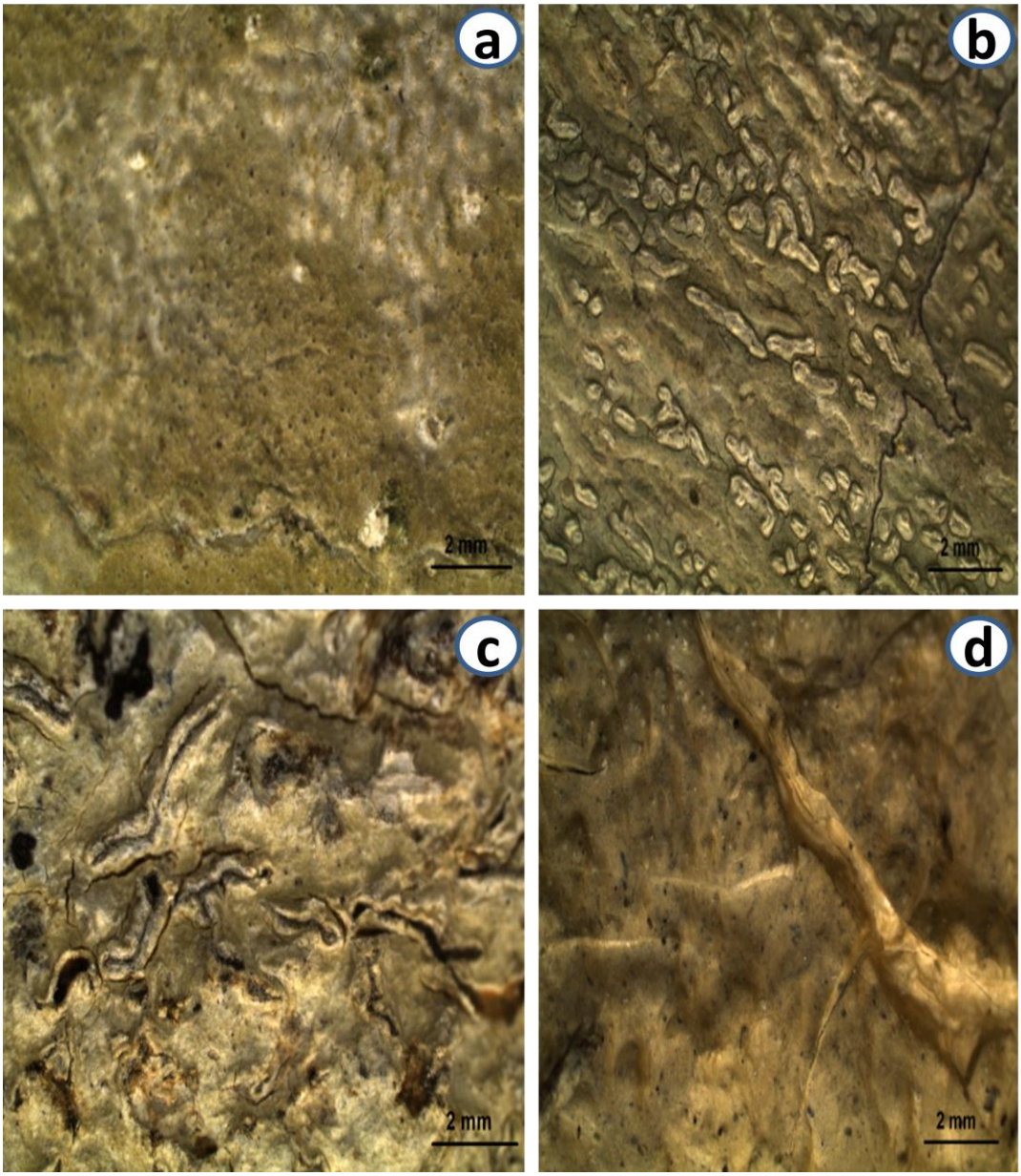


Figure 4.3 List of common Lichens recorded from Murlen National Park, Champhai district, Mizoram, (contd…)

(a) *Cryptothecia lunulata* (Zahlbr.) Makhija & Patw.

(b) *Diorygma hieroglyphicum* (Pers.) Staiger &Kalb.

(c) *Graphis* sp.

(d) *Myriotrema clandestinum* (Fée) Hale.

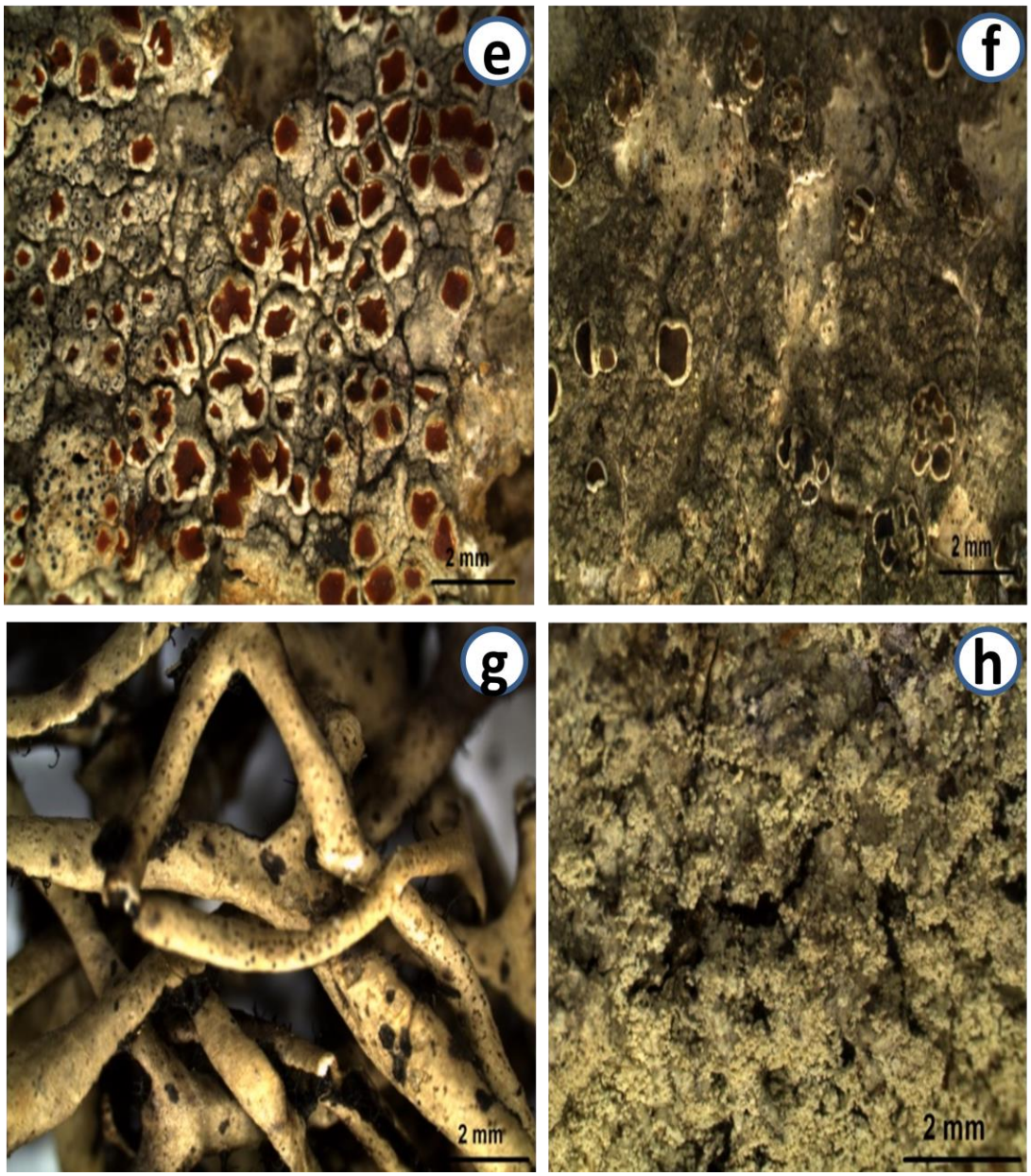


Figure 4.3 List of common Lichens recorded from Murlen National Park, Champhai district, Mizoram, (contd…)

(e) *Haematomma puniceum* (Sw.) A. Massal.

(f) *Lecidea granifera* (Ach.) Vain.

(g) *Everniastrum cirrhatum* (Fr.) Hale ex Sipman.

(h) *Myelochroa xantholepis* (Mont. & Bosch) Elix & Hale.

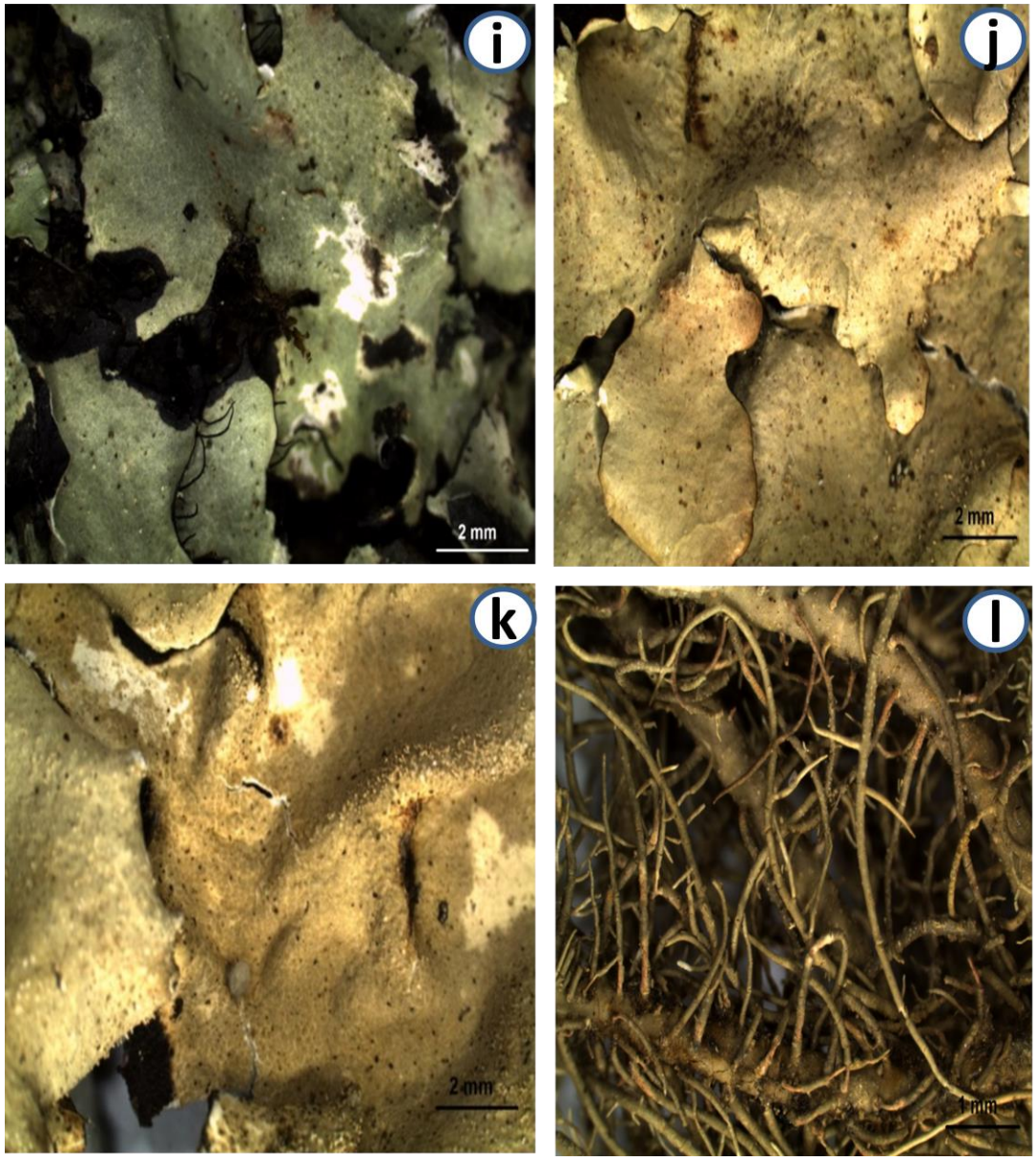


Figure 4.3 List of common Lichens recorded from Murlen National Park, Champhai district, Mizoram, (contd…)

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

(j) *Parmotrema saccatilobum* (Taylor) Hale.

(k) *Parmotrema tinctorum* (Despr. exNyl.) Hale.

(l) *Usnea baileyi* (Stirt.) Zahlbr.

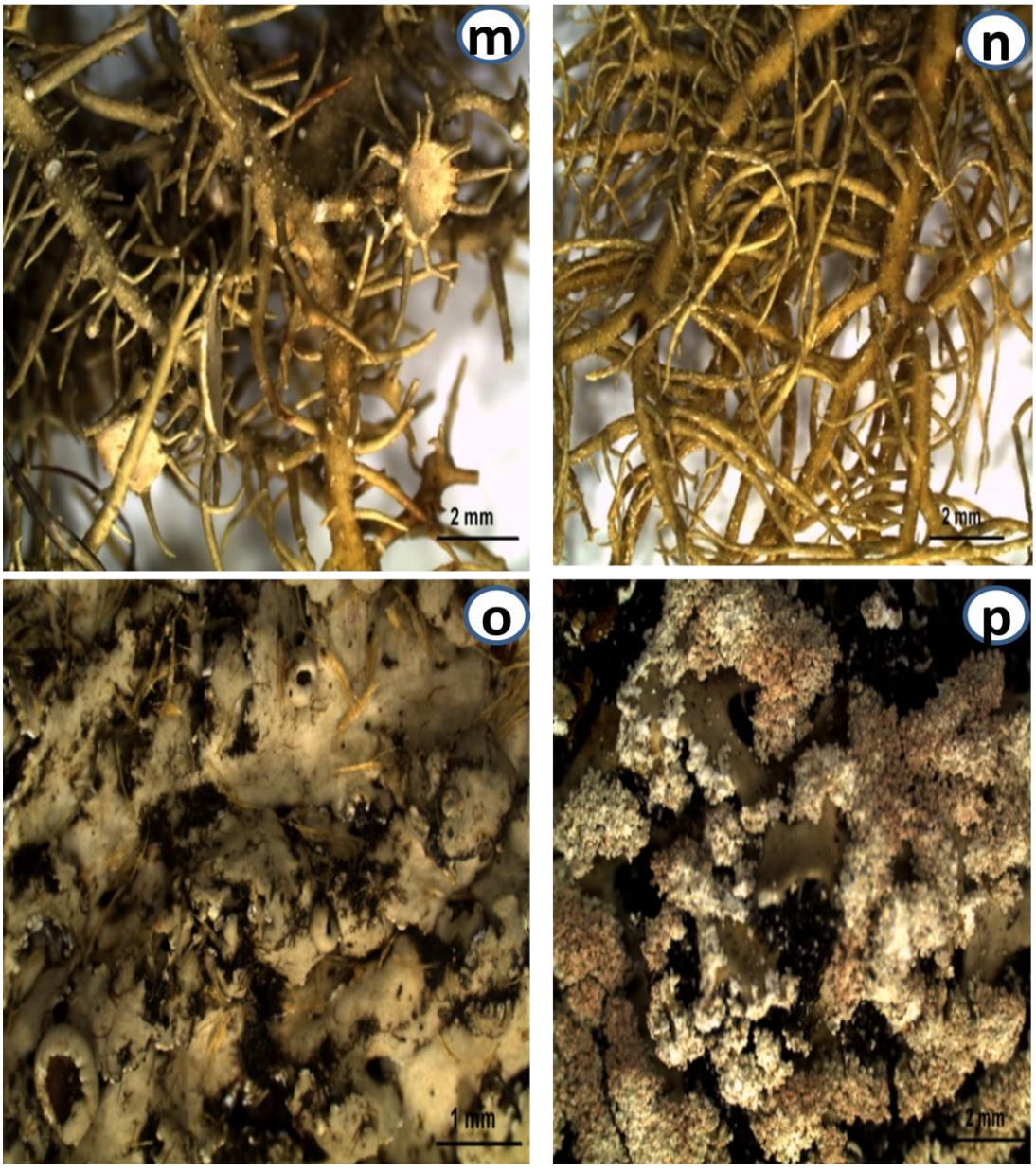


Figure 4.3 List of common Lichens recorded from Murlen National Park, Champhai district, Mizoram, (contd…)

(m) *Usnea orientalis* Motyka.

(n) *Usnea undulata* Stirt.

(o) *Heterodermia diademata* (Taylor) D. D. Awasthi.

(p) *Heterodermia speciosa* (Wulfen) Trevis.

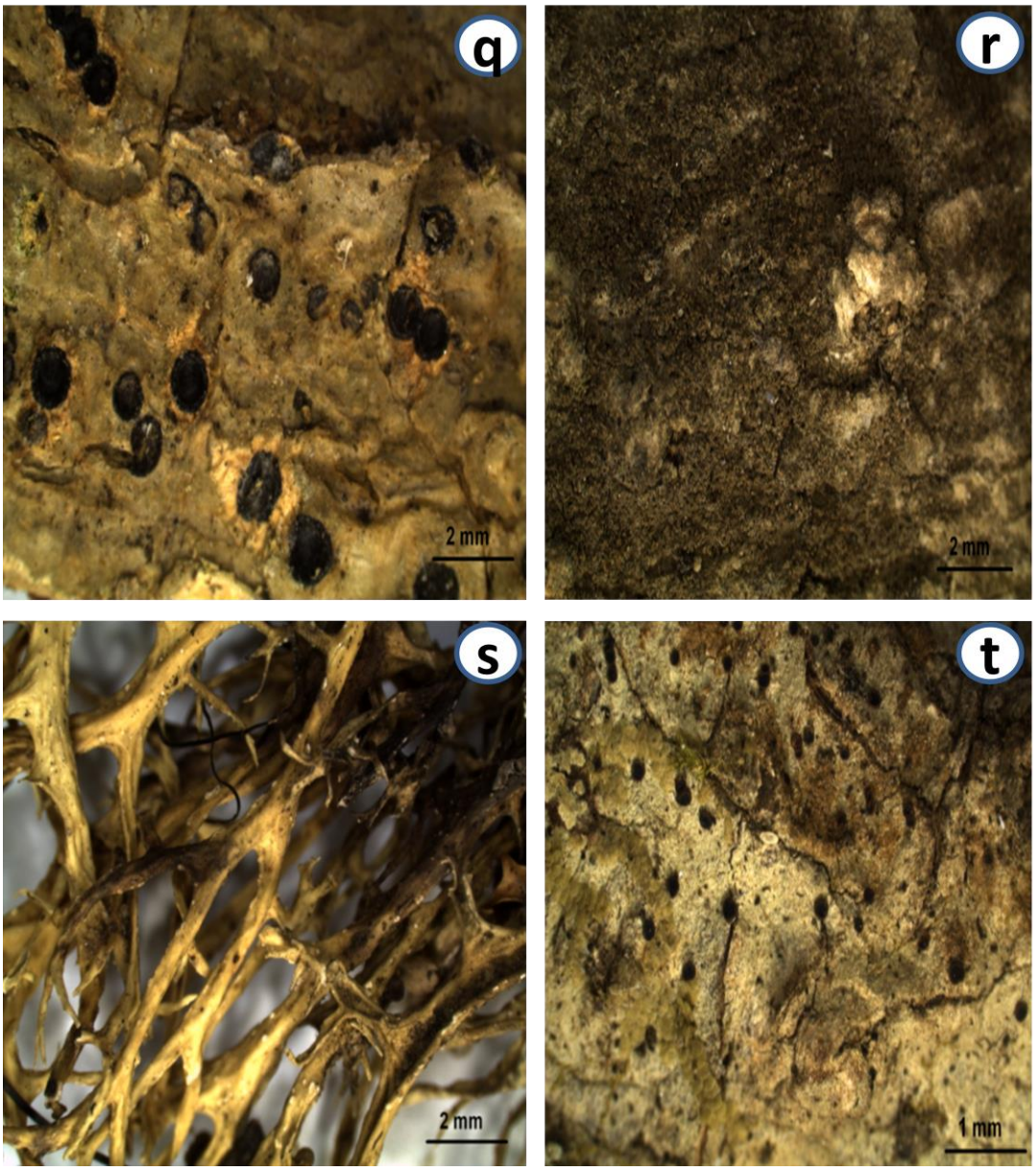


Figure 4.3 List of common Lichens recorded from Murlen National Park, Champhai district, Mizoram, (contd…)

(q) *Anthracothecium macrosporum* (Hepp) Müll. Arg.

(r) *Phyllopsora* sp.

(s) *Ramalina conduplicans* Vain.

(t) *Ramboldia manipurensis* (Kr. P. Singh) Kalb, Lumbsch & Elix.

4.3.4 LICHEN OF REIEK HILL

20 different species of lichens belonging to 11 genera and 8 families reported from the Reiek Hill, Mamit District of Mizoram state (Table 4.3) (Figure 4.4). Based on distribution of families, it was found that the family Parmeliaceae possessed highest number of species (9 sp.) followed by Graphidaceae (4 sp.), Pyrenulaceae (2 sp.) and Coccocarpiaceae, Letrouitiaceae, Pertusariaceae, Physciaceae and Roccellaceae were also contributed 1 sp. in each family.

Table 4.3Lichen flora from Reiek Hill, Mamit district, Mizoram

Sl.No.	Family	Genus	Species	Field No.
1	Coccocarpiaceae	*Coccocarpia*	*Coccocarpia palmicola* (Spreng.) Arv. & D.J. Galloway	18-036149
2	Graphidaceae	*Diploschistes*	*Diploschistes scruposus* (Schreb.) Norman	18-036148
3	Graphidaceae	*Diorygma*	*Diorygma hieroglyphicum* (Pers.) Staiger & Kalb	18-036136
4	Graphidaceae	*Diorygma*	*Diorygma macgregorii* (Vain.) Kalb, Staiger & Elix	18-036133
5	Graphidaceae	*Diorygma*	*Diorygma soozanum* (Zahlbr.) M. Nakan. & Kashiw.	18-036137
6	Letrouitiaceae	*Letrouitia*	*Letrouitia vulpina* (Tuck.) Hafellner & Bellem.	18-036151
7	Parmeliaceae	*Hypotrachyna*	*Hypotrachyna rhabdiformis* (Kurok.) Hale	18-036139
8	Parmeliaceae	*Parmotrema*	*Parmotrema crinitoides* J.C. Wei	18-036135
9	Parmeliaceae	*Parmotrema*	*Parmotrema hababianum* (Gyeln.) Hale	18-036146
10	Parmeliaceae	*Parmotrema*	*Parmotrema nilgherrense* (Nyl.) *Hale*	18-036138
11	Parmeliaceae	*Parmotrema*	*Parmotrema rampoddense*	18-036156

			(Nyl.) Hale	
12	Parmeliaceae	*Parmotrema*	*Parmotrema reticulatum* (Taylor) M. Choisy	18-036140
13	Parmeliaceae	*Parmotrema*	*Parmotrema tinctorum* (Despr. ex Nyl.) Hale	18-036155
14	Parmeliaceae	*Usnea*	*Usnea baileyi* (Stirt.) Zahlbr.	18-036153
15	Parmeliaceae	*Usnea*	*Usnea eumitrioides* Motyka	18-036154
16	Pertusariaceae	*Pertusaria*	*Pertusaria leucosorodes* Nyl.	18-036147
17	Physciaceae	*Heterodermia*	*Heterodermia diademata* (Taylor) D.D. Awasthi	18-036157
18	Pyrenulaceae	*Pyrenula*	*Pyrenula mastophoroides* (Nyl.) Zahlbr.	18-036152
19	Pyrenulaceae	*Pyrenula*	*Pyrenula andina* Aptroot	18-036132
20	Roccellaceae	*Chiodecton*	*Chiodecton leptosporum* Müll. Arg.	18-036150

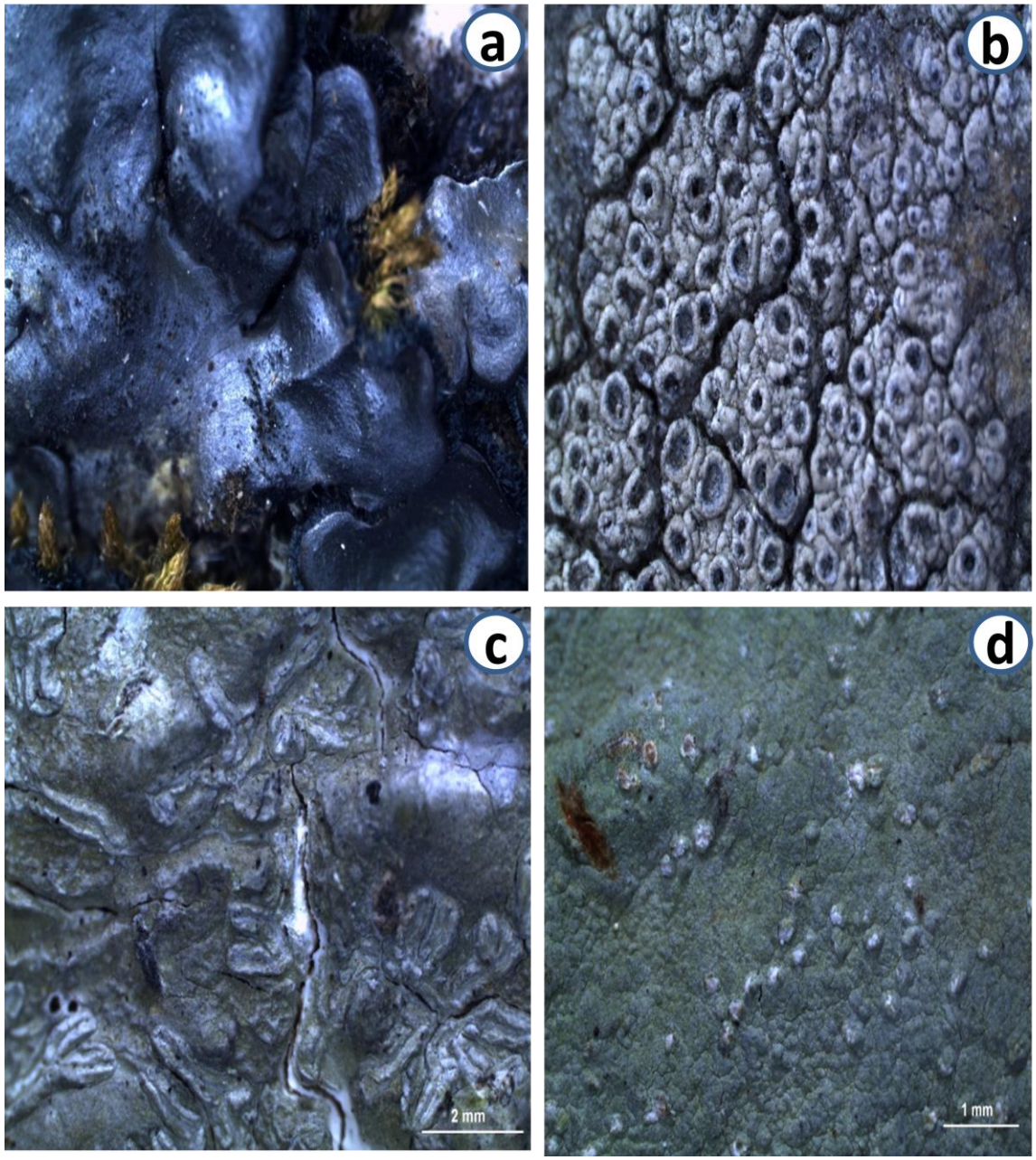


Figure 4.4 Lichen flora from Reiek Hill, Mamit district, Mizoram, (contd…)

(a) *Coccocarpia palmicola* (Spreng.) Arv. & D.J. Galloway

(b) *Diploschistes scruposus* (Schreb.) Norman

(c) *Diorygma hieroglyphicum* (Pers.) Staiger & Kalb

(d) *Diorygma macgregorii* (Vain.) Kalb, Staiger & Elix.

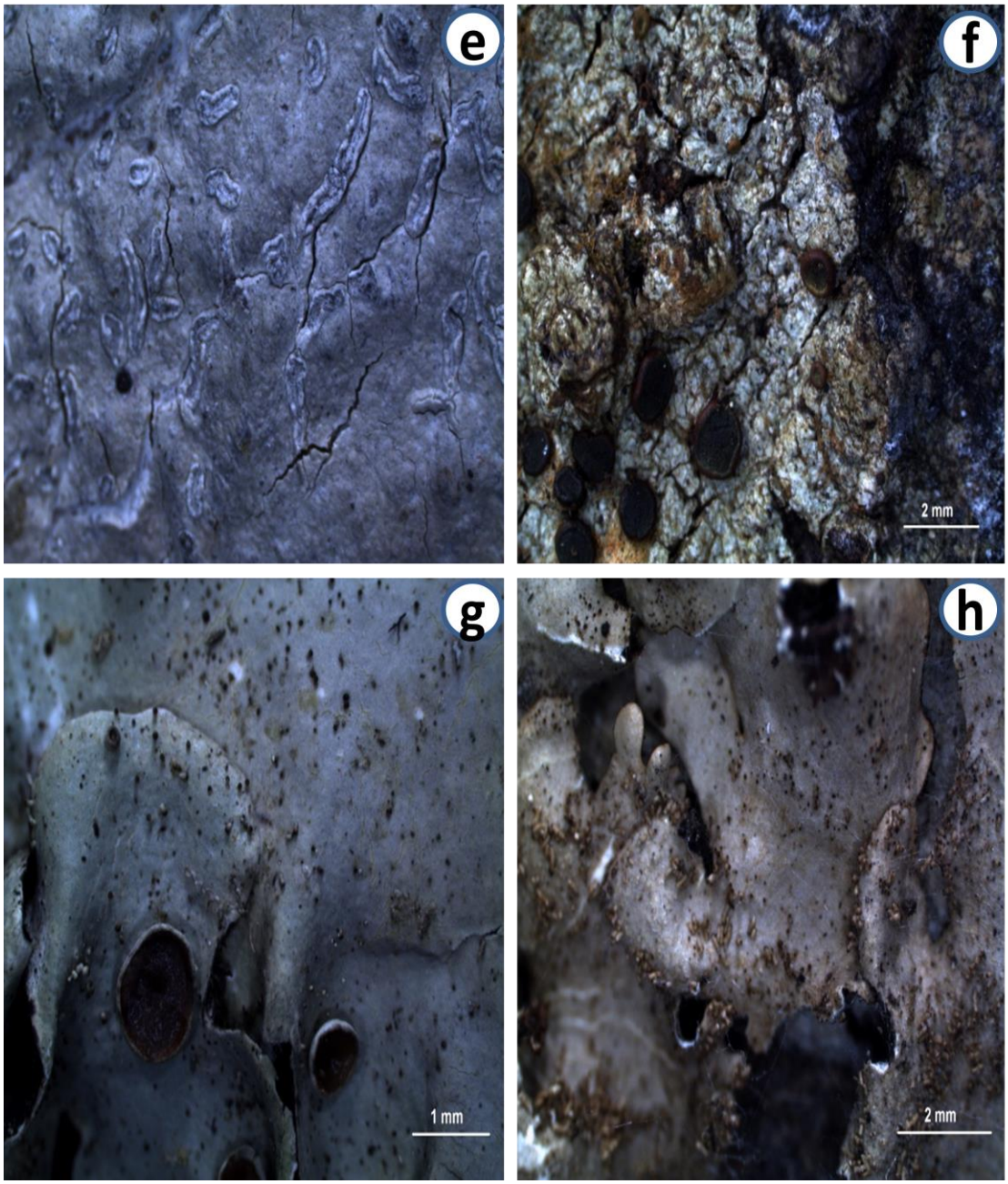


Figure 4.4 Lichen flora from Reiek Hill, Mamit district, Mizoram, (contd…)

(e) *Diorygma soozanum* (Zahlbr.) M. Nakan. & Kashiw.

(f) *Letrouitia vulpina* (Tuck.) Hafellner & Bellem.

(g) *Hypotrachyna rhabdiformis* (Kurok.)Hale

(h) *Parmotrema crinitoides* J.C. Wei

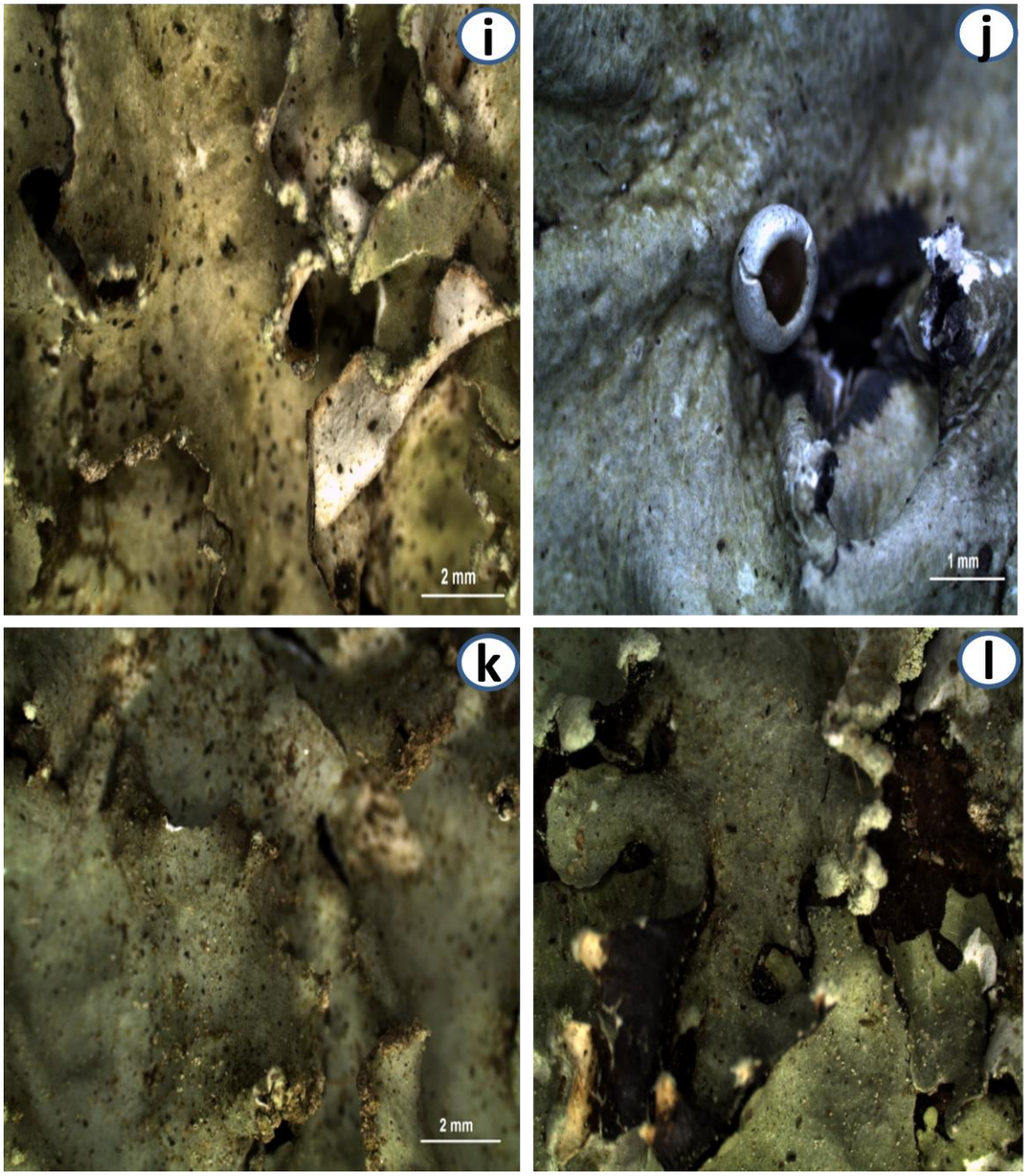


Figure 4.4 Lichen flora from Reiek Hill, Mamit district, Mizoram, (contd…)

(i) *Parmotrema hababianum* (Gyeln.) Hale

(j) *Parmotrema nilgherrense* (Nyl.) Hale

(k) *Parmotrema rampoddense* (Nyl.) Hale

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

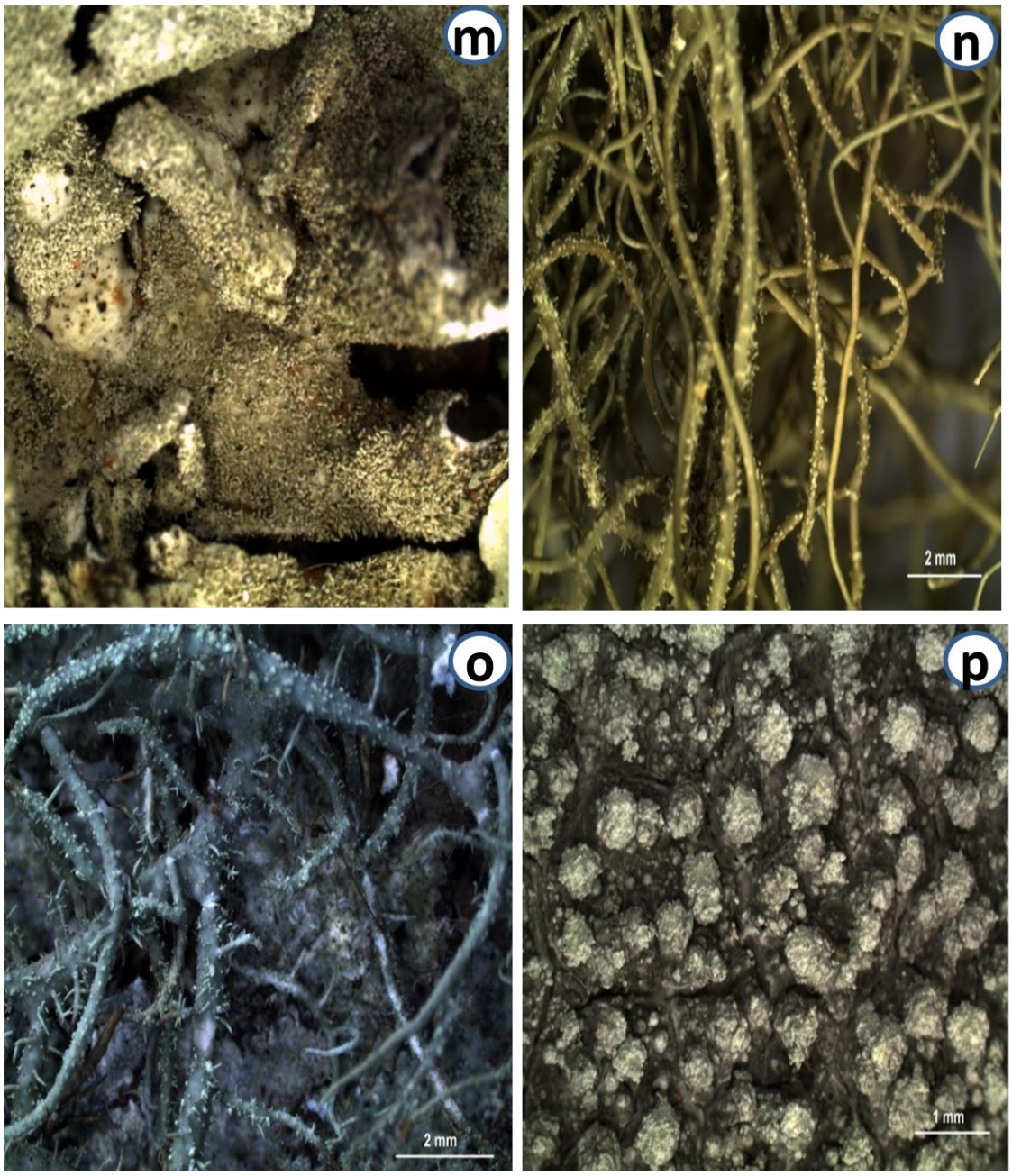


Figure 4.4 Lichen flora from Reiek Hill, Mamit district, Mizoram, (contd…)

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

(n) *Usnea baileyi* (Stirt.) Zahlbr.

(o) *Usnea eumitrioides* Motyka

(p) *Pertusaria leucosorodes* Nyl.

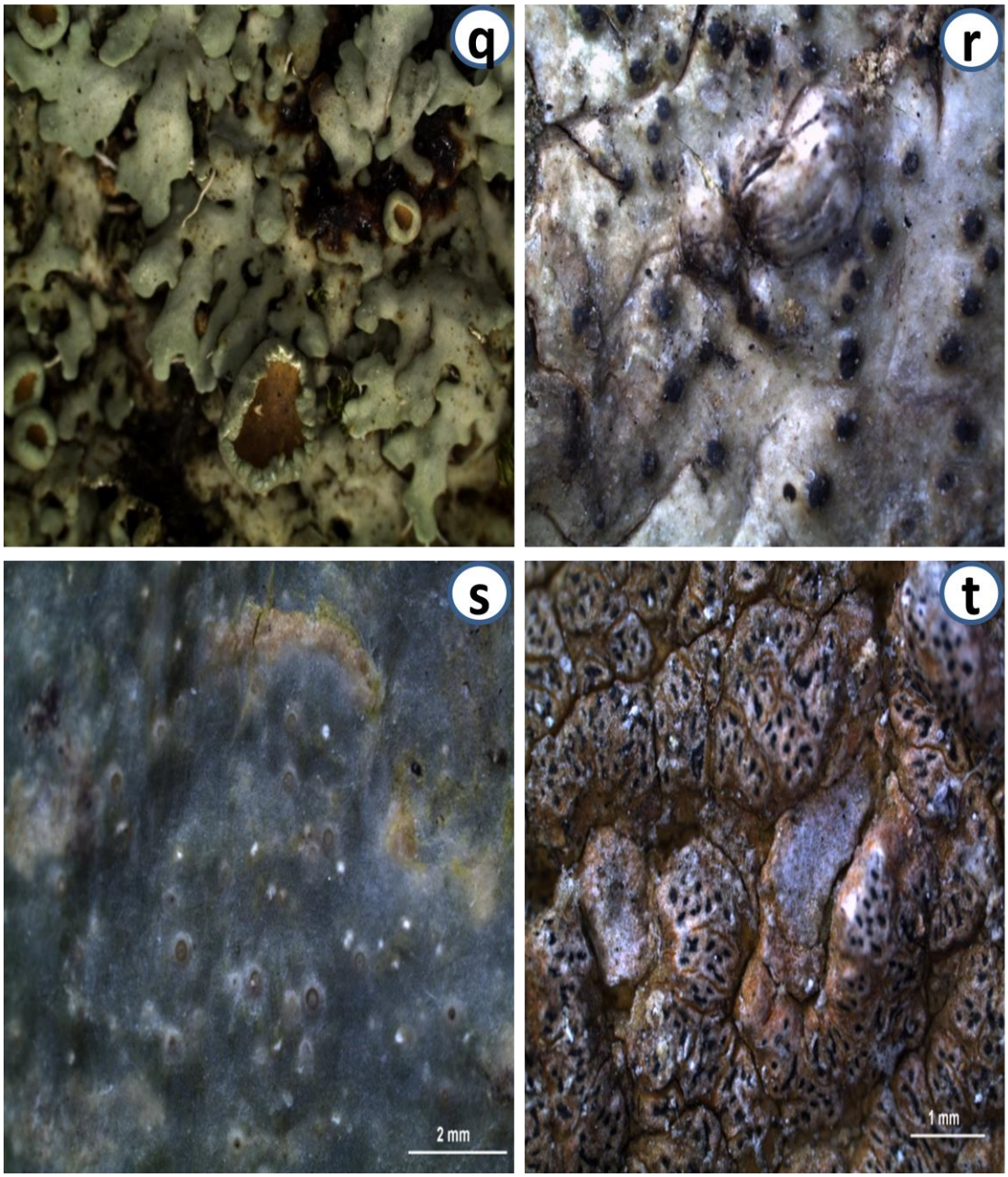


Figure 4.4 Lichen flora from Reiek Hill, Mamit district, Mizoram, (contd…)

(q) *Heterodermia diademat*a (Taylor) D.D. Awasthi

(r) *Pyrenula mastophoroides* (Nyl.) Zahlbr.

(s) *Pyrenula andina* Aptroot

(t) *Chiodecton leptosporum* Müll. Arg.

4.3.5 LICHENS OF MIZORAM UNIVERSITY CAMPUS, AIZAWL DISTRICT

In case of Mizoram University campus, 16 different species of lichens belonging to 8 genera and 6 families were reported (Table 4.4) (Figure 4.5).The distribution of families in the study site shown that Parmeliaceae were having the highest number of species (6 sp.) followed by Physciaceae (4 sp.), Caliciaceae and Graphidaceae both having 2 sp., whereas, family Malmideaceae and Pertusariaceae with 1 sp. were stands on lowest number.

Table 4.4 Lichen flora from Mizoram University Campus, Aizawl district, Mizoram

Sl.No.	Family	Genus	Species	Field No.
1	Caliciaceae	Dirinaria	*Dirinaria aegialita* (Afzel. ex Ach.) B.J. Moore	18-036166
2	Caliciaceae	Dirinaria	*Dirinaria consimilis* (Stirt.) D.D. Awasthi	18-036167
3	Graphidaceae	*Diorygma*	*Diorygma junghuhnii* (Mont. & Bosch) Kalb, Staiger & Elix	18-036131
4	Graphidaceae	*Diorygma*	*Diorygma soozanum* (Zahlbr.) M. Nakan. & Kashiw.	18-036134
5	Malmideaceae	Malmidea	*Malmidea granifera* (Ach.) Kalb, Rivas Plata & Lumbsch	18-036168
6	Pertusariaceae	Pertusaria	*Pertusaria leucosorodes* Nyl.	18-036161
7	Parmeliaceae	Bulbothrix	*Bulbothrix isidiza* (Nyl.) Hale	18-036159
8	Parmeliaceae	*Parmotrema*	*Parmotrema*	18-036158

			austrosinense (Zahlbr.) Hale	
9	Parmeliaceae	*Parmotrema*	*Parmotrema praesorediosum* (Nyl.) Hale	18-036160
10	Parmeliaceae	*Parmotrema*	*Parmotrema rampoddense* (Nyl.) Hale	18-036169
11	Parmeliaceae	*Parmotrema*	*Parmotrema tinctorum* (Despr. ex Nyl.) Hale	18-036170
12	Parmeliaceae	*Usnea*	*Usnea baileyi* (Stirt.) Zahlbr.	18-036171
13	Physciaceae	Heterodermia	*Heterodermia albidiflava* (Kurok.) D.D. Awasthi	18-036164
14	Physciaceae	Heterodermi	*Heterodermia diademata (*Taylor) D.D. Awasthi	18-036163
15	Physciaceae	Heterodermia	*Heterodermia hypochraea* (Vain.) Swinscow & Krog	18-036162
16	Physciaceae	Heterodermia	*Heterodermia obscurata* (Nyl.) Trevis.	18-036165

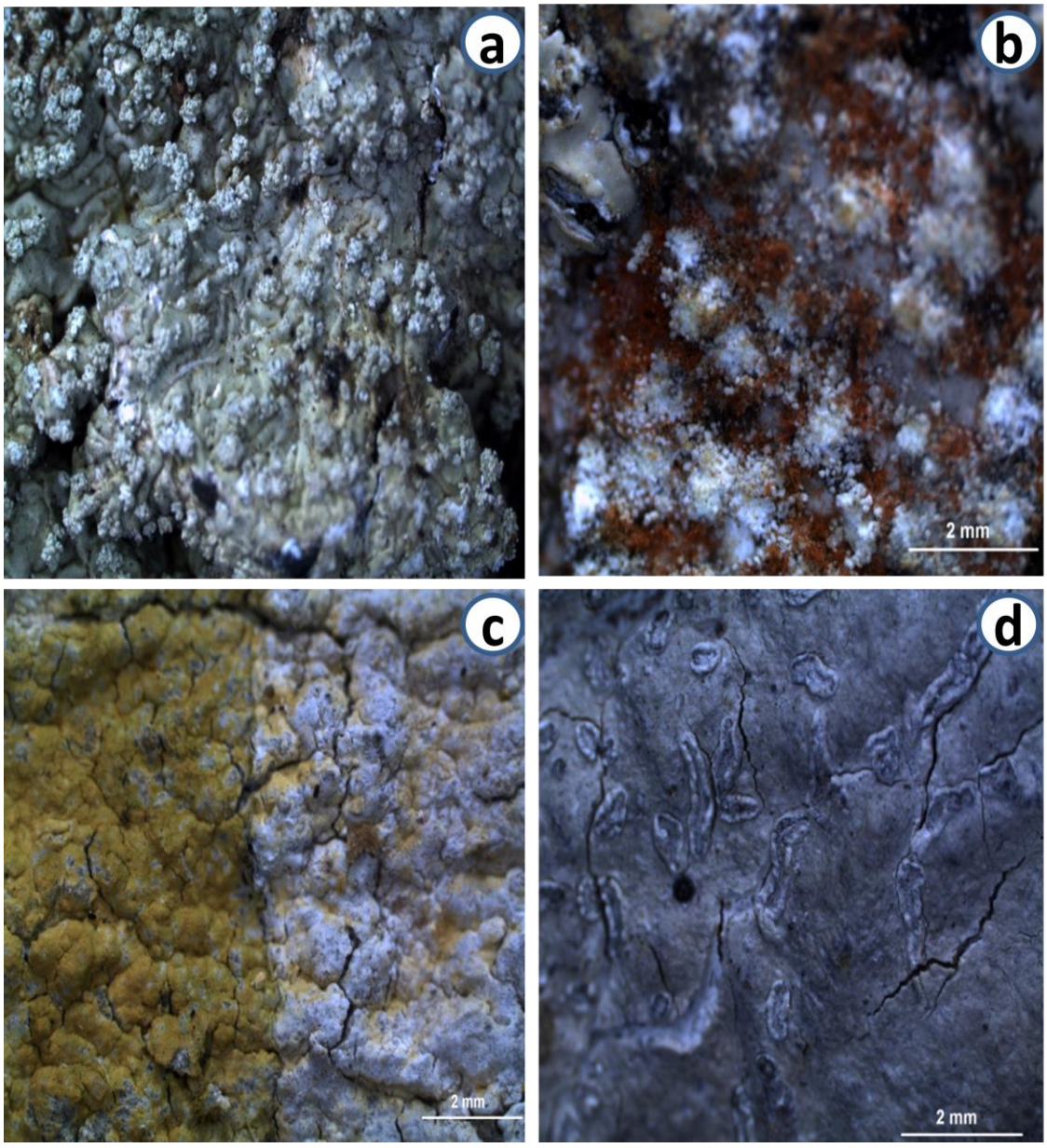


Figure 4.5 Lichen flora from Mizoram University Campus, Aizawl district, Mizoram (contd….)

(a) *Dirinaria aegialita* (Afzel. ex Ach.) B.J. Moore

(b) *Dirinaria consimilis* (Stirt.) D.D. Awasthi

(c) *Diorygma junghuhnii* (Mont. &Bosch) Kalb, Staiger & Elix

(d) *Diorygma soozanum* (Zahlbr.)M. Nakan. & Kashiw

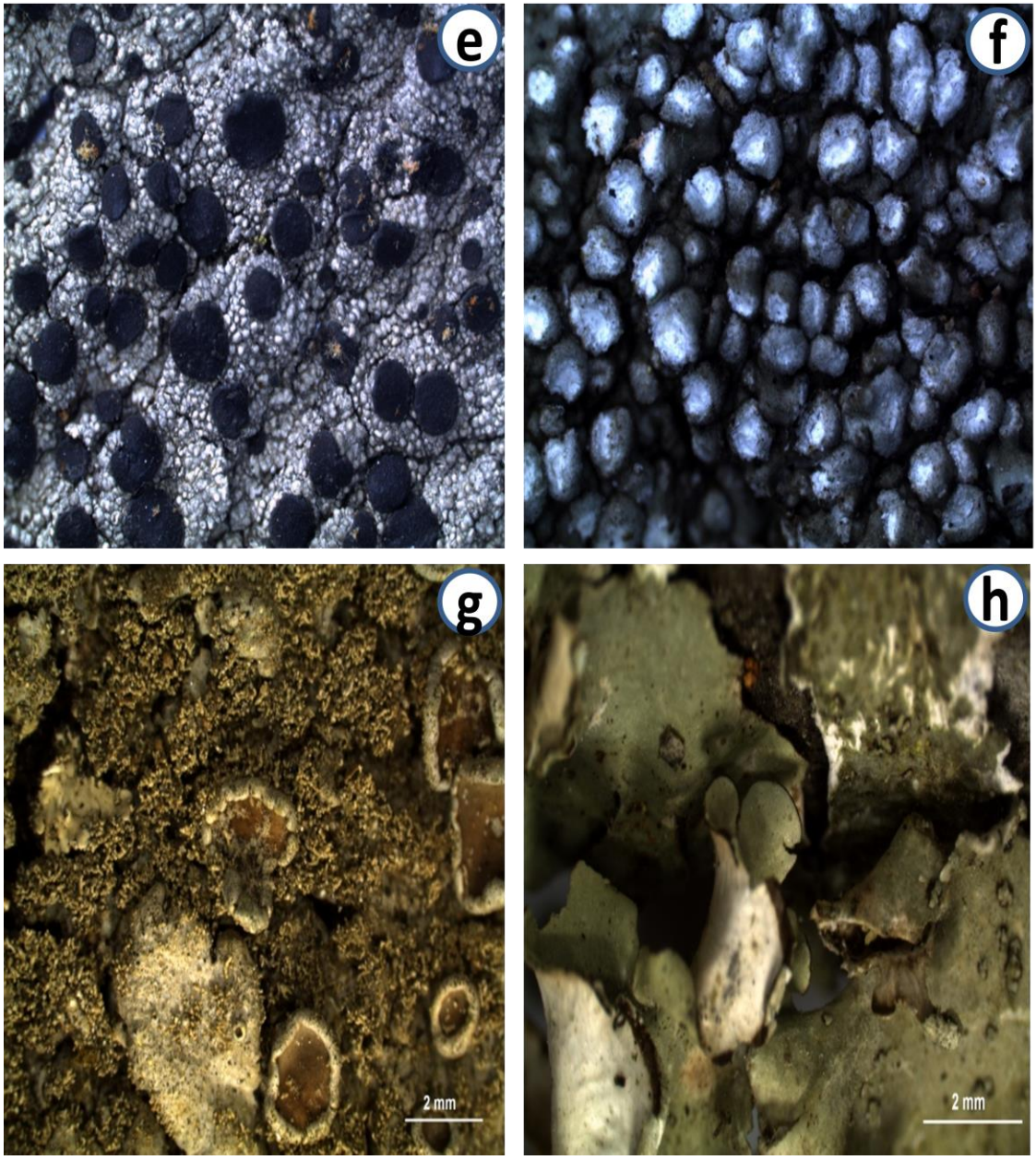


Figure 4.5 Lichen flora from Mizoram University Campus, Aizawl district, Mizoram (contd….)

(e) *Malmidea granifera* (Ach.) Kalb, Rivas Plata & Lumbsch

(f) *Pertusaria leucosorodes*Nyl.

(g) *Bulbothrix isidiza* (Nyl.) Hale

(h) *Parmotrema austrosinense* (Zahlbr.) Hale

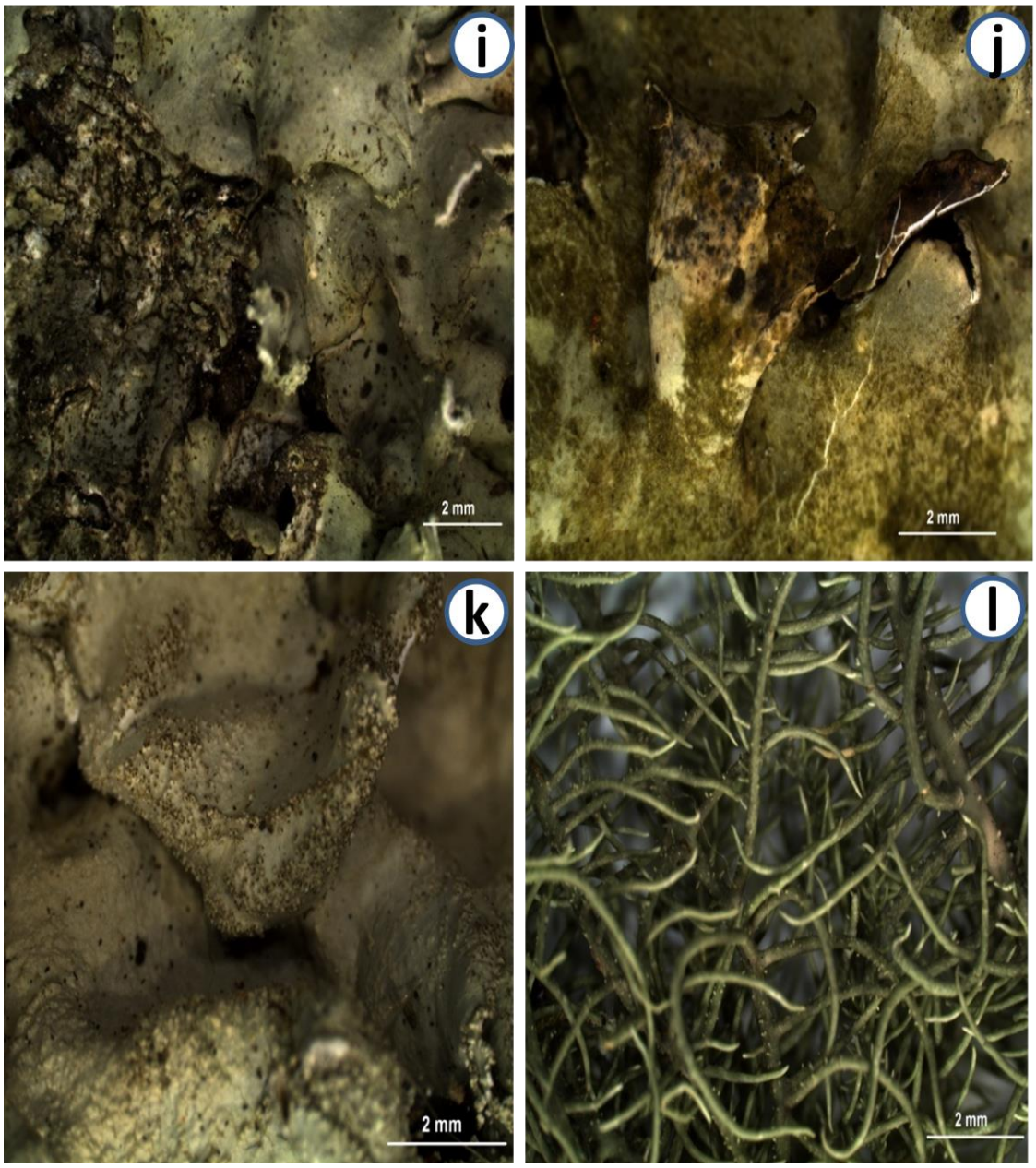


Figure 4.5 Lichen flora from Mizoram University Campus, Aizawl district, Mizoram (contd….)

(i) *Parmotrema praesorediosum* (Nyl.) Hale

(j) *Parmotrema rampoddense* (Nyl.) Hale

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

(l) *Usnea baileyi* (Stirt.) Zahlbr.

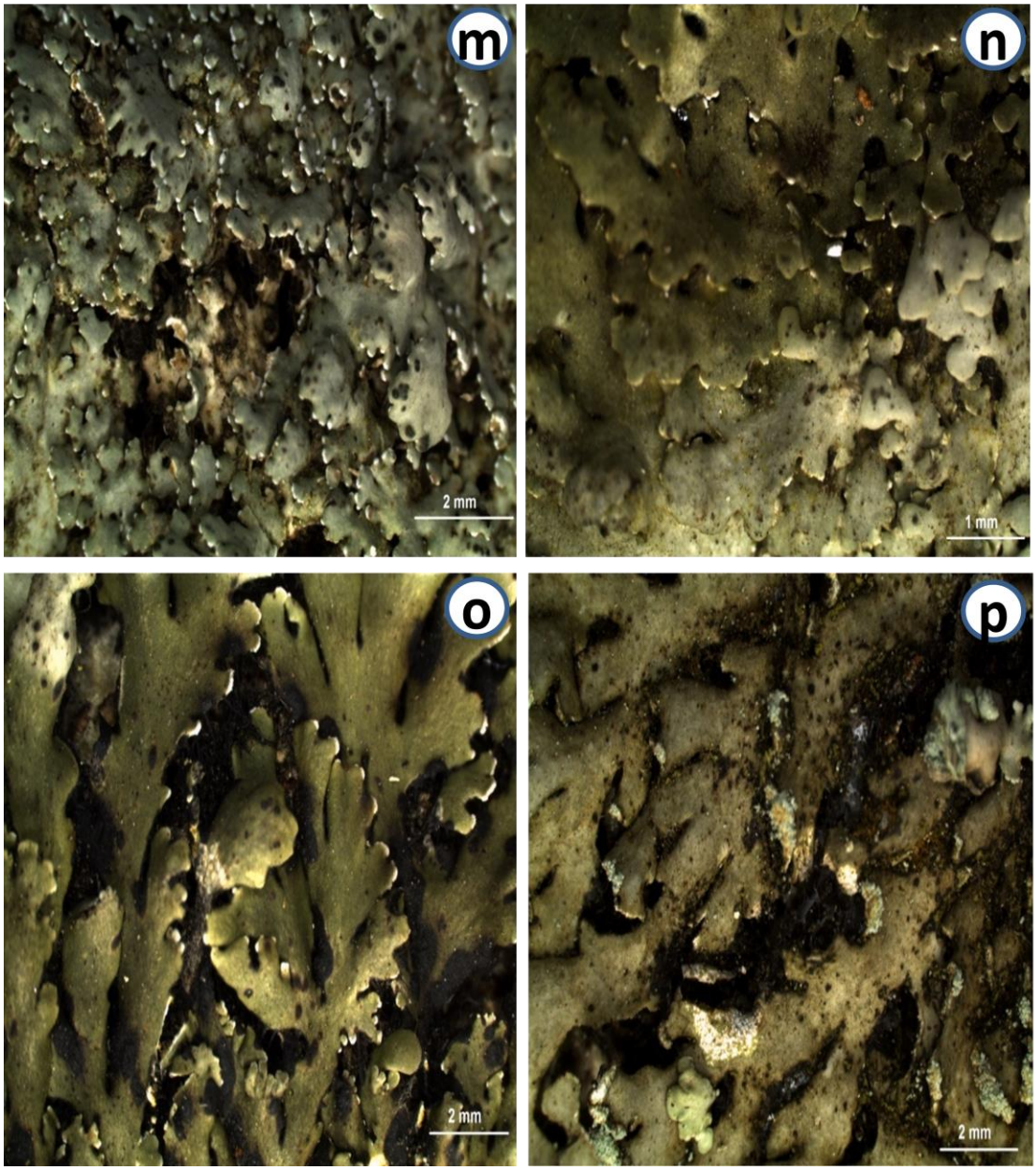


Figure 4.5 Lichen flora from Mizoram University Campus, Aizawl district, Mizoram (contd….)

(m)*Heterodermia albidiflava* (Kurok.) D.D. Awasthi

(n) *Heterodermia diademata* (Taylor) D.D. Awasthi

(o) *Heterodermia hypochraea* (Vain.) Swinscow & Krog

(p) *Heterodermia obscurata* (Nyl.)Trevis.

4.4 PHYTOCHEMICAL SCREENING

The phytochemical screening test of the acetone, aqueous and methanol extract of selected lichens viz., *Everniastrum cirrhatum*, *Parmotrema saccatilobum*, *Parmotrema reticulatum*, *Usnea baileyi*, *Parmotrema latissimum* and *Ramalina conduplicans* were analyzed on the basis of methods as described above in material and methods. The results of the test were made on the presence (+) and absence (-) of the phytochemical present in to the extracts; and the same was illustrated in table (4.5).

Table 4.5 Phytochemical screening of selected lichens of Mizoram

Lichen sample		**Tannins (*Ferric chloride Test)***	**Alkaloids (*Dragondroff's test)***	**Saponins (*Frothing test)***	**Glycosides (*Keller-kilani test)***	**Flavonoids (*NaOH solution test)***	**Proteins (*Xanthoproteic test)***	**Triterpenoids (*Salkowski Test)***	**Carbohydrates (*Benedict's test)***	**Steroids (*Liebermann-Burchard reaction)***
Everniastrum cirrhatum	Acetone	-	-	+	-	+	+	+	+	-
	Aqueous	-	-	-	-	+	+	-	+	-
	Methanol	+	+	+	-	+	+	+	+	-
Parmotrema saccatilobum	Acetone	-	+	-	-	+	-	+	-	-
	Aqueous	-	-	-	-	+	-	-	-	-
	Methanol	-	+	-	-	+	-	+	-	-
Parmotrema reticulatum	Acetone	-	-	-	-	+	+	-	-	-
	Aqueous	+	-	+	-	+	+	-	-	-
	Methanol	+	+	-	-	+	+	-	+	-
Usnea baileyi	Acetone	-	-	-	-	+	-	-	-	-
	Aqueous	-	-	-	-	+	-	-	-	-
	Methanol	-	+	-	-	+	-	-	-	-
Parmotrema latissimum	Acetone	-	-	+	+	+	-	+	-	-
	Aqueous	-	-	+	+	+	-	+	-	-
	Methanol	-	-	+	+	+	-	+	-	-

Ramalina conduplicans	Acetone	+	+	-	+	+	-	-	-	-
	Aqueous	-	-	-	-	-	-	-	-	-
	Methanol	+	+	+	+	+	+	+	-	-

The phytochemical screening of selected lichens were revealed that, methanol extract of *Evrniastrum cirrhatum* was of holding the first rank in terms of phytochemical like alkaloids, carbohytres, flavanoids, proteins, saponins tanninsand triterpenoids were present in the extract except glycosides and steroids. Similarly, acetone, aqueous and methanol extract of *Parmotrema reticulatum* was shown the presence of alkaloids, carbohytres, flavanoids, proteins, saponins and tannins. Whereas, acetone and methanol extract of *Ramalina conduplicans* shown the presence of all except carbohydrates and steroids. All the three solventsextract of *Parmotrema latissimum* indicated the presence of glycosides, flavanoids, saponins and triterpenoids, on the other hand, extract with the solvents of acetone and aqueous of *Usnea baileyi* observed the presence of flavanoids only and methanol extracts shown the presence of both flavanoids and alkaloids and acetone and methanol extract of *Parmotrema saccatilobum* were shown the presence of alkaloids, flavanoids and triterpenoids while the aqueous extract only with the presence of flavanoids.

4.5 ANTI-OXIDANT ACTIVITIES OF SELECTED LICHENS

The four lichen extracts were chosen for anti-oxidants activities on the basis of their phytoconstituents shown during their screening, and it was evaluated using 5 different protocols viz. DPPH free radical scavenging, Ferric reducing power capacity, Superoxide radical scavenging activity by alkaline DMSO method, total phenolic contents and total Flavonoids contents. The results were shown that the maximum number of lichens extract possessed the antioxidant properties; which reflect its potent medicinal values. The details were summarized as follows:

4.5.1 DPPH radical scavenging activity:

The scavenging DPPH radicals of4 selected lichen extractswhich made in 3 different solvents namely acetone, aqueous and methanol separately were evaluated for its scavenging activity, where, ascorbic acid was used as standard for comparative studies. The observed values of each extracts were sequentially shown in Table (4.7, 4.8, 4.9 and 4.10) including the standard (table 4.6) (fig. 4.6). The comparative graph of three different solvents were also made and depicted separately in figure (4.7; 4.8; 4.9 and 4.10).

Table 4.6 DPPH radical scavenging activity of standard Ascorbic acid

% scavenging activity of standard Ascorbic acid	
Concentration (µg/ml)	**% scavenging activity**
10	30.91
20	42.97
30	56.54
40	71.16
50	83.38
60	96.48
IC_{50}(µg/ml)	2.4

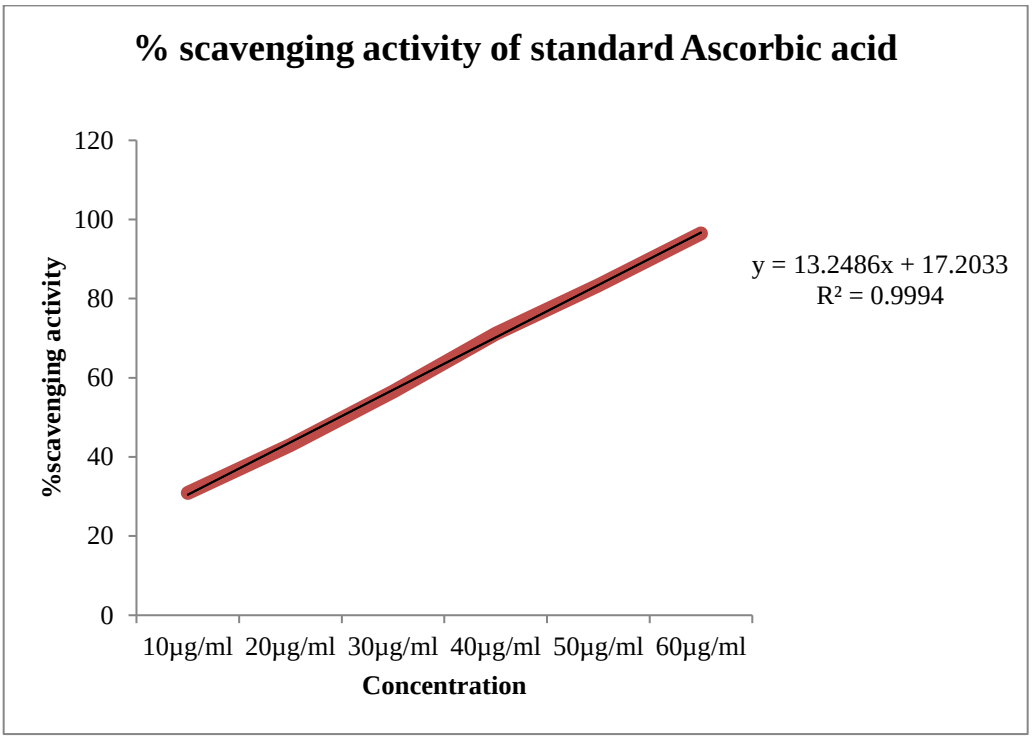


Figure 4.6 DPPH radical scavenging activity of standard Ascorbic acid

4.5.1.1 DPPH radical scavenging activity of *Usnea baileyi*

The scavenging effect of the above mentioned extractsof *Usnea baileyi* at different concentrations were evaluated, where the methanol extract has shown slightly higher percentage of inhibition (64.33%) in comparison to acetone (63.23%) and aqueous extract (60.8%) respectively at the highest concentration of 60 (µg/ml). The standard reference ascorbic acid had shown 96.48% of inhibition at the same concentration. In case of $IC_{50,}$ methanol has recorded values (3.7) as compare to acetone (4.5) and aqueous extracts (4.9).

Table: 4.7DPPH radical scavenging activity of *Usnea baileyi*

% scavenging activity of *Usnea baileyi*			
Concentration (µg/ml)	Acetone extract	Aqueous extract	Methanolic extract
10	11.47	10.09	8.62
20	28.67	19.3	14.04

30	37.56	28.49	21.12
40	44.97	32.89	32.21
50	51.96	48.91	47.68
60	63.23	60.8	64.33
IC_{50}(µg/ml)	4.5	4.9	3.7

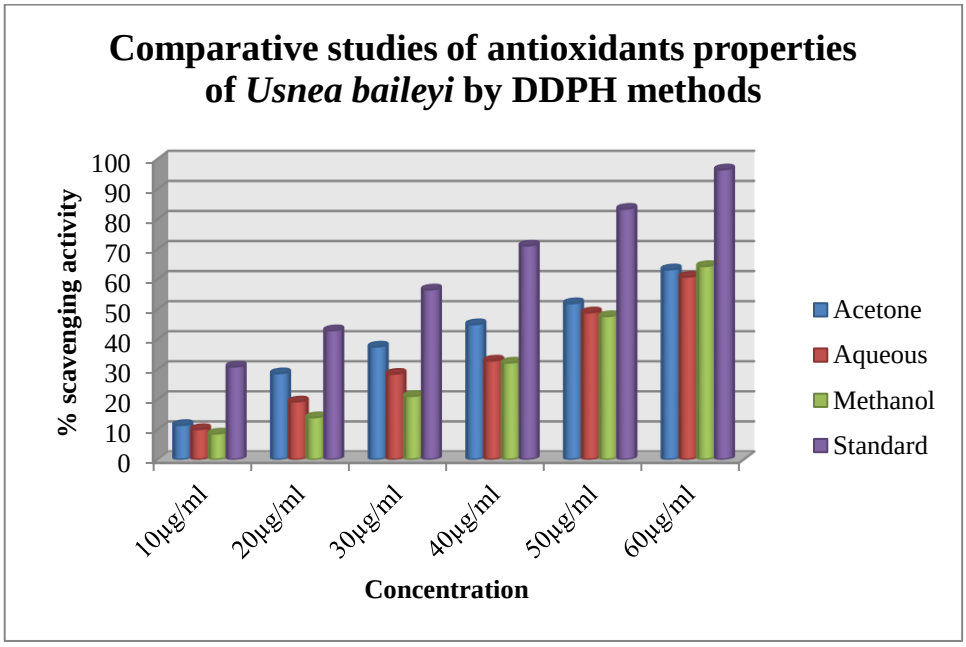


Figure 4.7 Comparative studies of antioxidants properties of *Usnea baileyi* by DDPH methods

4.5.1.2 DPPH radical scavenging activity of *Everniastrum cirhhatum*

The scavenging effect of the above mentioned extracts of *Everniastrum cirhhatum* at different concentrations were evaluated, where the methanol extract has shown more higher percentage of inhibition(71.81%) in comparison to acetone (64.78%) and aqueous extract (62.43%) respectively at the highest concentration of 60 (µg/ml). The standard reference ascorbic acid had shown 96.48% of inhibition at the same concentration. In case of $IC_{50,}$ methanol has recorded values (3.8) as compare to acetone (4.2) and aqueous extracts (4.3).

Table: 4.8 DPPHradical scavenging activity of *Everniastrum cirhhatum*

% scavenging activity of *Everniastrum cirhhatum*			
Concentration (µg/ml)	Acetone extract	Aqueous extract	Methanolic extract
10	15.45	10.12	13.48
20	25.95	23.1	28.26
30	36.71	42.04	43.71
40	49.74	50.33	53.44
50	60.28	57.38	63.94
60	64.78	62.43	71.81
IC_{50}(µg/ml)	4.2	4.3	3.8

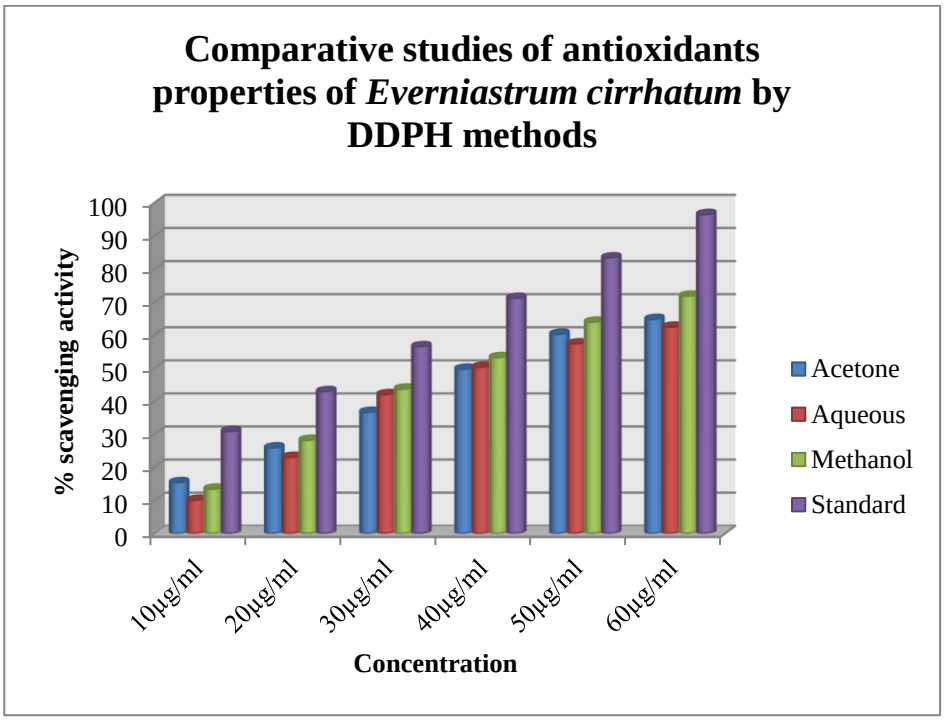


Figure 4.8 Comparative studies of antioxidants properties of *Everniastrum cirrhatum* by DDPH methods

4.5.1.3 DPPH radical scavenging activity of *Parmotrema reticulatum*

The scavenging effect of the above-mentioned extracts of *Parmotrema reticulatum* at different concentrations were evaluated, where the methanol extract has shown much higher percentage of inhibition (85.28%) in comparison to acetone (69.48%) and aqueous extract (61.86%) respectively at the highest concentration of 60 (µg/ml). The standard reference ascorbic acid had shown 96.48% of inhibition at the same concentration. In case of $IC_{50,}$ methanol has recorded values (2.7) as compare to acetone (3.8) and aqueous extracts (4.7).

Table 4.9 DPPH radical scavenging activity of *Parmotrema reticulatum*

% scavenging activity of *Parmotrema reticulatum*			
Concentration (µg/ml)	Acetone extract	Aqueous extract	Methanolic extract
10	13.11	8.05	27.17
20	31.82	20.03	38.13
30	47.45	39.71	56.25
40	51.73	45	68.94
50	62.91	48.75	78.99
60	69.48	61.86	85.28
IC_{50}(µg/ml)	3.8	4.7	2.7

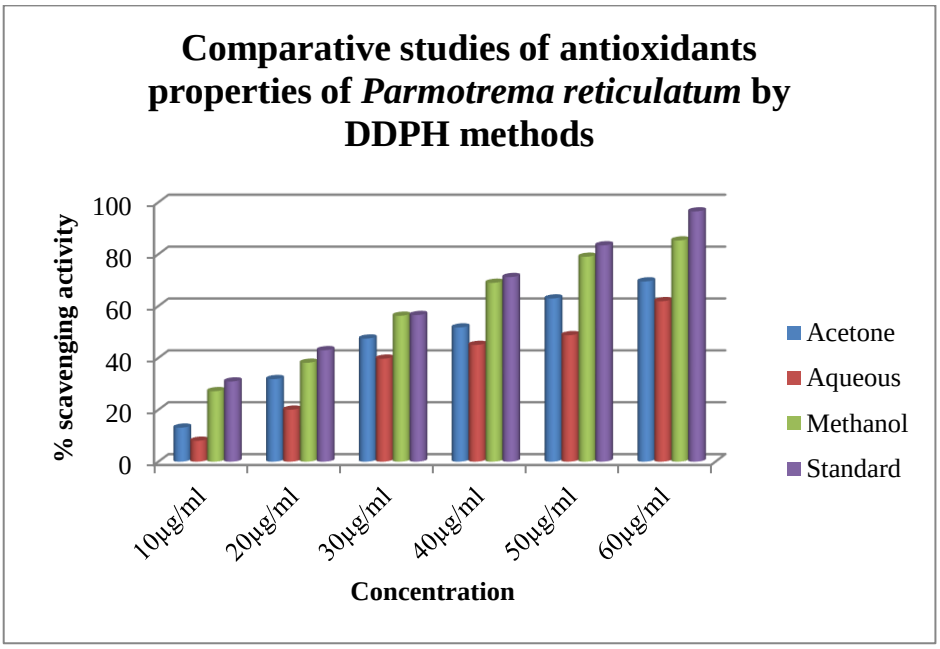


Figure 4.9 Comparative studies of antioxidants properties of *Parmotrema reticulatum* by DDPH methods

4.5.1.4 DPPH radical scavenging activity of *Ramalina conduplicans*

The scavenging effect of the above mentioned extracts of *Ramalina conduplicans* at different concentrations were evaluated, where the methanol extract has shown much higher percentage of inhibition(70.45%) in comparison to acetone (68.59%) and aqueous extract (64.4%) respectively at the highest concentration of 60 (µg/ml). The standard reference ascorbic acid had shown 96.48% of inhibition at the same concentration. In case of $IC_{50,}$ methanol has recorded values (3.31) as compare to acetone (4.33) and aqueous extracts (4.02).

Table: 4.10 DPPHradical scavenging activity of *Ramalina conduplicans*

% scavenging activity of *Ramalina conduplicans*			
Concentration (µg/ml)	Acetone extract	Aqueous extract	Methanolic extract
10	16.34	23.28	25.15
20	23.33	26.73	38.11
30	39.86	36.52	48.5

40	53.86	48.4	54.5
50	63.13	56.78	61.05
60	68.59	64.4	70.45
IC_{50} (µg/ml)	4.02	4.33	3.31

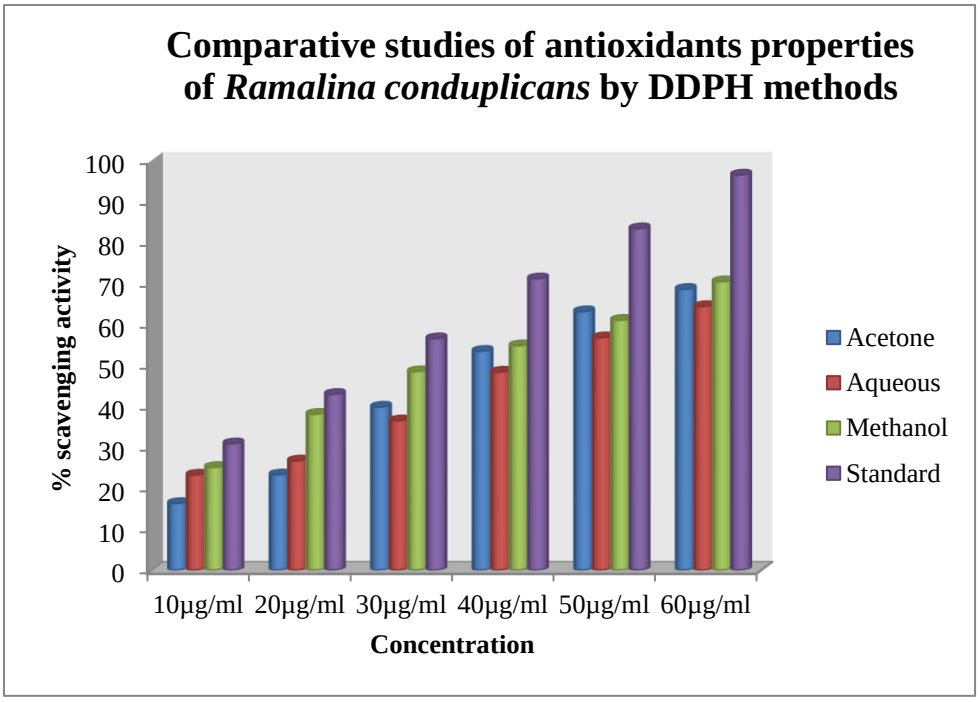


Figure 4.10Comparative studies of antioxidants properties of *Ramalina conduplicans* by DDPH methods

4.5.2 FERRIC REDUCING POWER:

The reducing power assays of the same selected lichen extracts werefurther evaluated, where ascorbic acid was remaining as a standard. In reducing power higher absorbance valuesindicates the higher reducing power. The resulted values at 700nm of each extract were represented in table (4.12, 4.13, 4.14 and 4.15) including the standard (table 4.11) (fig. 4.11). Accordingly, their comparative graph was also made and depicted separately in figure (4.12; 4.13; 4.14 and 4.15).

Table 4.11Reducing power of Standard Ascorbic acid

Reducing power of Standard Ascorbic acid (mean ±SD)	
Concentration	Reducing power of Ascorbic acid
5µg/ml	0.204±0.01
10µg/ml	0.351±0.02
15 µg/ml	0.517±0.02
30 µg/ml	0.742±0.01
60 µg/ml	0.915±0.00
100 µg/ml	1.080±0.04

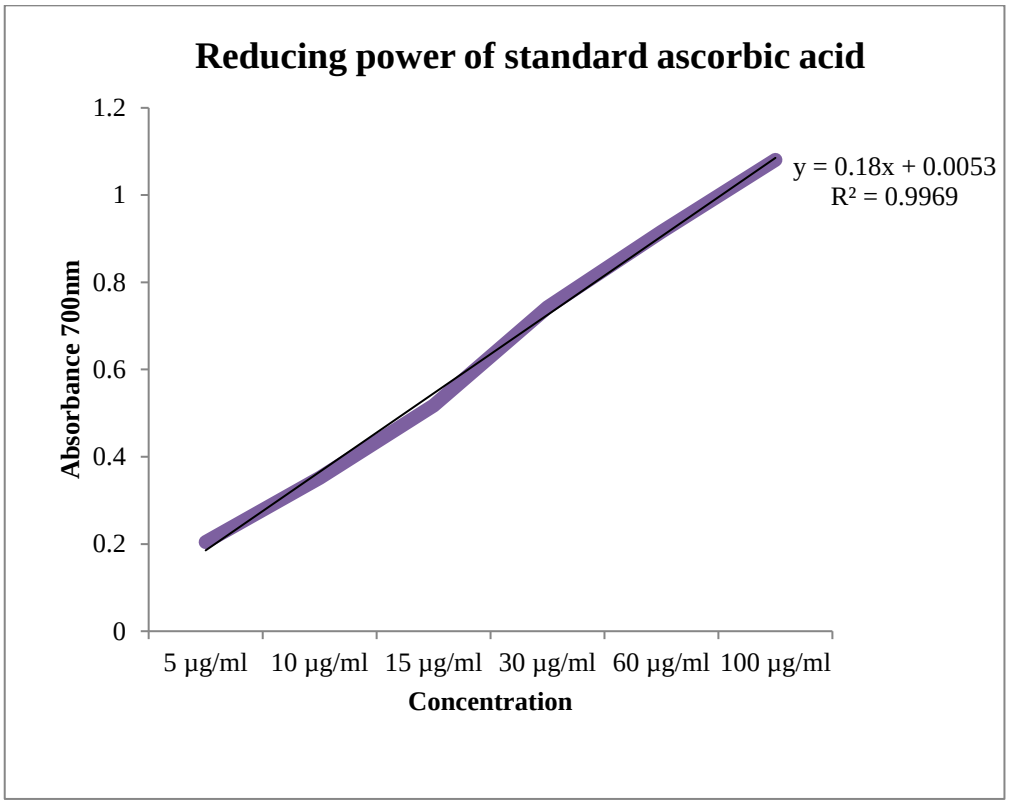


Figure 4.11 Reducing power assay of ascorbic acid

4.5.2.1 Reducing power of *Usnea baileyi*

The reducing power of the *Usnea baileyi* was found more active in methanol extract at concentration of 100µg/ml; and it was recorded with higher values 0.78±0.02

in comparison to acetone extract (0.69±0.01) and aqueous extract (0.62±0.02) respectively. On the other hand, the standard (ascorbic acid) has shown more potent reducing power (1.080±0.04) at same concentration as made for extracts.

Table: 4.12 Reducing power of *Usnea baileyi*

Reducing power of *Usnea baileyi* (mean ±SD)			
Concentration	Acetone extract	Aqueous extract	Methanolic extract
5µg/ml	0.04±0.00	0.03±0.01	0.16±0.02
10µg/ml	0.08±0.01	0.08±0.01	0.28±0.02
15 µg/ml	0.21±0.02	0.18±0.01	0.45±0.02
30 µg/ml	0.34±0.04	0.27±0.01	0.58±0.00
60 µg/ml	0.56±0.05	0.49±0.03	0.72±0.01
100 µg/ml	0.69±0.01	0.62±0.02	0.78±0.02

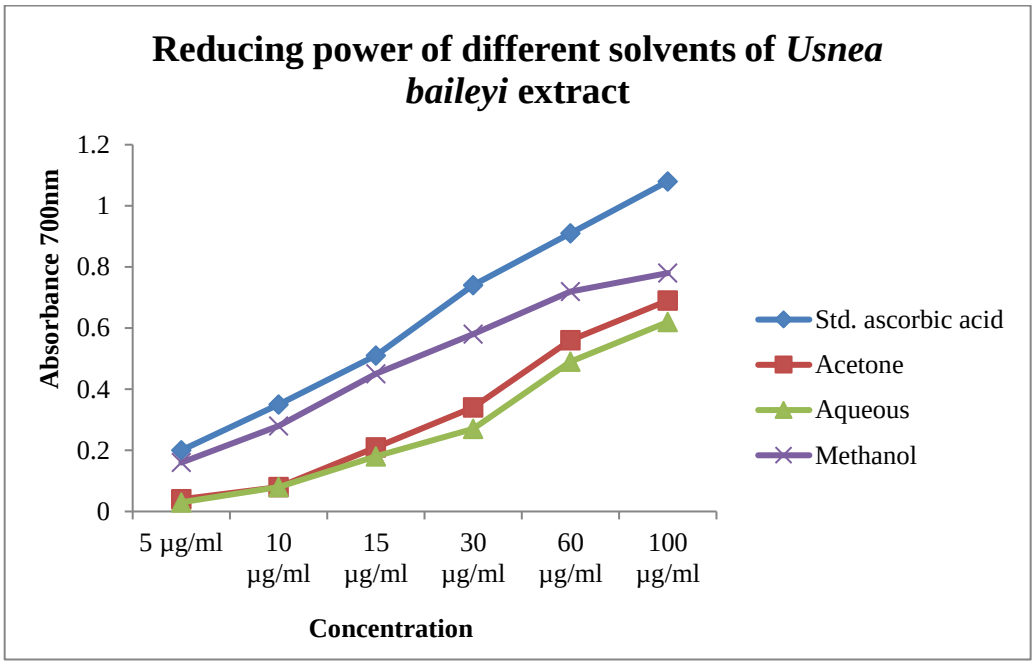


Figure 4.12 Reducing power of different solvents of *Usnea baileyi* extract

4.5.2.2 Reducing power of *Everniastrum cirhhatum*

The reducing power of the *Everniastrum cirhhatum* was found more active in methanol extract at concentration of 100µg/ml; and it was recorded with higher values 0.94±0.05 in comparison to acetone extract (0.78±0.03) and aqueous extract (0.61±0.03) respectively. On the other hand, the standard (ascorbic acid) has shown more potent reducing power (1.080±0.04) at same concentration as made for extracts.

Table 4.13Reducing power of *Everniastrum cirhhatum*

Reducing power of *Everniastrum cirhhatum* (mean ±SD)			
Concentration	Acetone extract	Aqueous extract	Methanolic extract
5µg/ml	0.09±0.03	0.03±0.02	0.12±0.02
10µg/ml	0.16±0.03	0.10±0.02	0.34±0.02
15 µg/ml	0.24±0.04	0.15±0.03	0.48±0.01
30 µg/ml	0.43±0.05	0.26±0.03	0.67±0.02
60 µg/ml	0.68±0.04	0.43±0.04	0.83±0.01
100 µg/ml	0.78±0.03	0.61±0.03	0.94±0.05

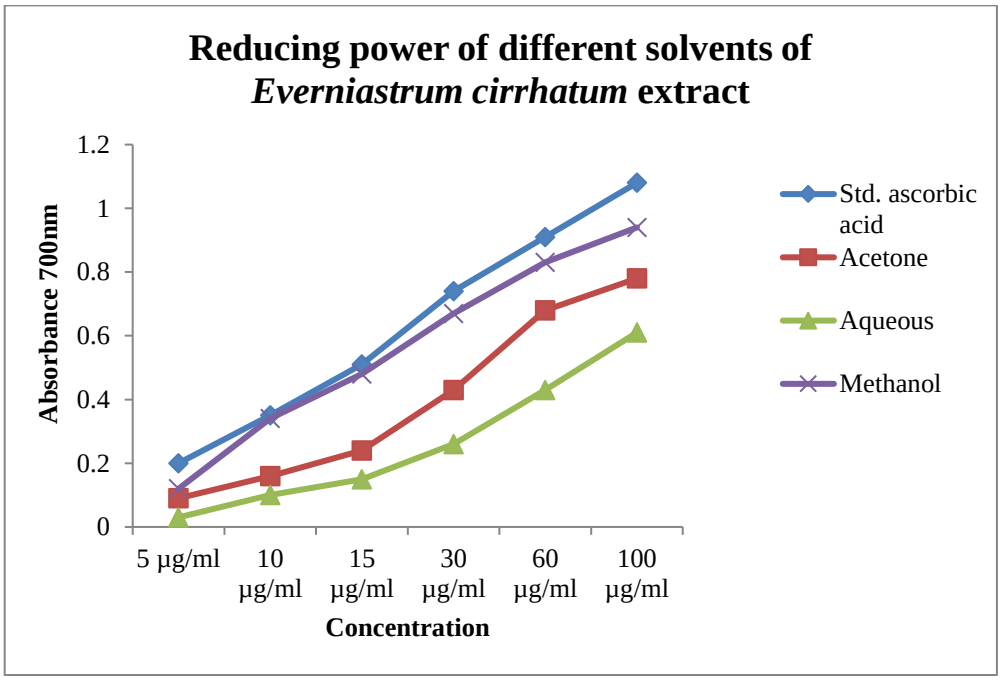


Figure 4.13Reducing power of different solvents of *Everniastrum cirrhatum* extract

4.5.2.3 Reducing power of *Parmotrema reticulatum*

The reducing power of the *Parmotrema reticulatum* was found more active in methanol extract at concentration of 100µg/ml; and it was recorded with higher values 0.85±0.02 in comparison to acetone extract (0.711±0.05) and aqueous extract (0.48±0.02) respectively. On the other hand, the standard (ascorbic acid) has shown more potent reducing power (1.080±0.04) at same concentration as made for extracts.

Table: 4.14 Reducing power of *Parmotrema reticulatum*

Reducing power of *Parmotrema reticulatum*(mean ±SD)			
Concentration	Acetone extract	Aqueous extract	Methanolic extract
5µg/ml	0.092±0.04	0.08±0.01	0.07±0.01
10µg/ml	0.197±0.03	0.11±0.02	0.15±0.02
15 µg/ml	0.243±0.02	0.17±0.03	0.32±0.04
30 µg/ml	0.417±0.06	0.25±0.04	0.62±0.03
60 µg/ml	0.630±0.04	0.32±0.03	0.72±0.03
100 µg/ml	0.711±0.05	0.48±0.02	0.85±0.02

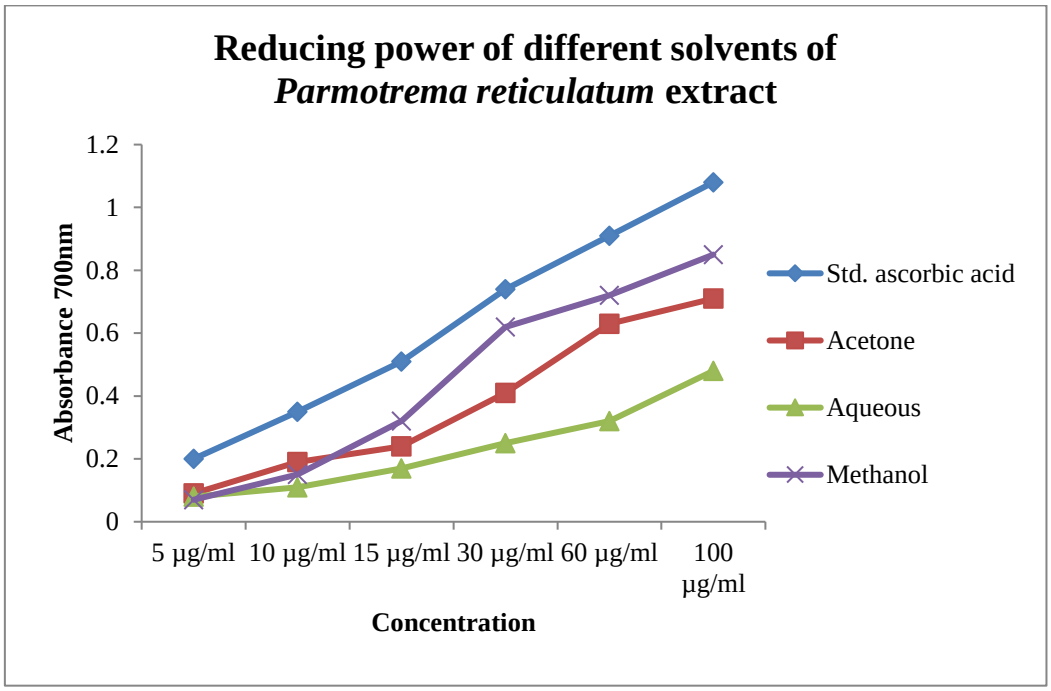


Figure 4.14 Reducing power of different solvents of *Parmotrema reticulatum* extract

4.5.2.4 Reducing power of *Ramalina conduplicans*

The reducing power of the *Ramalina conduplicans* was found more active in methanol extract at concentration of 100µg/ml; and it was recorded with higher values 0.61±0.04 in comparison to acetone extract (0.52±0.03) and aqueous extract (0.44±0.02) respectively. On the other hand, the standard (ascorbic acid) has shown more potent reducing power (1.080±0.04) at same concentration as made for extracts.

Table: 4.15 Reducing power of *Ramalina conduplicans*

Reducing power of *Ramalina conduplicans* (mean ±SD)			
Concentration	Acetone extract	Aqueous extract	Methanolic extract
5µg/ml	0.13±0.02	0.07±0.02	0.18±0.03
10µg/ml	0.16±0.02	0.12±0.03	0.26±0.02
15 µg/ml	0.24±0.02	0.16±0.03	0.33±0.02
30 µg/ml	0.29±0.02	0.21±0.02	0.43±0.04
60 µg/ml	0.39±0.05	0.33±0.03	0.54±0.05

100 µg/ml	0.52±0.03	0.44±0.02	0.61±0.04

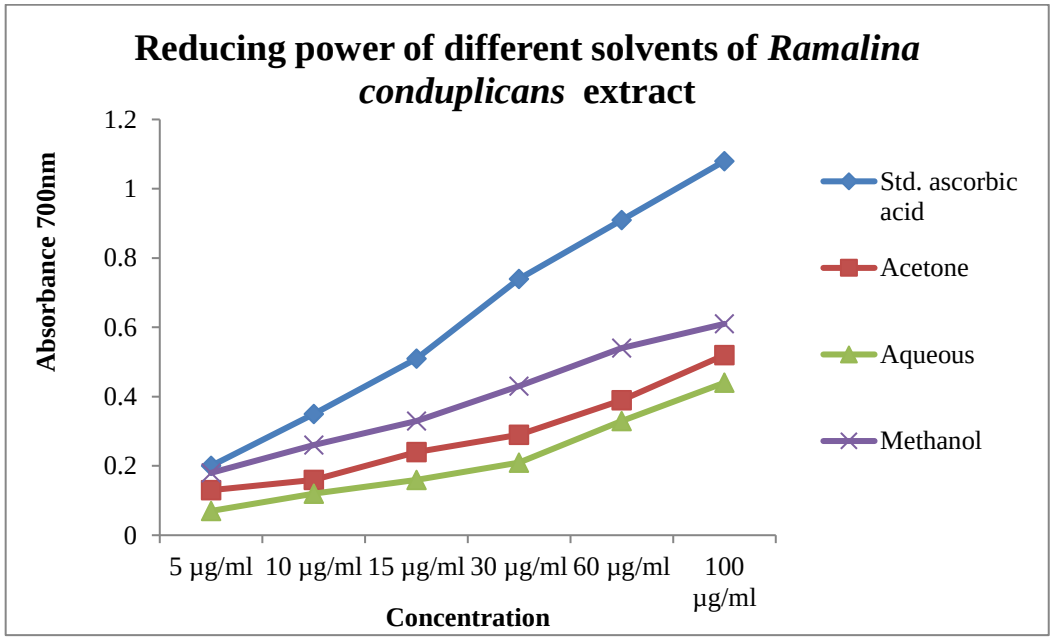


Figure 4.15 Reducing power of different solvents of *Ramalina conduplicans extract*

4.5.3 SUPEROXIDE ANION RADICAL SCAVENGING ACTIVITY

Identically, 4 selected lichen extracts which made in three different solvents were evaluated for its superoxide anion radical scavenging activity at different concentrations. Rutin was used as standard to compare the scavenging activity against lichens extracts at same concentrations. The scavenging activity of Rutin as a reference and lichens extracts were shown ascending in table (4.16, 4.17, 4.18, 4.19 and 4.20) with the standard graph in figure 4.16. The comparative graph of each extracts were also made and plotted separately in figure (4.17; 4.18; 4.19 and 4.20).

Table 4.16 Superoxide radical scavenging activity of standard *Rutin*

Superoxide radical scavenging activity of standard Rutin	
Concentration (µg/ml)	**% scavenging activity**
1	20.38
10	36.25

20	47.35
30	59.61
40	71.22
50	82.7
60	93.16
IC_{50}(µg/ml)	3.2

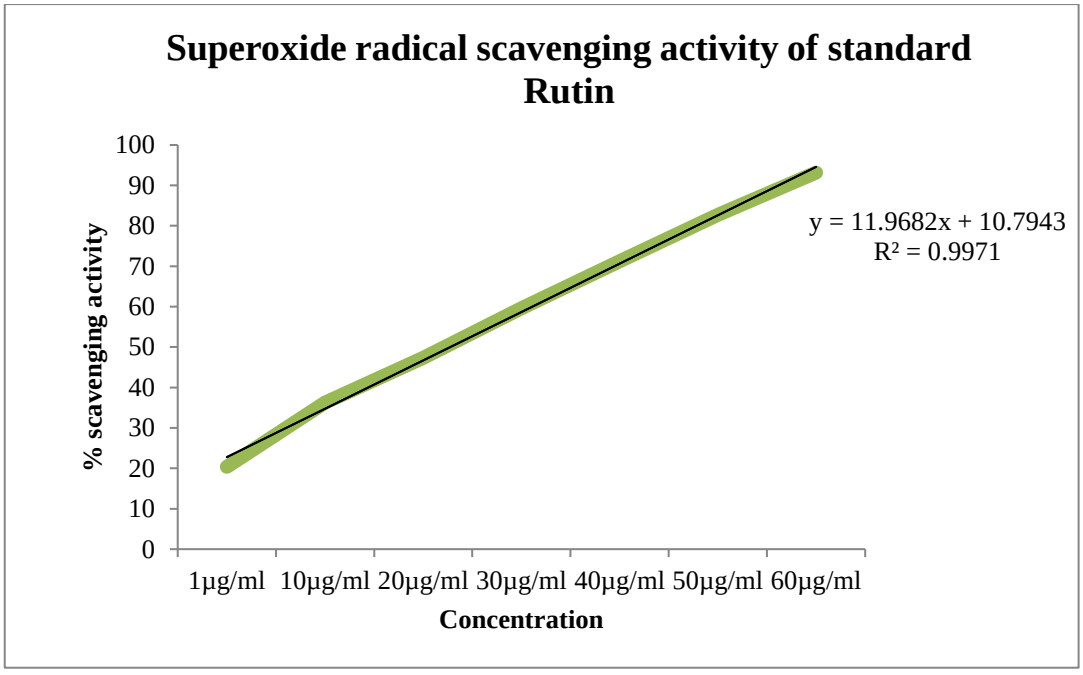


Figure 4.16 Superoxideradical scavenging activity of standard Rutin

4.5.3.1 Superoxide radical scavenging activity of *Parmotrema latissimum*

The superoxide radicals scavenging activity of *Parmotrema latissimum* was investigated and it was found that there was a significant difference between lichen extracts and Rutin (standard); because Rutin was showing more % inhibition (93.16%) as well as IC_{50} value (3.2µg/ml). In comparison to all three extracts, the methanol extract had resulted higher scavenging properties with 73.57% inhibition, however, acetone (72.87%) and aqueous (69.94%) extracts shown the less % of inhibition at concentration

of 60µg/ml. The IC_{50} value for methanol, acetone and aqueous extracts was observed as 3.7, 3.7 and 4.2respectively.

Table 4.17 Superoxide radical scavenging activity of *Parmotrema latissimum*

% scavenging activity of *Parmotrema latissimum*			
Concentration (µg/ml)	Acetone extract	Aqueous extract	Methanolic extract
1	8.28	5.65	7.75
10	20.26	17.33	16.1
20	33.5	24.78	25.82
30	48.45	31.48	38.24
40	55.03	39.93	48.99
50	61.69	58.35	61.97
60	72.87	69.94	73.57
IC_{50}(µg/ml)	3.72	4.24	3.72

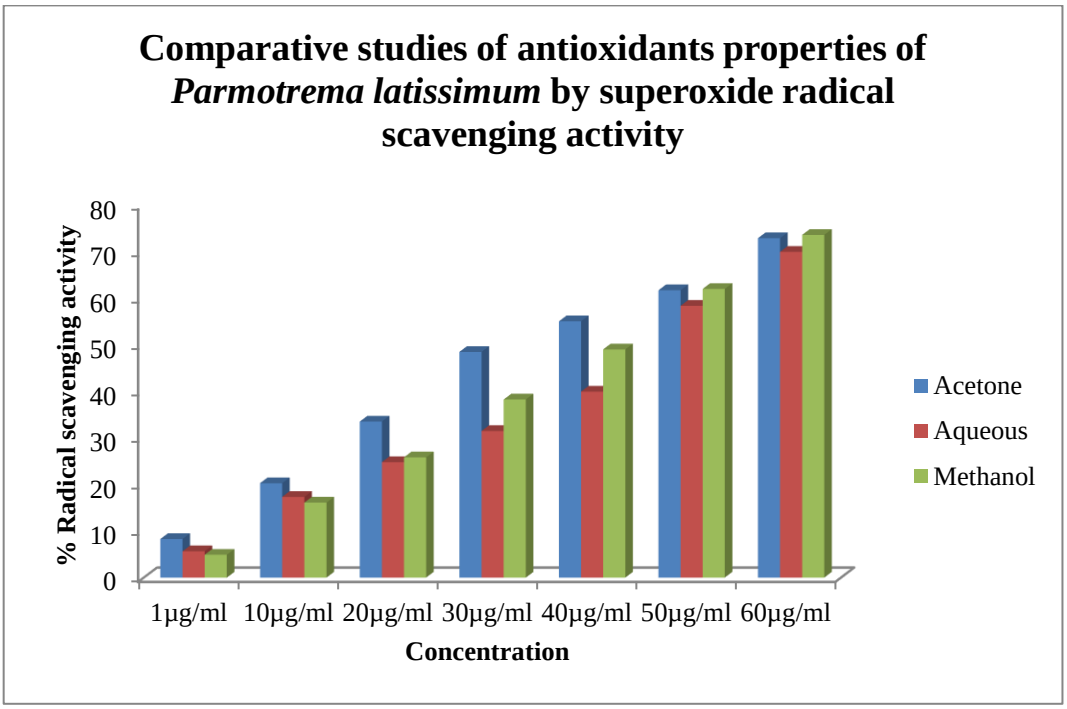


Figure 4.17 Comparative studies of antioxidants properties of *Parmotrema latissimum* by superoxide radical scavenging activity

4.5.3.2 Superoxide radical scavenging activity of *Parmotrema reticulatum*

The superoxide radicals scavenging activity of *Parmotrema reticulatum* was investigated and it was found that there was a significant difference between lichen extracts and Rutin (standard); because Rutin was showing more % inhibition (93.16%) as well as IC_{50} value (3.2µg/ml). In comparison to all three extracts, the methanol extract had resulted higher scavenging properties with 75.73% inhibition, however, acetone (69.77%) and aqueous (60.93%) extracts shown the less % of inhibition at concentration of 60µg/ml. The IC_{50} value for methanol, acetone and aqueous extracts was observed as 4.5, 5.0 and 5.4 respectively.

Table: 4.18 Superoxide radical scavenging activity of *Parmotrema reticulatum*

% scavenging activity of *Parmotrema reticulatum*			
Concentration (µg/ml)	Acetone extract	Aqueous extract	Methanolic extract
1	13.15	12.36	11.83

10	19.13	27.21	22.5
20	31.1	32.03	37.72
30	42.29	41.27	45.07
40	49.16	47.78	52.72
50	57.21	53.25	62.64
60	69.77	60.93	75.73
IC_{50}(µg/ml)	5.03	5.4	4.58

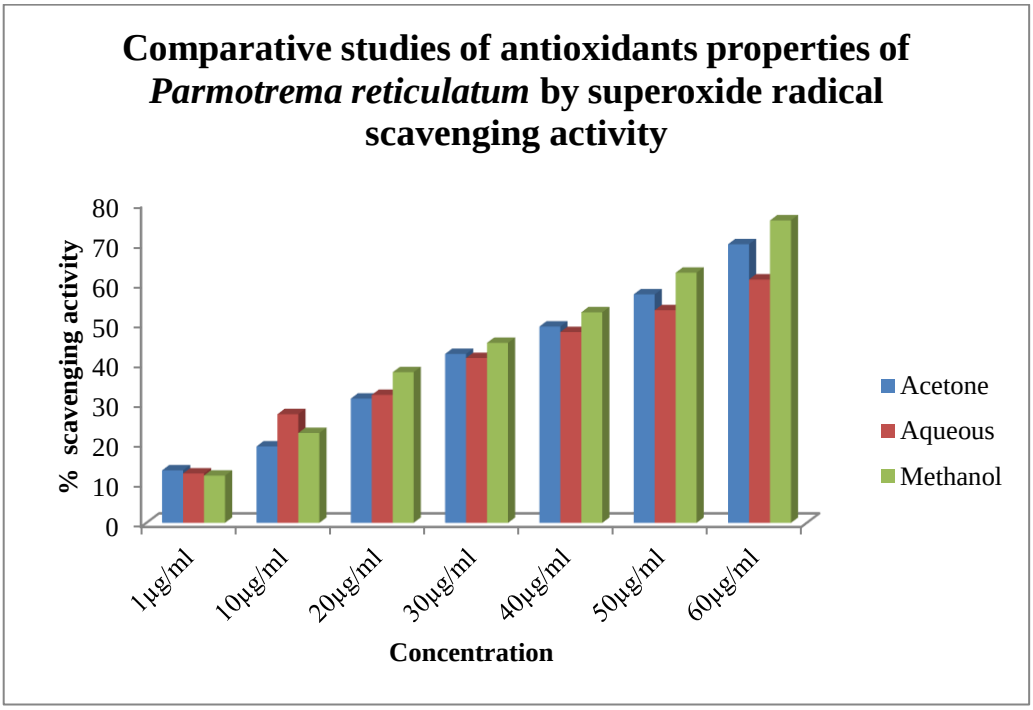


Figure 4.18 Comparative studies of antioxidants properties of *Parmotrema reticulatum* by superoxide radical scavenging activity

4.5.3.3 Superoxide radical scavenging activity of *Parmotrema saccatilobum*

The superoxide radicals scavenging activity of *Parmotrema saccatilobum* was investigated and it was found that there was a significant difference between lichen extracts and Rutin (standard); because Rutin was showing more % inhibition (93.16%) as well as IC_{50} value (3.2µg/ml). In comparison to all three extracts, the acetone extract had resulted higher scavenging properties with 74.69% inhibition, however, acetone

(64.06%) and aqueous (55.79%) extracts shown the less % of inhibition at concentration of 60µg/ml. The IC_{50} value for acetone, methanol and aqueous extracts was observed as 3.9, 4.9 and 4.9 respectively.

Table: 4.19 Superoxide radical scavenging activity of *Parmotrema saccatilobum*

% scavenging activity of *Parmotrema saccatilobum*			
Concentration (µg/ml)	Acetone extract	Aqueous extract	Methanolic extract
1	10.26	5.48	7.82
10	17.35	9.85	15.9
20	24.15	21.18	25.56
30	46.71	30.31	36.75
40	56.28	42.1	43.05
50	66.62	49.86	56.8
60	74.69	55.79	64.06
IC_{50}(µg/ml)	3.97	4.96	4.95

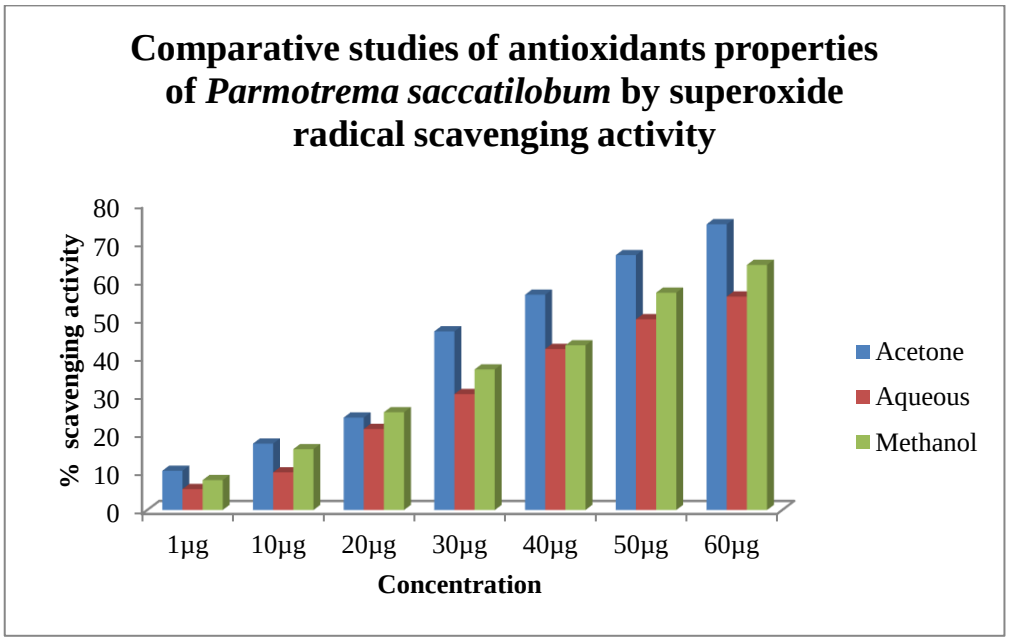


Figure 4.19.4 Comparative studies of antioxidants properties of *Parmotrema saccatilobum* by superoxide radical scavenging activity

4.5.3.4 Superoxide radical scavenging activity of *Everniastrum cirrhatum*

The superoxide radicals scavenging activity of *Everniastrum cirrhatum* was investigated and it was found that there was a significant difference between lichen extracts and Rutin (standard); because Rutin was showing more % inhibition (93.16%) as well as IC_{50} value (3.2µg/ml). In comparison to all three extracts, the methanol extract had resulted higher scavenging properties with 71.66% inhibition, however, acetone (60.48%) and aqueous (55.94%) extracts shown the less % of inhibition at concentration of 60µg/ml. The IC_{50} value for acetone, methanol and aqueous extracts was observed as 4.4, 5.4 and 6.1 respectively.

Table4.20 Superoxide radical scavenging activity of *Everniastrum cirrhatum*

% scavenging activity of *Everniastrum cirrhatum*			
Concentration (µg/ml)	Acetone extract	Aqueous extract	Methanolic extract
1	8.21	8.1	8.34
10	20	14.34	18.45

20	32.51	28.43	27.28
30	43.61	34.51	40.2
40	48.51	40.67	51.6
50	53.05	48.94	58.82
60	60.48	55.94	71.66
IC_{50}(µg/ml)	5.4	6.11	4.49

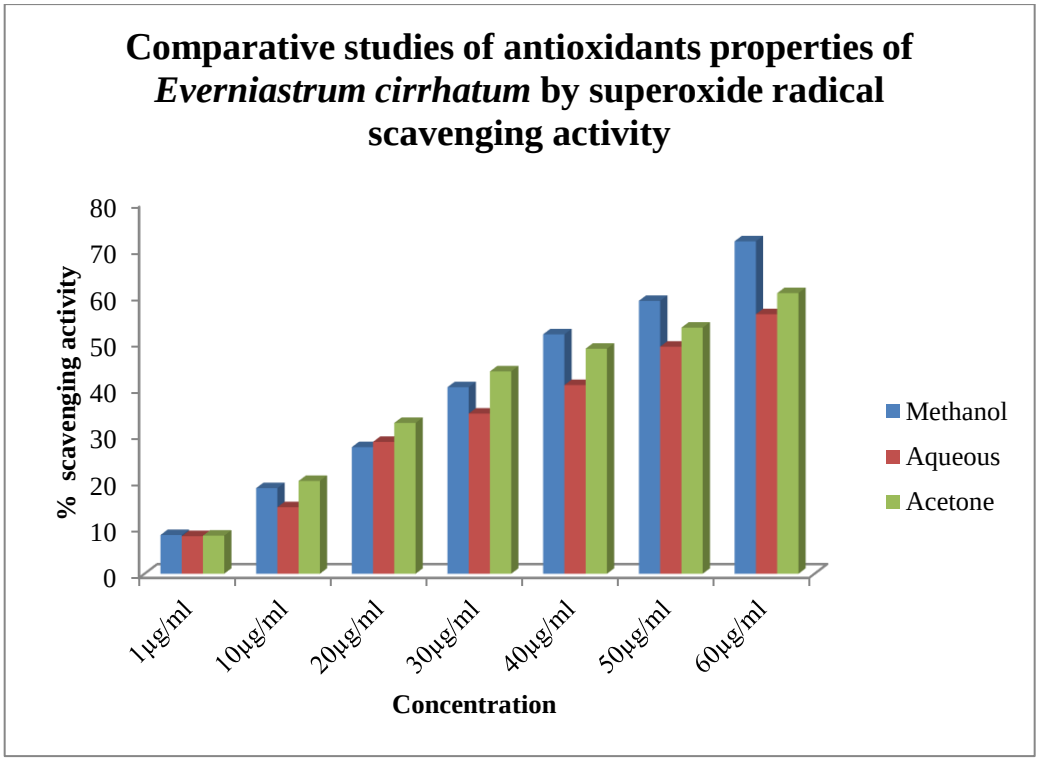


Figure 4.20 Comparative studies of antioxidants properties of *Everniastrum cirrhatum* by superoxide radical scavenging activity

4.5.4 TOTAL PHENOL CONTENTS

The total phenol contents of the four lichens extracts with three different solvents were evaluated by using the standard protocols as mentioned in chapter 3. The variations in their total phenolic contents were determined with standard graph obtained by Gallic acid ($y = 0.1785 x + 0.1090$, $r^2 = 0.9996$) as shown in figure 4.21.1

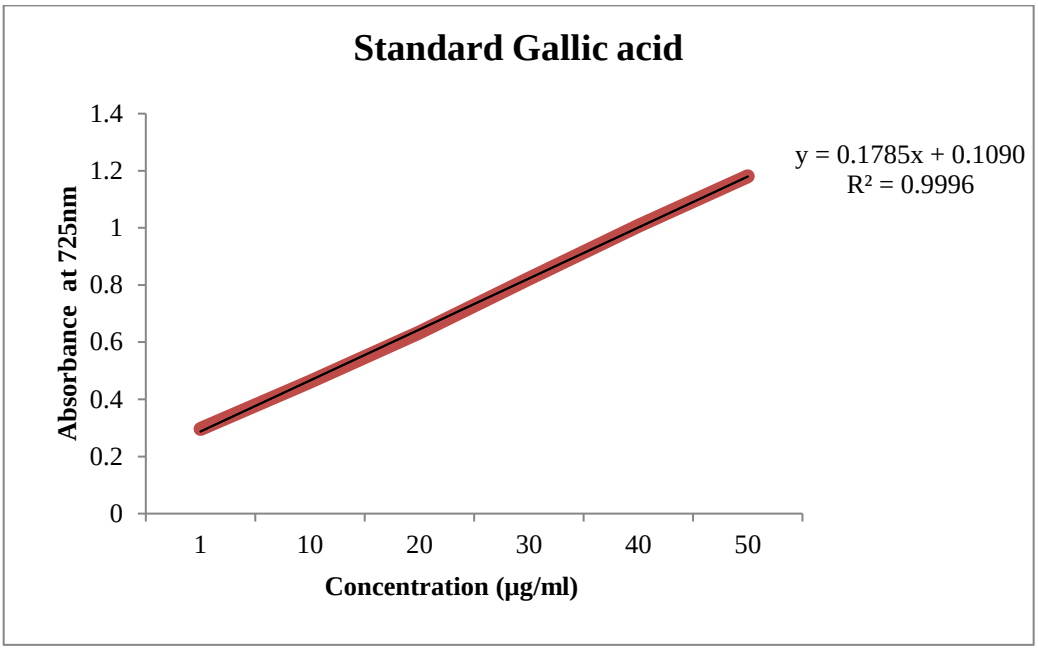


Figure 4.21.1Calibration curve of standard Gallic acid for the determination of total phenol contents

Table 4.21Total phenol contents of selected lichens extract

Lichens extract	TPC (µg GAE/mg of dry extract)		
	Acetone	**Aqueous**	**Methanol**
Parmotrema latissimum	4.80±0.11	2.82±0.14	7.95±0.06
Parmotrema reticulatum	5.99±0.07	4.49±0.06	8.78±0.07
Everniastrum cirrhatum	5.45±0.13	3.62±0.10	10.78±0.02
Parmotrema saccatilobum	9.37±0.13	5.06±0.17	9.54±0.12

Data are presented as mean +/- SD, n=3 experiments

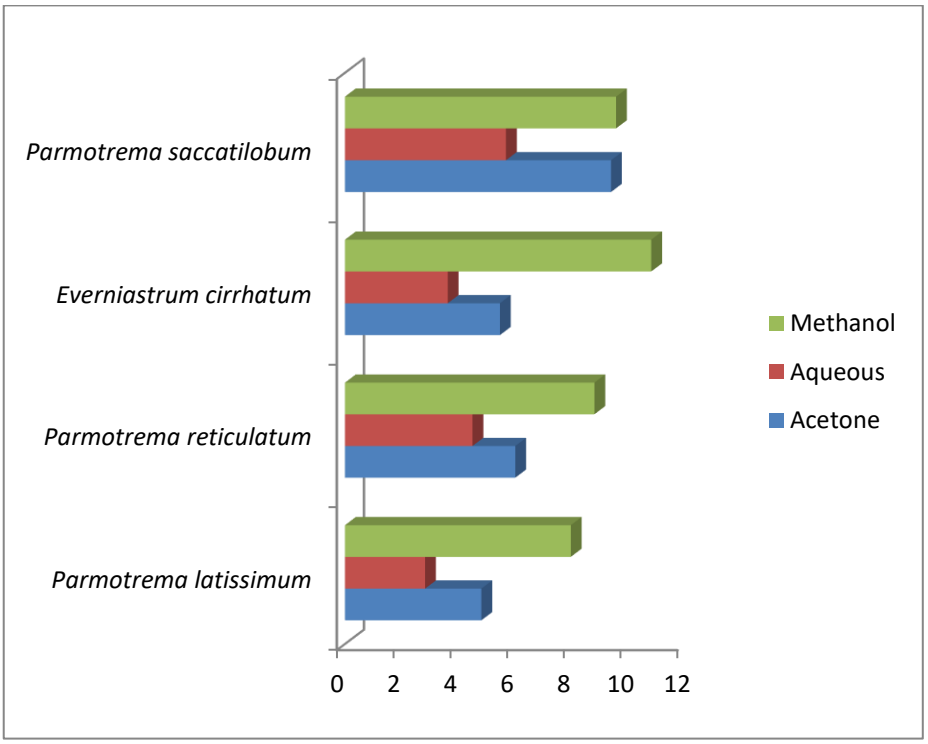


Figure 4.21.2 Comparative studies of the total phenol contents of acetone, aqueous and methanol extracts of 4 selected lichens.

The total phenol contents of all extracts of selected lichens were shown in (Table 4.21); where found that the extract of aqueous, acetone and methanol of *Parmotrema latissimum* have lowest value as 2.82, 4.80 and 7.95 µg GAE/mg of dry extract. *Everniastrum cirrhatum* extracts possessed the highest phenol contents with 3.62, 5.45 and 10.78 µg GAE/mg of dry extract followed by *Parmotrema saccatilobum* extracts with 5.06, 9.37 and 9.54 µg GAE/mg of dry extract and *Parmotrema reticulatum* extracts with 4.49, 5.99 and 8.78 µg GAE/mg of dry extract. Methanolic extracts had shown maximum contents followed by acetone and aqueous extracts comparatively (Fig 4.21.2).

4.5.5 TOTAL FLAVANOIDS CONTENTS

The total flavanoids contents of the four lichens extract with three different solvents were evaluated by using the standard protocols as mentioned in chapter 3. The

variations in their total flavanoids contents were determined with help of calibration curve of standard (Quercetin) (y = 0.3980 x + 0.0058, r^2= 0.9984) as shown in figure 4.22.1

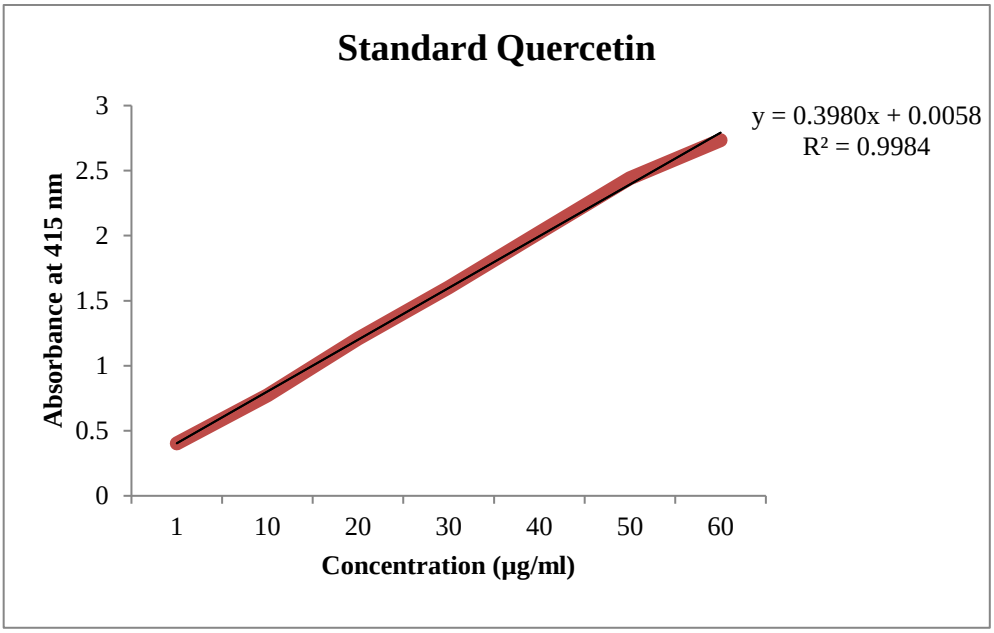


Figure 4.22.1 Calibration curve of standard Quercetin acid for the determination of total flavanoids contents

Table.4.22Total Flavanoids contents of some selected lichens

Lichens extract	TFC(µg QE/mg of dry extract)		
	Acetone	**Aqueous**	**Methanol**
Parmotrema latissimum	5.02±0.20	3.09±0.19	5.48±0.13
Parmotrema reticulatum	5.86±0.41	4.24±0.22	5.41±0.04
Everniastrum cirrhatum	3.61±0.16	2.41±0.06	4.65±0.12
Parmotrema saccatilobum	8.06±0.26	6.27±0.25	6.65±0.40

Data are presented as mean +/- SD, n=3 experiments

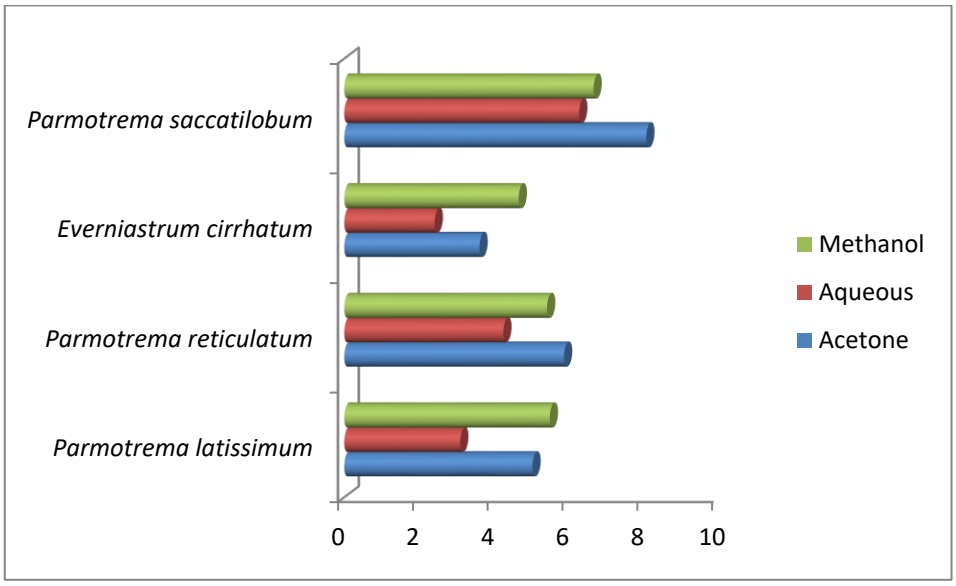


Figure 4.22.2 Comparative studies of the total flavanoids contents of acetone, aqueous and methanol extracts of 4 selected lichens.

The total Flavanoids contents of all extracts of selected lichens were shown in (Table 4.22); where found that the extract of aqueous, acetone and methanol of *Parmotrema latissimum* have lowest value as3.09, 5.02 and 5.48 µg QE/mg of dry extract. *Everniastrum cirrhatum* extracts possessed the lowest flavanoids contents with 2.41, 3.61 and 4.65 µg QE/mg of dry extract followed by *Parmotrema saccatilobum* extracts recorded the highest contents with 6.27, 8.06 and 6.65µg QE/mg of dry extract and *Parmotrema reticulatum* extracts with 4.24, 5.86 and 5.41 µg QE/mg of dry extract. Methanolic extracts had shown maximum contents in two extracts (*Parmotrema latissimum* and *Everniastrum cirrhatum)* and acetone (*Parmotrema reticulatum* and *Parmotrema saccatilobum)* comparatively (Fig 4.22.2).

4. 4 *IN*-VITRO ANTI-FUNGAL ACTIVITIES OF SOME SELECTED LICHENS

The 6 selected potential lichens extracts made in acetone, aqueous and methanol were tested against four human pathogenic fungi. The minimum inhibitory concentration (MIC) was evaluated on the basis of zone of inhibition by using Agar well diffusion assay.

Table 4.23 MIC and Zone of Inhibition of acetone extract of selected lichens against human pathogenic fungi

Lichens extract (Acetone)	Human Pathogenic Fungi							
	Epidermophyton floccosum		*Trichophyton mentagrophytes*		*Aspergillus flavus*		*Aspergillus fumigatus*	
	MIC	Zone of Inhibition	MIC	Zone of Inhibition	MIC	Zone of Inhibition	MIC	Zone of Inhibition
Standard drug (Meconazole)	50	22 ±1.20[a]	50	19 ±0.57[a]	50	18±0.33[a]	50	22 ±0.57[a]
Everniastrum cirrhatum	200	14 ±1.15[c]	200	12 ±1.15[e]	150	13 ±0.57[b]	200	12 ±0.88[f]
Parmotrema saccatilobum	200	13 ±1.33[d]	200	14 ±0.88[c]	200	11 ±0.57[c]	200	13 ±0.33[e]
Parmotrema reticulatum	150	14 ±0.88[c]	150	15 ±0.88[b]	150	10 ±1.20[d]	150	14 ±1.15[d]
Usnea baileyi	200	13 ±0.57[d]	200	10 ±0.88[f]	200	10 ±0.88[d]	200	12 ±1.15[f]
Parmotrema latissimum	200	14 ±0.57[c]	200	13 ±0.57[d]	200	11 ±0.57[c]	200	15 ±0.88[b]
Ramalina conduplicans	100	16 ±1.15[b]	100	14 ±1.15[c]	100	13 ±0.88[b]	100	17 ±0.57[b]

Standard drug (Meconazole) = 50mcg/disc; MIC for extract expressed in µg/ml.

**Each value represents Zone of inhibition mean ± SEM, n=3, Data were analyzed by using One-way ANOVA followed by Duncan Multiple rangetest ($p<0.05$).The same superscript letter in the same column are not significantly different at $p<0.05$.*

The acetone extract of *Everniastrum cirrhatum, Parmotrema saccatilobum, Parmotrema reticulatum, Usnea baileyi, Parmotrema latissimum* and *Ramalina conduplicans* shown the zone of inhibition (ZOI) against *Epidermophyton floccosum,* and it was recorded in between13-16 mm (mean value). In case of *Trichophyton*

mentagrophytes the mean value of ZOI in between 10-15 mm; whereas, 10-13 mm ZOI were observed against *Aspergillus flavus* and12 – 17 mm against *Aspergillus fumigatus.* On the other side, standard meconazole range from 18-22 mm, this showed the highest inhibition (fig.4.24).

Further, the Minimum Inhibition concentration (MIC) of the acetone extract of *Ramalina conduplicans* and *Parmotrema reticulatum* was found effective at the concentration of 100µg/ml and 150µg/ml respectively against *Epidermophyton floccosum, Trichophyton mentagrophytes, Aspergillus flavus* and *Aspergillus fumigatus*; while the acetone extract of *Everniastrum cirrhatum, Parmotrema saccatilobum, Usnea baileyi* and *Parmotrema latissimum* were found effective at the concentration of 200µg/ml against same pathogens, and compared with reference standard drug meconazole at 50mcg/disc (Table 4.23).

Six acetone lichens extracts along with standard (meconazole) were carried out at different concentration on mentioned fungi. It was observed that there was a significant difference between antifungal activities. In post hog test of Duncan multiple range test shown that the acetone extracts of 6 lichens including standard drugs form two homogeneous groups in accordance with the zone of inhibition values against *Epidermophyton floccosum;* three homogeneous groups were formed against *Trichophyton mentagrophytes* and *Aspergillus flavu s*whereas four homogeneous groups were formed against *Aspergillus fumigatus* respectively (Table 4.23).

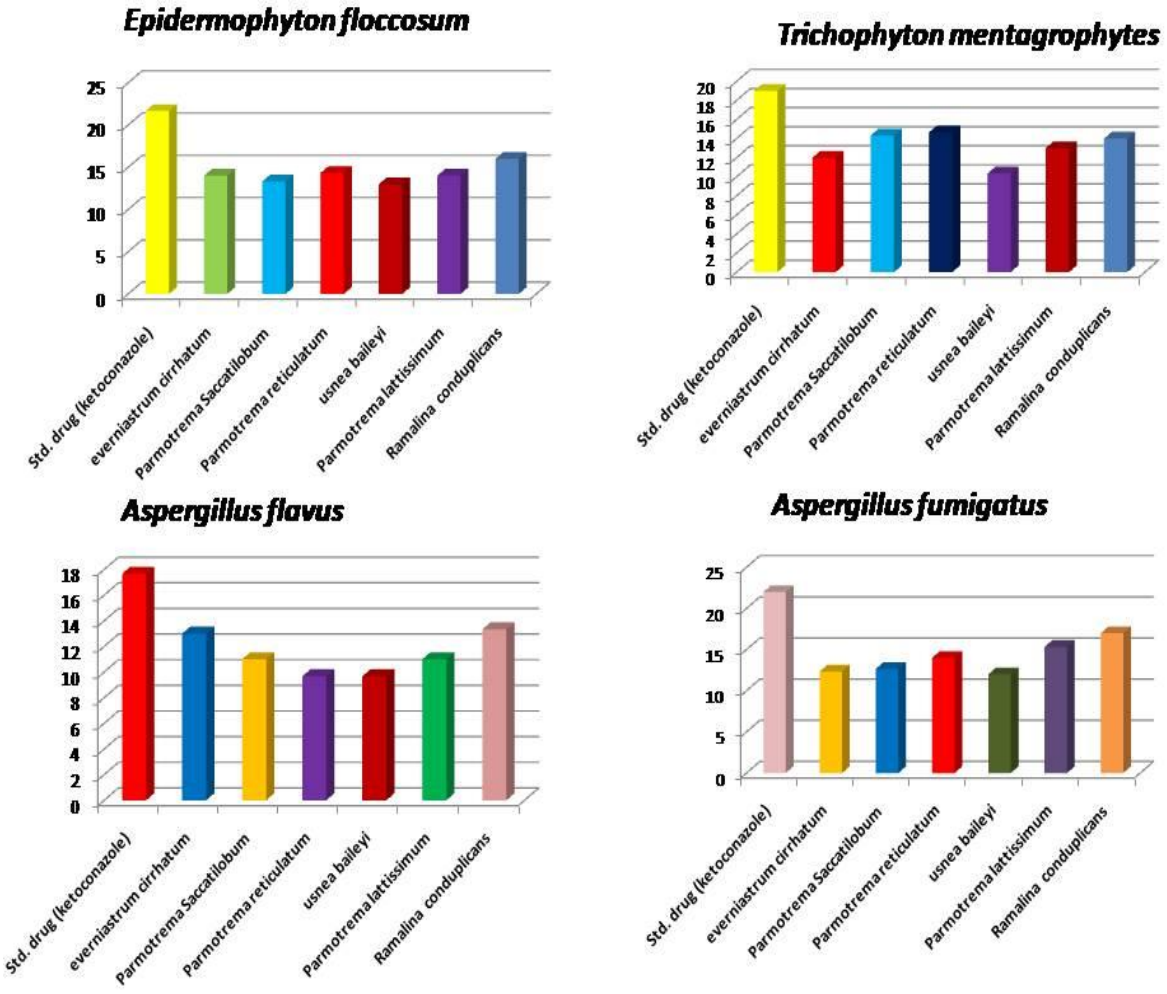


Figure 4.23 Zone of Inhibition by acetone extracts of selected lichens against the pathogenic fungi.

Table 4.24MIC and Zone of Inhibition of aqueous extract of selected lichens against human pathogenic fungi

Lichens extract (Aqueous)	Human Pathogenic Fungi							
	Epidermophyton floccosum		***Trichophyton mentagrophytes***		***Aspergillus flavus***		***Aspergillus fumigatus***	
	MIC	**Zone of Inhibition**	**MIC**	**Zone of Inhibition**	**MIC**	**Zone of Inhibition**	**MIC**	**Zone of Inhibition**
Std drug (Meconazole)	50	22 ±0.33[a]	50	22 ±1.20[a]	50	19 ±0.88[a]	50	19 ±1.15[a]
Everniastrum cirrhatum	200	7 ±0.57[d]	200	8 ±0.88[d]	150	7 ±0.88[e]	200	7 ±0.88[f]
Parmotrema saccatilobum	200	8 ±0.57[c]	200	9 ±0.57[c]	200	8 ±1.20[d]	200	9 ±1.15[d]
Parmotrema reticulatum	150	6 ±0.33[e]	150	8 ±0.66[d]	150	9 ±0.88[c]	150	11 ±0.57[b]
Usnea baileyi	200	6 ±0.33[e]	200	7 ±0.88[e]	200	7 ±0.57[e]	200	10 ±0.88[c]
Parmotrema latissimum	200	11 ±0.57[b]	200	11 ±0.57[b]	200	9 ±0.57[c]	200	8 ±0.57[e]
Ramalina conduplicans	100	NA	100	11 ±0.66[b]	100	11 ±1.15[b]	100	7 ±0.88[f]

Standard drug (Meconazole) = 50mcg/disc; MIC for extract expressed in µg/ml.

**Each value represents Zone of inhibition mean ± SEM, n=3, Data were analyzed by using One-way ANOVA followed by Duncan Multiple rangetest ($p<0.05$). The same superscript letter in the same column are not significantly different at $p<0.05$.*

The aqueous extract of *Everniastrum cirrhatum*, *Parmotrema saccatilobum*, *Parmotrema reticulatum*, *Usnea baileyi*, *Parmotrema latissimum* and *Ramalina conduplicans* shown the zone of inhibition (ZOI) against *Trichophyton mentagrophytes*, and it was recorded highest in between 8-11 mm (mean value). In case of *Aspergillus*

flavus and Aspergillus fumigates the mean value of ZOI in between 7-11 mm; whereas, 6-11 mm ZOI were observed against *Epidermophyton floccosum*.On the other side, standard meconazole range from 19-22 mm, this showed the highest inhibition (fig.4.24).

Further, the Minimum Inhibition concentration (MIC) of the aqueous extract of *Ramalina conduplicans* and *Parmotrema reticulatum* was found effective at the concentration of 100µg/ml and 150µg/ml respectively against *Epidermophyton floccosum, Trichophyton mentagrophytes, Aspergillus flavus* and *Aspergillus fumigatus*; while the aqueous extract of *Everniastrum cirrhatum, Parmotrema saccatilobum, Usnea baileyi* and *Parmotrema latissimum* were found effective at the concentration of 200µg/ml against same pathogens, and compared with reference standard drug meconazole at 50mcg/disc (Table 4.24).

Six aqueous lichens extracts along with standard (meconazole) were carried outat different concentration on mentioned fungi. It was observed that there was a significant difference between antifungal activities. In post hog test of Duncan multiple range test shown that the acetone extracts of 6 lichens including standard drugs form two homogeneous groups in accordance with the zone of inhibition values against *Aspergillus flavus;* three homogeneous groups were formed against *Trichophyton mentagrophytes* and *Aspergillus fumigatus* whereas five homogeneous groups were formed against *Epidermophyton floccosum* respectively (Table 4.24).

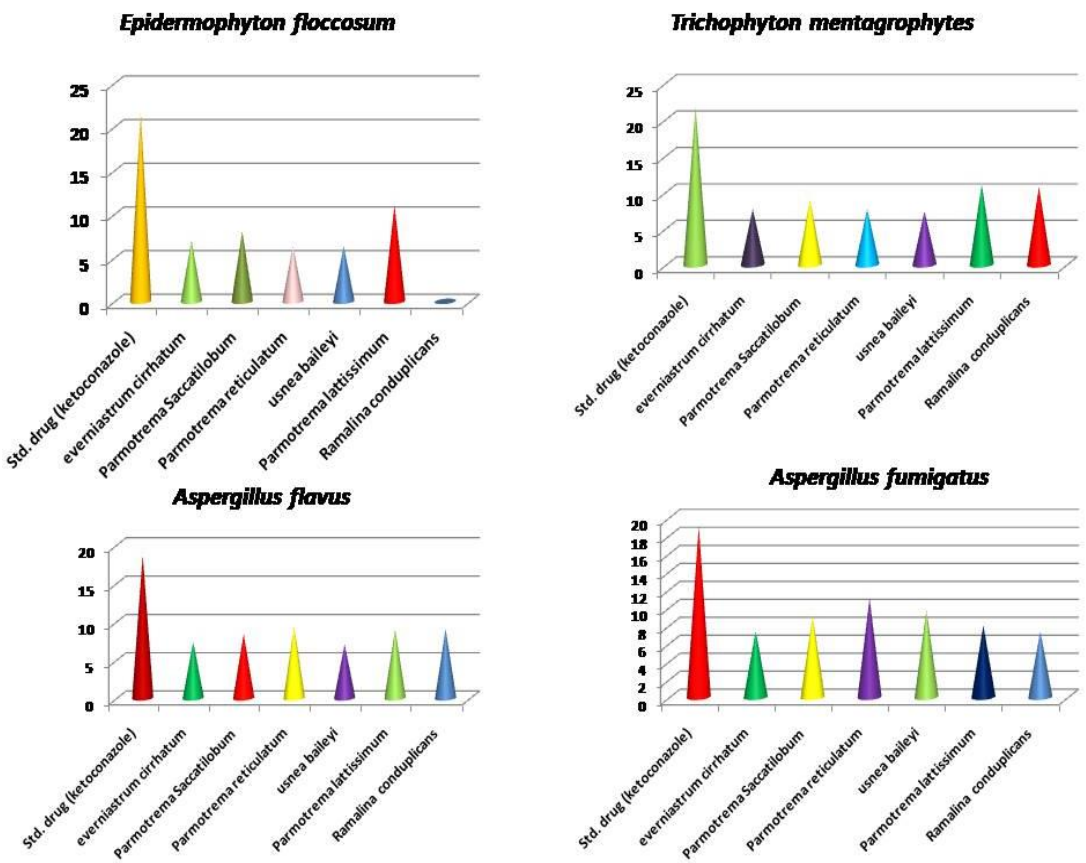


Figure 4.24 Zone of Inhibition by aqueous extracts of selected lichens against the pathogenic fungi.

Table 4.25 MIC and zone of Inhibition of methanol extract of selected lichens against human pathogenic fungi

Lichens extract (Methanol)	Human Pathogenic Fungi							
	Epidermophyton floccosum		***Trichophyton mentagrophytes***		***Aspergillus flavus***		***Aspergillus fumigatus***	
	MIC	**Zone of Inhibition**	**MIC**	**Zone of Inhibition**	**MIC**	**Zone of Inhibition**	**MIC**	**Zone of Inhibition**
Standard drug (Meconazole)	50	28±0.88[a]	50	24±0.88[a]	50	21 ±0.88[a]	50	21±0.88[a]
Everniastrum cirrhatum	150	17 ±0.88[c]	200	8±0.57[f]	200	14 ±0.88[c]	150	16±1.20[c]
Parmotrema saccatilobum	200	15 ±1.20[e]	150	17±0.88[b]	200	11 ±1.15[e]	200	12±1.52[e]
Parmotrema reticulatum	150	18 ±0.88[b]	200	14±1.20[c]	150	17 ±0.88[b]	150	14±1.85[d]
Usnea baileyi	200	15 ±0.57[e]	200	9 ± 1.33[e]	200	11 ±0.57[e]	200	8 ±0.88[f]
Parmotrema latissimum	200	16 ±0.66[d]	200	12±0.88[d]	200	12 ±1.20[d]	150	17 ±1.52[b]

Standard drug (Meconazole) = 50mcg/disc; MIC for extract expressed in µg/ml.

**Each value represents Zone of inhibition mean ± SEM, n=3, Data were analyzed by using One-way ANOVA followed by Duncan Multiple rangetest ($p<0.05$).The same superscript letter in the same column are not significantly different at $p<0.05$.*

The methanol extract of *Everniastrum cirrhatum, Parmotrema saccatilobum, Parmotrema reticulatum, Usnea baileyi, Parmotrema latissimum* and *Ramalina conduplicans* shown the zone of inhibition (ZOI) against *Epidermophyton floccosum,* and it was recorded highest in between 15-18 mm (mean value). In case of *Aspergillus fumigates* and *Trichophyton mentagrophytes* the mean value of ZOI in between 8-17 mm; whereas, 11-17 mm ZOI were observed against *Aspergillus flavus.* On the other

side, standard meconazole range from 21-28 mm, this showed the highest inhibition (fig.4.25).

Further, the Minimum Inhibition concentration (MIC) of the methanol extract of *Ramalina conduplicans* was found effective at the concentration of 100µg/ml, whereas, *Everniastrum cirrhatum* and *Parmotrema reticulatum* was found effective at the concentration of 150µg/ml against *Epidermophyton floccosum, Trichophyton mentagrophytes, Aspergillus flavus* and *Aspergillus fumigatus*; while the methanol extract of *Parmotrema saccatilobum, Usnea baileyi* and *Parmotrema latissimum* were found effective at the concentration of 200µg/ml against same pathogens, and compared with reference standard drug meconazole at 50mcg/disc (Table 4.25).

Six methanol lichens extracts along with standard (meconazole) were carried outat different concentration on mentioned fungi. It was observed that there was a significant difference between antifungal activities. In post hog test of Duncan multiple range test shown that the methanol extracts of 6 lichens including standard drugs form three homogeneous groups in accordance with the zone of inhibition values against *Aspergillus flavus* and *Epidermophyton floccosum;* whereas, four homogeneous groups were formed against *Trichophyton mentagrophytes* and *Aspergillus fumigatus* respectively (Table 4.25).

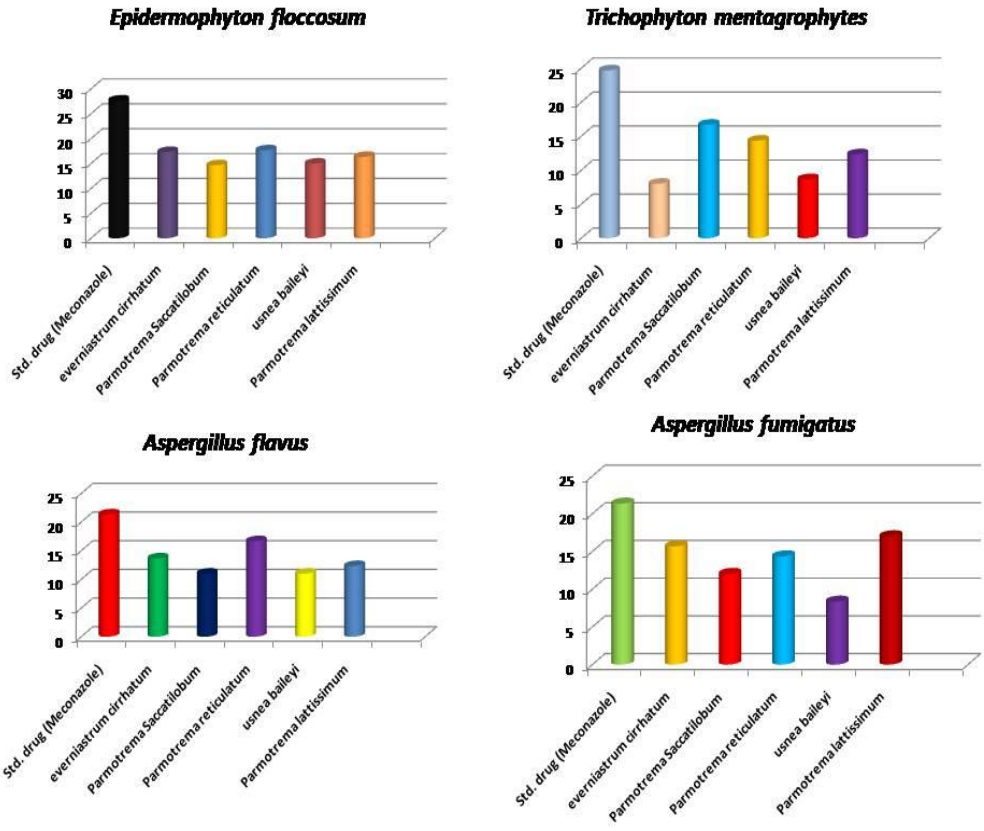


Figure 4.25 Zone of Inhibition by methanol extracts of selected lichens against the pathogenic fungi.

COMPARATIVE EFFICACY OF LICHENS EXTRACTS WITH SYNTHETIC ANTIFUNGAL DRUGS

As the above tables have already shown the good antifungal activity against the test fungi along with standard drug; similarly, the selected lichens extracts (after 12 months) and two known synthetic antifungal drugs such as Phytoral and K2 were evaluated against same fungi to check out their comparative efficacy. Even after 12 months the extracts showed good activity, which means the shelf life of extracts were very high. The observed minimum effective concentration (MEC) was highlighted in the table 4.26; where, *Ramalina conduplicans* was reported for highest MEC against the test fungi.

Table4.26 Comparative Efficacy of the lichens extracts with two known synthetic antifungal drugs

Synthetic antimycotic drugs	**Active Ingredients**	**Expiry duration (months)**	**Minimum Effective Concentration (mg/ml)**			
			Epidermophyton floccosum	*Trichophyton mentagrophytes*	*Aspergillus flavus*	*Aspergillus fumigatus*
Everniastrum cirrhatum	Evernic acid, Divaricatic acid, Protolichesterinic acid and Salazinic acid	12	0.15	0.15	0.15	0.15
Parmotrema saccatilobum	Atranorin, chloroatranorin and Protocetraric acid	12	0.2	0.2	0.2	0.2
Parmotrema reticulatum	Atranorin, Protocetraric acid, Usnic Acid and consalazinic	12	0.15	0.15	0.15	0.15
Usnea baileyi	Stictic acid, Usnic acid	12	0.2	0.2	0.2	0.2
Parmotrema latissimum	Atranorin and usnic acid	12	0.2	0.2	0.2	0.2
Ramalina conduplicans	Usnic acid, Salazinic acid and Sekikaic acid, norstictic	12	0.1	0.1	0.1	0.1
Phytoral	Ketoconazole IP 200mg	24	0.04	0.04	0.04	0.04
K2	Ketoconazole IP 2.0% w/w	24	0.08	0.08	0.08	0.08

4.7 GASTRO-PROTECTIVE ACTIVITY

4.7.1 Ethanol-induced Gastric ulcer

The gastroprotective effects of methanolic extract of *Usnea baileyi* at 50, 100 and 200 mg/kg body wt. on ethanol-induced gastric damage in wistar albino rats with standard protocols was determined. The detailed investigations were performed by measurement of gastric jucie pH, acidity, ulcer index, percentage of preventive index and its histological evaluation of gastric lesion(table 4.27 & 4.28)..

4.7.2 Measurement of gastric juice pH and acidity

The results for the pH of gastric juice and estimation of total acidity for evaluation of antisecretory effect of methanolic extract of *Usnea baileyi* were shown below (table 4.27).

Table 4.27 Measurement of gastric juice pH and acidity

Treatment	No. of animal	pH of the Gastric Juice	Acidity (mEq/L)
Ulcer Control	5	1.68±0.59	144.16±3.72
Standard (Omeprazole)	5	5.51±0.32*	40.29±4.47*
Low dose (50mg)	5	1.80±0.46	115.48±4.71*
Moderate dose (100mg)	5	2.28±0.52	77.83±6.09*
High dose (200mg)	5	4.02±0.48*	55.23±6.61*

Each value is the mean ± SEM for 5 observations. Data were analyzed by using One-way ANOVA followed by Duncan's test.

** Indicates significance difference at p< 0.05 compared to ulcer group.*

After the administration of ethanol in ulcer group showed the adverse effect by decreasing pH of the gastric juice (1.68±0.59) and higher increase of total acidity (144.16±3.72mEq/L), which proved the cause of ulcer. On the contrary, the group with the treatment of methanolic extract at the dose of 50, 100 and 200mg/kg shown improvement by increasing the pH of gastric juice and the values recorded as 1.80±0.46, 2.28±0.52 and 4.02±0.48; while, in case of total acidity, it was found that

the decreasing of values as 115.48±4.71, 77.83±6.09 and 55.23±6.61mEq/L. Parallelly, in control omeprazole a standard drug applied on treated group where, it observed that pH of gastric juice was increasing with 5.51±0.32 and total acidity was decreasing with 40.29±4.47mEq/L.

4.7.3 Ulcer index and preventive index

The ulcer index and their preventive index percentage were carried out as per protocol mentioned in the chapter 3; where ulcer index was recorded on basis of damage encounter with ethanol in ulcer treated group. The following table (table 4.28) elaborated the details of gastroprotective effect of the methanolic extract along with standard drug by calculating their preventive index percentage.

Table 4.28 Gastroprotective effect of the methanolic extract of *Usnea baileyi* on ethanol-induced gastric lesions in rats

Treatment	No of animal	Mean ulcer index ± SEM	Preventive index %
Ulcer Control (Treated)	5	353±13.45	----
Omeprazole Standard	5	115±7.27*	67.42
Low dose (50mg)	5	287±11.41*	18.69
Moderate dose (100mg)	5	212±9.19*	39.94
High dose (200mg)	5	155±7.67*	56.09

Each value is the mean ± SEM for 5 observations. Data were analyzed by using One-way ANOVA followed by Duncan's test.

** Indicates significance differenc at p< 0.05 compared to ulcer group.*

Since, the observed value of ulcer index was 353±13.45 showed very high damage in the gastric mucosa in ethanol treated ulcer group. Besides this, methanolic extract treated groups at the dose of 50, 100, 200mg/kg shown the improvement of gastric mucosa (ulcer formation) with the ulcer index value of 287±11.41, 212±9.19 and 155±7.67 respectively; while, omeprazole treated group showed lesser values as 115±7.27 in comparison to extract at different doses.

Moreover, the methanol extract of *Usnea baileyi* at the graded dose (50-200mg/kg) had shown the preventive index percentage of 18.69%, 39.94% and 56.09% respectively; which resulted that the higher dose of the extract was more preventive in accordance with standard drug at the dose of 20mg/kg at 67.42%.

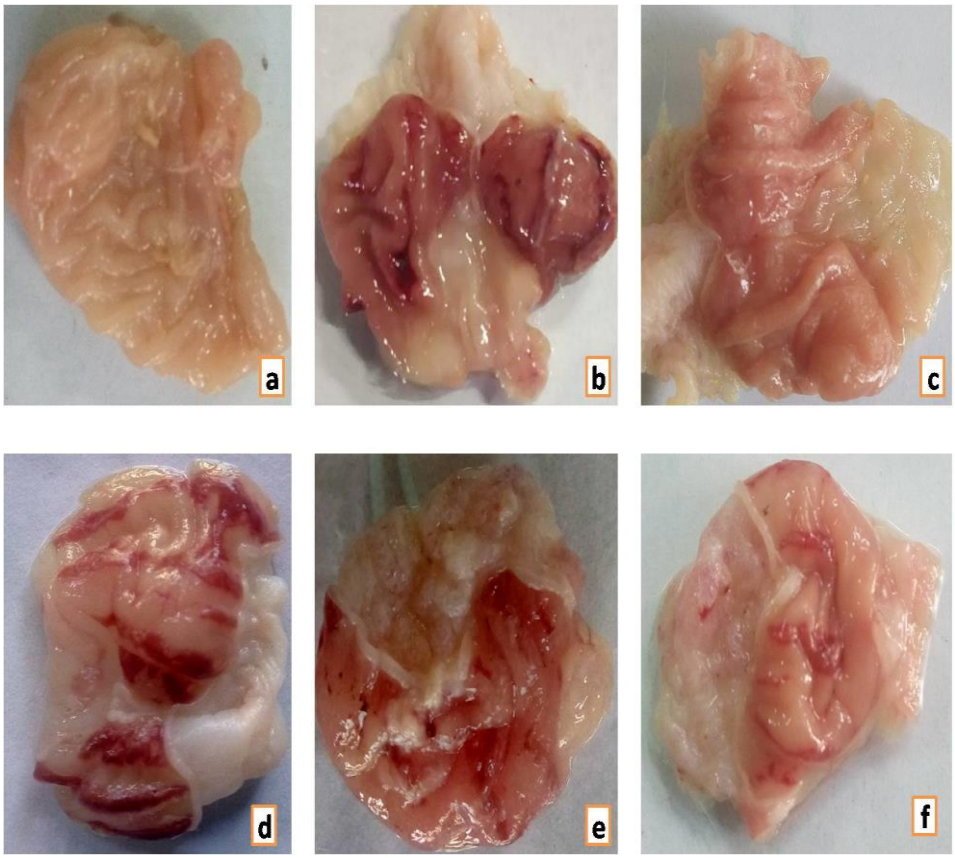


Figure 26 (a) normal group, (b) Ulcer Group, (c) Omeprazole treated group, (d) 50 mg extract treated group, (e) 100 mg extract treated group and (f) 200 mg extract treated group.

4.5.4 Histological evaluation of gastric lesions

In histological observation of ethanol induced gastric lesion in ulcer groups had shown a lot of damage on the gastric mucosa having deep injury, edema, leukocyte infiltration and hemorrhagic red bands. After treatment with different doses like low dose at 50mg/kg, moderate dose 100mg/kg and high dose of 200mg/kg b.wt of methanolic extract, the animal observed with gradual improvement. That means it was totally dose dependent where reducing in ulcer area, hemorrhagic red bands, reduced in submucosal oedema and leucocytes

infiltration. In contrast, the treatment of standard drugs omeprazole at 20mg/kg had shown more protection (Figure 4.27).

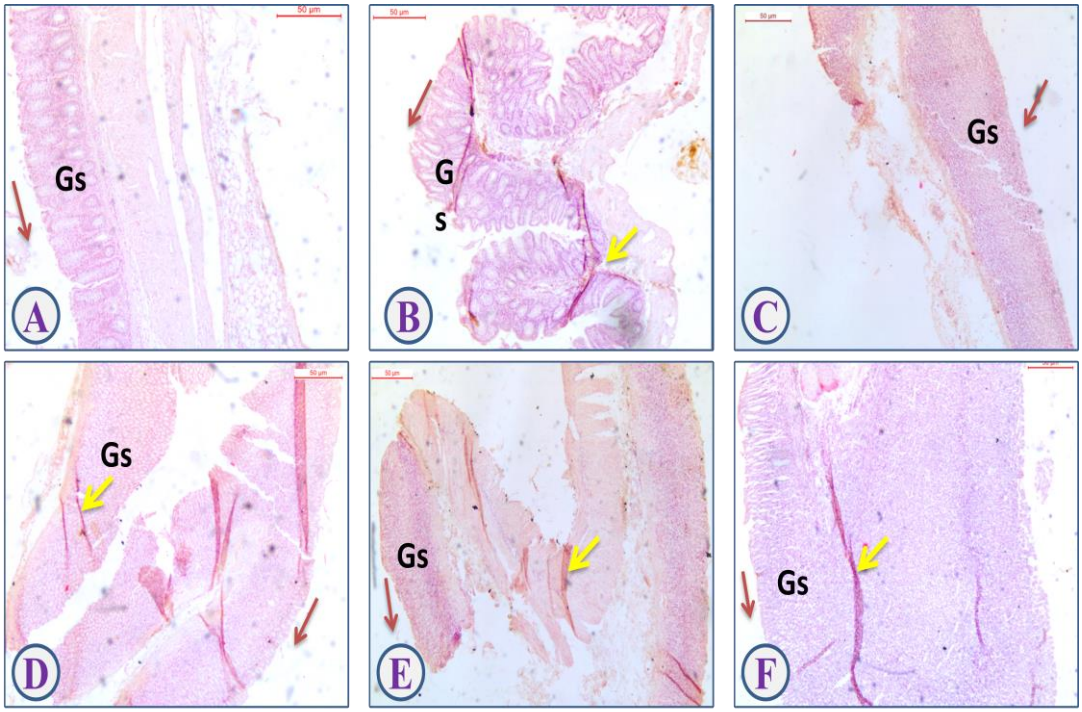


Figure 27 (a) normal group, no injuries to gastricmucosa layer are seen (b) Ulcer Group showing severe destruction to surface epithelium and necrotic lesions are present (c) Omeprazole treated group showing less disruption to gastricmucosalayer withmild leucocyte infiltration and edema (d) 50 mg extract treated group shows with improve in disturbanceto gastricmucosa layer with some hemorrhage (e) 100 mg extract treated group show less disturbanceto gastricmucosa layer and leucocyte infiltration in sub mucosal layer and (f) 200 mg extract treated group proved that the extract with higher dose can protect the gastrointestinal with a few disturbance to gastricmucosa layer.

Gs: gastric glands, yellow arrow: the hemorrhage, brown arrow: disruption to the surface epitheliumand deep mucosa

Photoplate 2: Field visit and Collection of Lichens samples

Photoplate 3: Processing, Morphological and Anatomical Identification of Lichens

Photo plate 4: Extraction process of lichens sample by using rota evaporatary

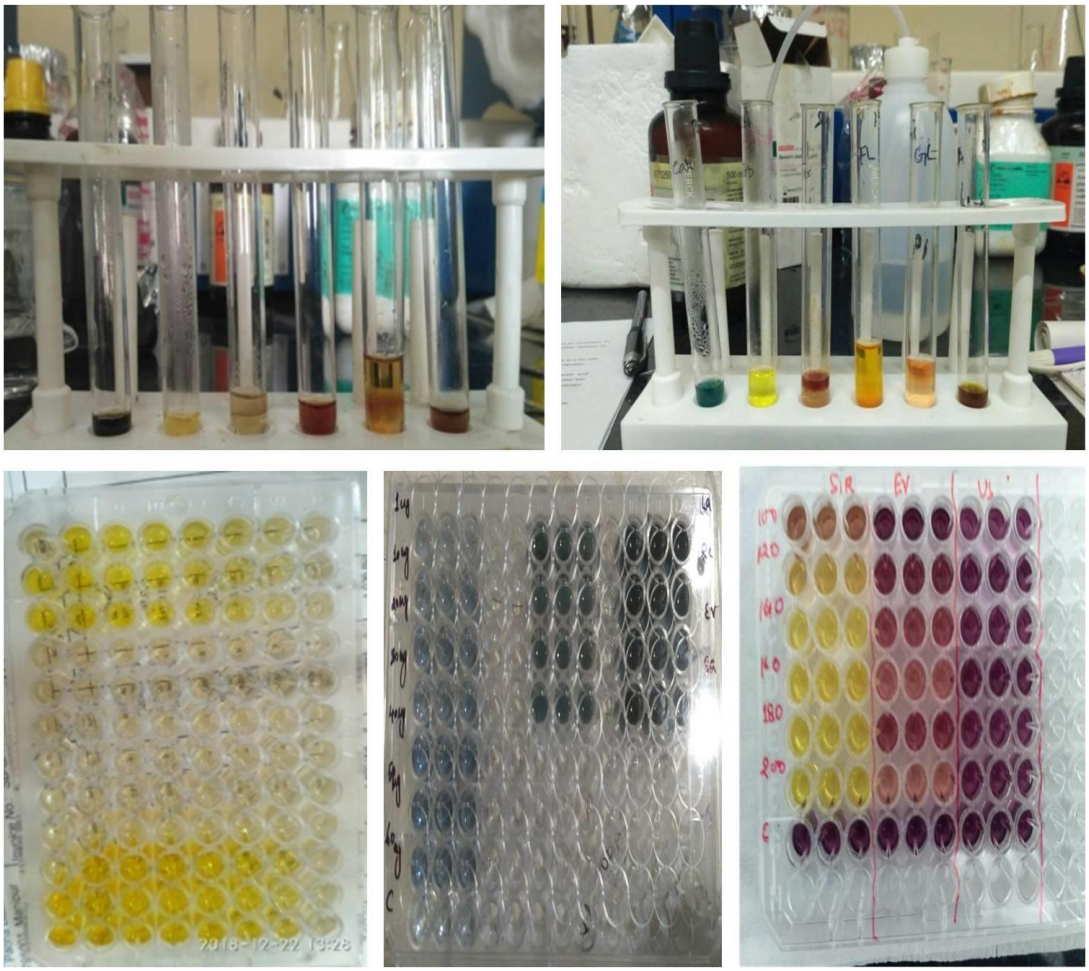


Photo Plate 5: Phytochemical screening and antioxidants activity of lichen extracts

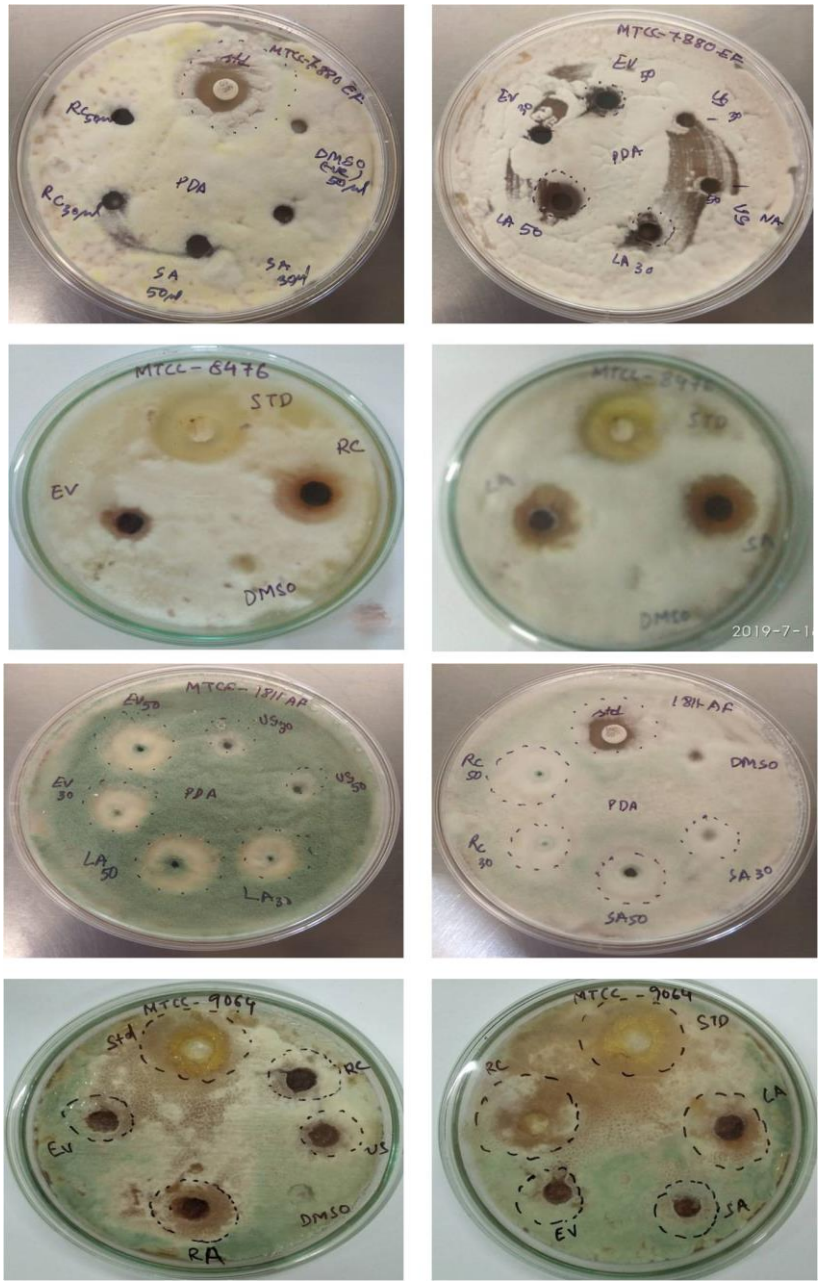


Photo plates 6

RC: *Parmotrema reticulatum*, SA: *Parmotrema saccatilobum*, EV: *Everniastrum cirrhatum*, LA: *Parmotremalatissimum*, US: *Usnea baileyi*, RA: *Ramalina conduplicans.*

Zone of Inhibition of selected lichens against the tested dermatophytic fungi*Epidermophyton floccosum* (MTCC7880), *Trichophyton mentagrophytes* (MTCC8476), *Aspergillus flavus* (MTCC9064) and *Aspergillus fumigatus* (MTCC 1811).

Photo plates 7

Experimental work of *In-vitro* Anti fungal and *In-vivo* gastroprotective in Rats

CHAPTER -5

Discussion

5.1 SYSTEMATIC INVESTIGATION

According to annotated checklist by Singh and Sinha (2010) the total lichen species were 2303 in India, but, as per the latest report made by singha et al., (2018) the number has increased with addition of 411 species and it is 2714. The studies of lichens are called as the lichenology and Dr. D.D awasthi was known as the father of Indian lichenology, who initiated the scientific studies on lichens. The well organized studies of lichens were started in the northern Himalayas of India; the exploration of lichens in northeastern parts of India is quite less compared to other parts of India. Therefore, the present study was formulated for new exploration of lichens in the north eastern region with special reference to Mizoram. In this study, the *in vitro* pharmacological investigation of selected lichens was also made against some known human pathogenic fungi along with *in vivo* study. The new systematic studies on lichens of Mizoram state were originated recently when Logeshet al. (2017) has reported 159 lichen species as new distributional records from the Mizoram, out of which 14 species were described as a new records for India; and it was based on the finding made by Chinlampianga et al. (2013), where he reported 10 lichen species from Aizawl. Recently, Thangjam et al. (2019) has been reported 20 lichen species with addition of their diversity and distribution from Indo-Burma hotspot region of district Champhai, Mizoram.

The present study documented a total of 78 species under 30 genus and 19 families from the 3 different studies sites of various districts of Mizoram. Genus *parmotrema* is one of the most dominated in the region with 15 species followed by *Heteroderimia* with 7 species. Among the families, Parmeliaceae was the most dominant and it was growing luxuriantly with 30 species followed by Graphidaceae and Physciaceae with 10 and 8 species respectively (Table 4.1–4.4) (Fig 4.1 – 4.5). Furthermore, Parmeliaceae was the family which possessed the highest number of species in the eastern parts of Himalaya (Sinha and Ram 2011; Thangjam et al., 2019). In addition to previous findings, present study also enumerates 42 species from a small region of National Park, 20 species from a tourist spot and 16 species

from a university campus, which clearly indicates the richness of lichens diversity in state.

The new record of *Pyrenula* species also indicated the potential of this region; and it inviting the researchers to come forward for exploration of lichens. In India, Upreti (1990, 1991) listed few species of *Pyrenula*, where he found *Pyrenula* species having *subducta* and *brucea* spore type. Most of reported on *Pyrenula* species were carried out from eastern Himalayan region as well as from Western Ghats of India (Ingle et al. 2018). The genus *Pyrenula* is crustose lichen mostly found in evergreen forest; which normally grows on smooth and shaded bark of the higher plants. According to the latest key (Taxonomic details) of the lichens species, there are 226 species of *Pyrenula* was reported from all over the world (Aptroot 2012); out of this 77 species were reported from India (Ingle et al. 2018) and the present record from Mizoram has raised its number to 78.

The sites of present study exhibited luxuriant growth of both micro lichens (crustose) and macro lichens (foliose and fruticose) due to moist and tropical climate. The occurrence of crest forming lichen like genera *Acanthothecis, Biatora, Brigantiaea, Cryptothecia, Porina* and *Pyrenula* together with cyanolichens genera *Leptogium* clearly indicateda moist shady habitat of the study area,which is suitable for lichens colonization. Most of the crustose genera encountered in the study area were known for their wide distribution in tropical evergreen forest of the country (Pinokiyo et al., 2008, Rout et al., 2010).

The region is flourished with the diversity of genus *Parmotrema* viz. *Parmotrema reticulatum* (Taylor) M. Choisy, *Parmotrema saccatilobum* (Taylor) Hale, and *Parmotrema tinctorum* (Despr. exNyl.) Hale; which usually grow in the moist area mostly covered with trees canopy in forest getting less sunlight (Dey et al., 2015). Since it is known that the lichens are the indicator of pollution particularly *Usnea* sp. highly sensitive to sulfur dioxide (Gupta et al., 2016). Similarly, the occurrence of both foliose parmelioid lichens, *Bulbothrix, Hypotrachyna, Myelochroa* neighboring genus *Usnea,* further indicated a pollution free environmental condition in the study area. Moreover, this finding, if correlated with other previous reports on lichens documentation and distribution, it gave

detailed overview of lichens found in the state. And this could become a good tool for environmental biomonitoring and other bioprospecting studies.

5.2 PHYTOCHEMICAL SCREENING

The preliminary phytochemical screening on selected some lichen species in three different solvents was performed to known the presence of secondary metabolites by which lichens species were evaluated for its medicinal properties. Most of the lichens species showed the presence of secondary metabolites (Table 4.5).The above observation was also supported with a study made by Swathi et al. (2010), where the phytochemical study of *Everniastrum cirrhatum* (Fr.) Hale shown the presence of alkaloids, saponins, tannins and terpenoids, therefore, *E. cirrhatum* can be used for different bioactivities. Since, the phytochemical screening of lichens was stated in last decades, but there are lots of studies made by many researches were shown different kinds of secondary metabolites. For example, the extract of *Flavoparmelia caperata* showed the various metabolites like Tannins, Proteins, Carbohydrates, Flavonoids,Triterpenes and steroids, while alkaloids was reported in most of the *Parmotrema* species like *Parmotrema grayanum, Parmotrema reticulatum, Parmotrema tinctorum* and *Teloschistes flavicans*. Saponins and flavanoids were observed in case of *Usnea subflorida* (Rashmi et al., 2014). Similarly, Anupama et al. (2017) had also reported presence of carbohydrates, phenols, flavanoids, tannins, terpenoids, coumarins and saponins in *Parmotrema tinctorum*; whereas, *Parmotrema grayanum* and *Parmotrema reticulatum* were shown the presence of steroids only (Devi et al., 2017). All previous cited findings observations were also supported the findings of present study. Henceforth, it can say that the diverse range of secondary metabolites makes lichen more important in field of Pharmacology as well as for the drug discovery.

5.3 ANTI-OXIDANT ACTIVITIES OF SELECTED LICHENS OF MIZORAM

Antioxidants are those compounds which had the potential to inhibit the free radicals; the free radicals are produced by chemical reaction of oxidation process which leads to various diseases by damaging the cells in human body. There are many sources by which body get the antioxidants; but antioxidants obtained from

the natural resources are important factors for human health. Since many chronic diseases such as cardiovascular, diabetes and neurodegenerative were initiated by the oxidative stress, thus, production of new chemical with the antioxidants properties aremore in priority for pharmaceutical companies to development of novel drugs (White et al., 2014). In fact, many researchers have investigated lichen species like *Cetraria islandica, Parmelia saxatilis, Ramalina pollinaria, Ramalina dumeticola, Usnea ghattenis, Usnea rubrotincta, Usnea longissima* for their antioxidants properties and some of them were reported for good antioxidant activities too (Sharma and Kalikotay, 2012; Gunasekaran et al., 2016).

Similarly in the present study, some lichens were selected for evaluation of antioxidants properties. The DPPH scavenging activity of methanol extract of *Usnea baileyi, Everniastrum cirhhatum, Parmotrema reticulatum* and *Ramalina conduplicans* has shown higher percentage in radical scavenging activity from the range of 64 – 85 %and the acetone extract were slight lower in its scavenging activities with the range from 63–69 %; whereas, Aqueous extracts were reported for least activity (60–64 %) in comparison to other extracts (Table 4.6–4.10). In case of reducing power activities (Table 4.11–4.15); the same lichens species have shown a very strong reducing power and the activities were in between 0.61–0.94, while standard ascorbic acid showed the activity as 1.08. Moreover, the superoxide radical scavenging activity of *Parmotrema latissimum, Parmotrema reticulatum,* and *Everniastrum cirrhatum* were shown higher by methanol extract with 71–75 percent of inhibition, while *Parmotrema saccatilobum* acetone extract have shown higher percentage of inhibition ie., 74.69% (Table 4.16 – 4.20). Overall, it was observed that the standard antioxidants had higher scavenging activity at all the tested concentrations than the extracts, but, still extracts showed good free radical scavenging activities. On the other hand, Kosanić et al. (2011) assessed the acetone, aqueous and methanol extract of some selected lichens as *Cladonia furcata, Hypogymnia physodes, Lasallia pustulata, Parmelia caperata* and *Parmelia sulcata* for DPPH radical scavenging, superoxide anion radical scavenging, reducing power, determination of total phenolic compounds and total flavonoid content; and reported that *Lasallia pustulata* has shown higher antioxidant activities of inhibition and very

strong reducing power. Besides this, lichens from family Parmeliaceae have shown effective antioxidants properties as *Parmotrema saccatilobum* (4.38 μmolTE/mg sample) and *Usnea ghattensis* (4.74 μmol TE/mgsample) (Fernández-Moriano et al., (2015).The most phenolic compounds like physodalic acid and evernic acid were found in *Pseudevernia furfuracea* and *Evernia prunastri* and reported with the strong antioxidant properties with strong scavenging properties with high phenolic contents for the first time from the morocco (Aoussar et al., 2017). Likewise, in present finding also the total phenol and total flavanoids contents of selected lichens viz. *Parmotrema latissimum, Parmotrema reticulatum, Parmotrema saccatilobum* and *Everniastrum cirrhatum* had shown a very high content (table 4.21 and 4.22). Indeed, the finding was concluded that the superoxide anion radical scavenging and strong relationships between total phenolic and flavonoid contents. Additionally, Shendge et al. (2017) experiment out the antioxidant properties of *Everniastrum cirrhatum,* and reported for scavenging potential.While DPPH and ferric reduction activities of *Parmotrema reticulatum* has shown the positive response with 19μg/ml IC_{50} value as well as higher absorbance value (0.643±0.003) (Ganesan et al., 2017). Similarly, other lichen species like *Ramalina pacifica* and *Usnea longissima* was reported for strong antioxidant activities like DPPH radical scavenging activity, reducing power assay and total phenolic content and total flavonoid contents (Smitha and Garampalli, 2016; Aydin et al., 2018).

5.4 *IN-VITRO* ANTI-FUNGAL ACTIVITIES OF SOME SELECTED LICHENS

A part from the taxonomical studies and antioxidants properties; lichens were also reported for its very effective potential as antibacterial, antifungal and against many more human problems as well as other antibiotic properties. The studies of lichens as an antibiotic was began in 1973 by Vartia a scientist, where he reported that nine crystalline lichenic acids tested against the bacteria (Halam and Van Haluwin, 2004). As it has already been reported recently that, the microbes are becoming more resistant to the broad spectrum of antibiotics, so then, it is the high time for the researcher to bring out the novel bioactive compounds that can inhibits the growth of microorganism; as the same statement was earlier mentioned by

Timbreza et al.(2017). Thereupon, the present study was also carried out with extraction of 6 selected lichens species like *Everniastrum cirrhatum, Parmotrema saccatilobum, Parmotrema reticulatum, Usnea baileyi, Parmotrema latissimum* and *Ramalina conduplicans* with three different solvents like acetone, aqueous and methanol and tested against 4 dermatophytic fungi such as *Epidermophyton floccosum, Trichophyton mentagrophytes, Aspergillus flavus* and *Aspergillus fumigatus.*

As a result, the minimum inhibitory concentration (MIC) of acetone and aqueous extracts was observed higher in *Ramalina conduplicans* at 100µg/ml and *Parmotrema reticulatum* at 150µg/ml, while the rest at 200µg/ml(Table 4.23-4.25); likewise, methanol extract of *Parmotrema reticulatum* shown the MIC at concentration 150µg/ml. In addition to this, methanol extract of *Everniastrum cirrhatum* had shown the MIC at same concentration (150µg/ml); while the other methanolic extracts were reported for their MIC at concentration200µg/ml. Further, in case of their zone of inhibition (ZIC), the acetone extracts was found maximum ZIC against *Aspergillus fumigatus*; while in aqueous extracts, the ZIC was found maximum against *Trichophyton mentagrophytes.* However, the methanol extracts showed the highest ZIC against *Epidermophyton floccosum.*

There are many research findings which are correlated with present studies. For example, acetone, methanol and aqueous extracts of the *Lecanora atra, Lecanora muralis, Parmelia saxatilis, Parmelia sulcata* and *Parmeliopsis ambigua* was evaluated for their antimicrobial activity against the 6 bacterial species and 10 species of fungi and determined the MIC by using disc diffusion method. Further, extract prepared in acetone and methanol solvents were accounted for their effectiveness, while aqueous extracts haven't shown any antimicrobial activities (Ranković et al., 2009; Ranković and Kosanić, 2012). According to another finding, there was a huge variation were observed in the MIC of *Parmotrema reticulatum* extract against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* at concentration in between 100-300µg/ml (Jain et al., 2016). Conversely, the finding of Pandeyet al. (2013) had shown the MIC of *Cladia aggregata* against human pathogenic fungi *Malassezia furfur, M. globosa* and *M.*

sympodialis as 2.72, 0.63 and 1.28 mg/ml respectively at different concentrations, but a synthetic drug fluconazole was found more effective in their investigation. Correspondingly, there were several established results on the antifungal activity performed by different lichen species. Like, *Everniastrum cirrhatum* minimum fungicidal concentration (60µl/ml) was reported against dermatophytic fungi such as *Epidermophyton floccosum, Microsporum gypseum, M. canis, M. audounii, Trichophyton rubrum, T. mentagrophytes, T. violaceum,* and *T. tonsurans* (Shahi et al., 2000). More or less, broad spectrum antifungal properties with value 80µl/ml were observed in case of *Parmelia cirrhatum* aqueous extract against some human and plant pathogens (Shahi et al., 2003). Identically, *Heterodermia leucomelos* and other lichens extracts of *Parmelia tinctorum, Ramalina* sp., *Teloschistes flavicans,* and *Usnea undulate* was also reported in past for their antifungal activities. (Dikshit, 1991; Shahi et al., 2001). The results of present study are also in accordance with the other previous findings.

Moreover, due to more effectiveness in their biological activities, currently the researches now concentrated on nanoparticles which have to advance a herbo-metallic colloidal nano-formulation. Consequently, Singh et al. (2015) did the experiment in same line by using extract of *U. longissima* along with Swarna nanoparticles by using anti-quorum sensing (QS) property against *Streptococcus mutans.* Further, they also concluded that the presence of polyphenols once combined with Swarna nanoparticles, automatically the antimicrobial activities was increased. Since, many studies have also proven about the antimicrobial activities are due to the presence of different classes of secondary metabolites like phenols, alkaloids, terpenoids, flavanoids, glycosides, saponins and other active principles. For this reason, the phenolic compounds and their derivatives as well as other metabolites of lichen were evaluated against different pathogenic microorganisms. Actually, these substances have more power to acidify the microbial cell wall and thus, rupture in cytoplasmic membrane, immobilization of the enzymes activities, and interfere with physiological functions such as electrons transport and oxidative phosphorylation and finally disturbing thestructures and rendering them more permeable leading to leakage of ions and other cell contents which decreased the

intracellular ATP pool of bacterial or fungal cell and also increased extracellular ATP, which altogether cause the death of the microorganisms (Zhao et al., 2001; Müller, 2001; Randhir et al., 2004; Vattem et al., 2004;Kumar et al., 2012).

5.5GASTROPROTECTIVE ACTIVITY

The gastroprotective activities of some potent secondary metabolites contents in extracts of lichens species has been already conducted with the effective results (Lakshmi et al., 2013; Sepulveda et al., 2013; Sharma et al., 2014). For this purpose, the *Usnea baileyi* of family Parmeliaceae was evaluated for its gastroprotective activity, because, under genus *Usnea,* this particular species still not yet explored. Following the protocol of Sagun et al. (2016), where ethanol induced gastric ulcer in rats model with the administration of plants extract were explained that ethanol treated groups produced numerous necrotic lesions and deep ulcers and upsurge the gastric acidity due to the depletion of bicarbonates secretion and mucus production while the pretreated of extracts and Omeprazole reduced as a very reverse observation. Based on the same, the present observation as depicted in Table 4.28 and 4.29 and Figure 4.30 and 4.31, the gastric lesions were reduced significantly at the dose dependent manner with the oral administration of methanolic extract of *Usnea baileyi* at dose 50, 100 and 200 mg/kg body weight. In contrast to extract, standard drug Omeprazole was used as proton pump inhibitor has shown high activity. Odabasoglu et al. (2006) was used usnic acid (extracted from genus *Usnea*) for gastroprotective effects; and reported that, the effect of usnic acid must be attributed to its reducing effect on the oxidative damage and neutrophil infiltration in tissues. In addition to this, Sepulveda et al. (2013) studies some of the secondary metabolites obtained from the lichens and reported that depsidone and depside have the action to prevents the gastric lesion on ethanol induce mice model. Moreover, lobaric acid, atranorin and psoromic acid shown the reduced of gastric lesions with 76%, 63% and 65% and variolaric acid, diffractaic acid and perlatolic acid with 32%, 14% and 45%, respectively.

Based on previous as well as present findings it summarized that, the variation in pharmacological activities of plants including lichens may be related to the concentration and nature of constituents, their respective composition, the

functional groups,the structural configuration of the components and their possible synergistic interaction.

CHAPTER -6

Summary and Conclusion

Lichens are considered as a constant and self-sustaining relationship between fungi as mycobionts and the algal partners as photobionts. As the mycobiont is sole in the symbiotic relationship and it is typically controls the relationship (Ranković and Kosanić, 2015). Lichens are widely distributed in all the geographical region of the world. The total amount of lichens species was reported that number of accepted species is 19,387 in 995 genera, 115 families. (Lücking et al., 2017).Singha et al., (2018) reported the addition of 411 species to the list of Annotated Checklist 2010; therefore the total number of lichens recorded from India became 2714 species. In the context of eastern Himalayas; Singh et al., (2018) reported 1047 species Further, Logesh et al., (2017) reported 159 species of lichens in Mizoram including Thangjam et al., (2019).

Lichens are known for its unique secondary metabolites, it can synthesize a great variety of metabolites (Richardson, 1988; Lawrey, 1989; Elix, 1996). Lichens were believed that it is widely used in different traditional system of medicines (TSM) such as Ayurveda, Chinese medicine (TCM), Homeopathic and Unani for the treatment of various ailments (Saklani and Upreti, 1992). They play a very important role in the ancient and traditional system of medicinal practices. Lichens are also used in different other purpose *viz.* food, fodder, spices, dyes, cosmetics, perfumery. Lichens as whole and its identified metabolites are also having numerous biological activities as antimicrobial, antiprotozoal, antiviral, antiproliferative, anti-inflammatory, analgesic, antipyretic, antitermite, antioxidant, cytotoxic, enzyme inhibitory, insecticidal, wound healing ,antitumor as well as enzyme inhibitory (Yılmaz et al., 2004, Kosanić et al., 2013, Rajan et al., 2016).

Despite of all this, The use of lichens and their products in medicine still hold a considerable interest as alternative medicine to cure various diseases in different parts of the world. More than 50% lichen species known from the world exhibited presence of peculiar antibiotic substances. Thus, there is a lot of scope for carrying out such studies in India and particularly in the state of Mizoram where lichen grows luxuriantly. Therefore, the main target of this research purpose is to find out the antifungal effects to reinforce bioprospecting of lichens as herbal

medicine. The first phase of the experiment was involve the collection of the lichens species and their identification, preparation of the extracts and its phytochemical properties followed by determination of antioxidants, antifungal activity against the common pathogenic fungi; In addition to this, extract was also evaluated for its gastroprotective activity.

The present study documented a total of 78 species under 30 genus and 19 families from the 3 different studies sites of various districts of Mizoram. Genus *parmotrema* is one of the most dominated in the region with 15 species followed by *Heteroderimia* with 7 species. Among the families, Parmeliaceae was the most dominant and it was growing luxuriantly with 30 species followed by Graphidaceae and Physciaceae with 10 and 8 species respectively. In addition to previous findings, the investigation also enumerates 42 species from a small region of National Park, 20 species from a tourist spot and 16 species from a university campus, which clearly indicates the richness of lichens diversity in state. Moreover, the new record of *Pyrenula* species (*Pyrenula dissimulans*) also indicated the potential of this region; and it inviting the researchers to come forward for exploration of lichens.

Apart from taxonomic studies, the preliminary phytochemical screening on selected some lichen species in three different solvents was performed to known the presence of secondary metabolites by which lichens species were evaluated for its medicinal properties. Most of the lichens species showed the presence of secondary metabolites. All previous cited findings observations were also supported the findings of present study. Henceforth, it can say that the diverse range of secondary metabolites makes Lichen more important in field of Pharmacology as well as for the drug discovery. For this purpose, selected lichens were evaluated for antioxidants properties. The DPPH scavenging activity of methanol extract of *Usnea baileyi*, *Everniastrum cirhhatum, Parmotrema reticulatum* and *Ramalina conduplicans* has shown higher percentage in radical scavenging activity from the range of 64 – 85 % and the acetone extract were slight lower in its scavenging activities with the range from 63–69 %; whereas, aqueous extracts were reported for least activity (60–64 %) in comparison to other extracts (Table 4.6–4.10). In case of reducing power activities (Table 4.11–4.15); the same lichens species have shown a

very strong reducing power and the activities were in between 0.61–0.94, while standard ascorbic acid showed the activity as 1.08. Moreover, the superoxide radical scavenging activity of *Parmotrema latissimum, Parmotrema reticulatum,* and *Everniastrum cirrhatum* were shown higher by methanol extract with 71–75 percent of inhibition, while *Parmotrema saccatilobum* acetone extract have shown higher percentage of inhibition ie., 74.69% . Likewise, present finding also the total phenol and total flavanoids contents of some selected lichens viz. *Parmotrema latissimum, Parmotrema reticulatum, Parmotrema saccatilobum, Everniastrum cirrhatum* had shown a very high contents. Furthermore, Antifungal studies also carried out in carried out in extraction of 6 selected lichens species like *Everniastrum cirrhatum, Parmotrema saccatilobum, Parmotrema reticulatum, Usnea baileyi, Parmotrema latissimum* and *Ramalina conduplicans* with three different solvents like acetone, aqueous and methanol and tested against 4 dermatophytic fungi viz. *Epidermophyton floccosum, Trichophyton mentagrophytes, Aspergillus flavus* and *Aspergillus fumigates.* Accordingly, the minimum inhibitory concentration of the acetone and aqueous extracts was observed higher in *Ramalina conduplicans* at 100µg/ml and *Parmotrema reticulatum* at 150µg/ml while the rest at 200µg/ml. similarly; in the methanol extract it was observed that *everniastrum cirrhatum* and *Parmotrema reticulatum* was effective the MIC at 150µg/ml while the other methanolic extract of lichen species at 200µg/ml. Further; in the acetone extracts, the zone of inhibition (ZIC) was found maximum inhibition against *Aspergillus fumigates*. While in aqueous extract, (ZIC) was found maximum inhibition against *Trichophyton mentagrophytes.* In the methanol extracts; (ZIC) was observed the highest inhibition against *Epidermophyton floccosum.*

Along with the invitro studies, invivo studies was also carried out on the methanolic extracts of *Usnea baileyi* of family Parmeliaceae for its gastroprotective activity, the gastric lesions were reduced significantly at the dose dependent manner with the oral administration of methanolic extract of *Usnea baileyi* at dose 50, 100 and 200 mg/kg body weight. In contrast to extract, standard drug Omeprazole was used as proton pump inhibitor has shown high activity.

Altogether, it is summarized that lichens are the key primary producers with important linkage to nutrient cycling and forest food chain, moreover, their genetic richness is also more accountable in protected forest area with respect to biodiversity bioprospection (Jovan 2008, Rout et al. 2010). The results also clearly indicates that the state has rich diversity of lichens and there is need of further intensive and extensive survey and documentation in different geographical parts of Mizoram to get a clear picture of lichen diversity in the state. The future lichen exploration in the state will not only bring out some other new lichen taxa to the science but also provide a base for carrying out environmental biomonitoring and other bioprospecting studies filed of lichenology.

Moreover, it is an urgent need to studies on different others fungi to bring out the effective properties of antimicrobial in lichens and need to isolate each and every compounds present in the extract to find out the novel compound in the future. Further, some of these observations may help in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings. Additionally, conservation strategies must be developed for its sustainable harvesting and future researches.

AL-Wajeeh NS, Halabi MF, Hajrezaie M, Dhiyaaldeen SM, Bardi DA, Salama SM, and Ali HM (2016). The gastroprotective effect of Vitex pubescens leaf extract against ethanol-provoked gastric mucosal damage in Sprague-Dawley rats. *PloS one*. 11(9):1-21.

Anupama TV, Sheela KB and Saji G (2017). Phytochemical screening and proximate composition of lichen *Parmotrema tinctorum* (Nyl.) Hale (Parmeliaceae) from Wayanad, Kerala. *International Journal of Chemical Studies*. 5(5):1003-1007.

Aoussar N, Manzali R, Nattah I, Rhallabi N, Vasiljevic P, Bouksaim M and Mellouki F (2017). Chemical composition and antioxidant activity of two lichens species (*Pseudevernia furfuracea* L and *Evernia prunastri* L) collected from Morocco. *Journal of Materials and Environmental Science*. 8(6):1968-1976.

Aptroot A (1997). Species diversity in tropical rainforest ascomycetes: lichenized versus non-lichenized; foliicolous versus corticolous. *Abstracta Botanica*. Pp.37-44.

Aptroot A and Feijen FJ (2002).Annotated checklist of the lichens and lichenicolous fungi of Bhutan. *Fungal Diversity*. 11:21-48.

Aptroot A (2012). A world key to the species of *Anthracothecium* and *Pyrenula*. *The Lichenologist*.44(1):5-53.

Asahina Y and Shibata S (1954).Chemistry of lichen substances. *Japan Society for the Promotion of Science*, Tokyo.

Awasthi DD (1961). Some foliose and fruticose lichens from Assam and North-East Frontier Agency of India. *In Proceedings of the Indian Academy of Sciences-Section B*. 54:1-24.

Awasthi DD (1965). Catalogue of the lichens from India, Nepal, Pakistan, and Ceylon.

Awasthi DD (1993). A key to the microlichens of India, Nepal and Sri Lanka. *Lichenologist*. 25:101-104.

Awasthi DD (2007). Compendium of the Macrolichens from India, Nepal and Sri Lanka. Bishen Singh Mahendra Pal Singh, India.

Ayatollahi Mousavi SA and Kazemi A (2015).In vitro and in vivo antidermatophytic activities of some Iranian medicinal plants. *Medical mycology*. 53(8): 852-859.

Aydin S, Kinalioğlu K and Sökmen BB (2018).Antioxidant, anti-urease and anti-elastase activities of *Usnea longissima* Ach. *Bangladesh Journal of Botany*. 47(3):429-435.

Baniya CB, Solhøy T, Gauslaa Y and Palmer MW (2010).The elevation gradient of lichen species richness in Nepal. *The Lichenologist*. 42(1):83-96.

Behera BC, Morey MV and Gaikwad SB (2016).Anti-lipoxygenase, radical scavenging and antimicrobial activities of lichen species of genus *Heterodermia* (Physciaceae). *Botanica Pacifica: a journal of plant science and conservation*. 5(1):79-85.

Bisht S, Sharma S, Kumar V, Kumar M, Bisht SS and Nautiyal BP (2014).Assessment of antimicrobial efficacy of secondary metabolites of lichen species from Uttarakhand temperate Himalayas, India. *Journal of natural products*. 7:168-176.

Blois MS (1958). Antioxidant determinations by the use of a stable free radical.*Nature*. 181(4617):1199-1200.

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

Breuss O and John V (2004).New and interesting records of lichens from Turkey. *Österreichische Zeitschriftfür Pilzkunde*. 13:281-294.

Büdel B and Henssen A (1983).*Chroococcidiopsis* (Cyanophyceae), a phycobiont in the lichen family Lichinaceae. *Phycologia*. 22(4):367-375.

Chapman AD (2009). Numbers of Living Species in Australia and the World.2nd edition. Australian Government, Department of the Environment, Water, Heritage and the Arts.

Chinlampianga M, Logesh AR, Dubey U, Shukla AC and Upreti DK (2013) Preliminary studies on lichen flora of Mizoram. *Sci Tech J*. 1:22-25.

Choudhary V (2018) Murlen National Park complete detail-updated, Abhinav Nature Conservation, http://natureconservation.in/murlen-national-park-complete-detail-updated/ . Accessed 1August 2018.

Committee for the Purpose of Control and Supervision on Experiments on Animals.(2003). CPCSEA Guidelines for laboratory animal facility. Indian J. Pharmacol., 35(4).

Crawford SD (2019). Lichens used in traditional medicine. Lichen Secondary Metabolites, Bioactive Properties and Pharmaceutical Potential. Springer, Champ. pp. 31-97.

da Silva Cáceres, ME and Aptroot A (2017). Lichens from the Brazilian Amazon, with special reference to the genus *Astrothelium*. *The Bryologist*. 120(2):166-183.

Daimari R, Hazarika N, Hoque RR, Nayaka S and Upreti DK (2014).New records of epiphytic lichens from three districts of Assam, India. *Indian Forester,*140(8): 807-811.

Debnath R, Khare R, Gogoi L, Upreti DK and Rout J (2018). New additions of macrolichens to the lichen flora of Arunachal Pradesh, India in Eastern Himalaya. *Studies in Fungi*. 3(1):100–114.

Devi A, Mohabe S, Reddy M, Akkulanna S, Nayaka S (2017). Qualitative phytochemical analysis of three solvents extracts of some selected

macrolichens from Seshachalam Biosphere Reserve, Andhra Pradesh. *Cryptogam Biodiversity and Assessment*. 2(1):19–25. DOI: https://doi.org/10.21756/cab.v2i1.8391

Devi AB, Mohabe S, Reddy MA and Nayaka S (2015).Efficacy of a potential lichen Parmotremaandinum (Müll. Arg.) Hale against pathogenic microorganisms. *Journal on New Biological Reports*. 4(2):149-156.

Devi RK, Rout J, Upreti DK, Nayaka S, Pinokiyo A (2015). New records of lichens from Manipur State, North-eastern India. *Mycosphere*. 6:796-813.

Dey AK, Mishra GK, Rout J, Upreti DK (2015). An enumeration of epiphytic lichens from Hojai sub-division of Nagaon district, Assam, India. *Int. J. Adv. Res. Biol. Sci*. 2(10): 111–115.

Dikshit A (1991). Antifungal activity of some macrolichens. *In Proceedings of the International Conference on Global Environment and Diversification of Plants through Geological Time* (p. 53).

Divakar PK, Upreti DK (2005). Parmelioid Lichens in India (a Revisionary Study) Dehra Dun. Bishen Singh Mahendra Pal Singh, India.

Elix JA and Stocker-Wörgötter E (1996).Biochemistry and secondary metabolites. *Lichen biology*. 1:154-180.

Elix JA and McCarthy PM (1998).Catalogue of the lichens of the smaller Pacific Islands.

Esslinger TL and Egan RS (1995).A sixth checklist of the lichen-forming, lichenicolous, and allied fungi of the continental United States and Canada. *Bryologist*.467-549.

Esslinger TL (2018). A Cumulative Checklist for the Lichen-Forming, Lichenicolous and Allied Fungi of the Continental United States and Canada, Version 22. *Opuscula Philolichenum*. 17:6-268.

Fernández-Moriano C, Divakar PK, Crespo A and Cuadrado MPGS (2015). Antioxidant and cytoprotective potentials of Parmeliaceae lichens and identification of active compounds. *In Anales de la Real Academia Nacional de Farmacia*. 81(2):164 -178.

Firdous SS, Khan S, Dar MEUI, Shaheen H, Habib T and Ullah TS (2017).Diversity and distribution of lichens in different ecological zones of Western Himalayas Pakistan. *Bangladesh Journal of Botany*. 46(2):805-811.

Galloway DJ (1991). Phytogeography of southern hemisphere lichens. In Quantitative approaches to phytogeography.Springer, Dordrecht. pp. 233-262.

Galloway DJ (1992). Lichens of Laguna San Rafael, ParqueNacional'Laguna San Rafael', southern Chile: indicators of environmental change. *Global Ecology and Biogeography Letters*.37-45.

Galloway DJ (1994).Biogeography and ancestry of lichens and other ascomycetes.In Ascomycete Systematics.Springer, Boston, MA. pp. 175-184

Ganesan A, Thangapandian M, Ponnusamy P and Sundararaj JP (2017). Antibacterial and antioxidant activity of *Parmotrema reticulatum* obtained from Eastern Ghats, Southern India. *Biomedical Research*. 28 (4):1593-1597.

Gogoi, R, Joseph S, Nayaka S and Yasmin F (2019).Additions to the lichen biota of Assam State, India. *Journal of Threatened Taxa*. 11(6):13765-13781.

Groombridge B (1992) Global biodiversity: status of the earth's living resources. Chapman & Hall, London.

Gunasekaran S, Rajan VP, Ramanathan S, Murugaiyah V, Samsudin MW and Din LB (2016). Antibacterial and antioxidant activity of lichens *Usnea rubrotincta, Ramalina dumeticola, Cladonia verticillata* and their chemical constituents.*Malaysian Journal of Analytical Sciences*. 20(1):1-13.

Gupta S, Khare R, Bajpai O, Rai H, Upreti DK, Gupta RK and Sharma PK (2016). Lichen as Bioindicator for Monitoring Environmental Status in Western Himalaya, India. *International Journal of Environment*. 5(2):1-15.

Halama P and Van Haluwin C (2004).Antifungal activity of lichen extracts and lichenicacids. *BioControl*. 49(1):95-107.

Hawksworth DL and Hill DJ (1984). The lichen-forming fungi.Blackie Academic and Professional.

Honegger R (2000). Simon Schwendener (1829-1919) and the dual hypothesis of lichens. *The Bryologist*. 103(2):307-313.

Huneck S (1999). The significance of lichens and their metabolites. *Naturwissenschaften*.86(12):559-570.

Hussain H, Badawy A, Elshazly A, Elsayed A, Krohn K, Riaz M and Schulz B (2011). Chemical constituents and antimicrobial activity of *Salix subserrata*. *Rec. Nat. Prod*, 5(2):133-137.

Ingelfinger R, Henke M, Roser L, Ulshöfer T, Calchera A, Singh G, Parnham, MJ, Geisslinger G, Fürst R, Schmitt I and Schiffmann S(2020). Unraveling the pharmacological potential of lichen extracts in the context of cancer and inflammation with a broad screening approach. *Frontiers in pharmacology*. 11. https://doi.org/10.3389/fphar.2020.01322.

Ingle KK, Uppadhyay V, Nayaka S, Trivedi S and Sahoo D (2018).New records and an updated key of *Pyrenula*from India.*Cryptogam Biodiversity and Assessment*.doi: https://doi.org/10.21756/cab.esp7

Ivanova D and Ivanov D (2009). Ethnobotanical use of lichens: Lichens for food Review. *Scripta Scientifica Medica*. 41(1):11-16.

Jain AP, Bhandarkar S, Rai G, Yadav AK and Lodhi S (2016).Evaluation of *Parmotrema reticulatum* taylor for antibacterial and anti-inflammatory activities. *Indian journal of pharmaceutical sciences*. 78(1):94-102.

Jayakumar K, Babu PG, Theja CP, Kumari SG, Veena D and Paramageetham CH (2016). Biomedical Potentials of *Talaromycestratensis* - A New Endolichenic Fungi Associated with High Altitude Curstose Lichen *Lecanora* sp. *Research journal of pharmaceutical biological and chemical sciences*. 7(5):1412-1419.

Jørgensens PM (1983). Distribution patterns of lichens in the Pacific region. *Australian Journal of Botany Supplementary Series*. 13(10):43-66.

Joshi Y, Nguyen TT, Wang XY, Lőkös L, Koh YJ and Hur JS (2011).Contribution to the lichen mycota of South Korea. *Mycotaxon*. 116(1): 61-74.

Jovan S (2008). Lichen bioindication of biodiversity, air quality, and climate: baseline results from monitoring in Washington, Oregon, and California. Gen. Tech. Rep. PNW-GTR-737. Portland, OR: US Department of Agriculture, Forest Service, Pacific Northwest Research Station. 115:737.

Kambar Y, Vivek MN, Manasa M, Vinayaka KS, Mallikarjun N and Kekuda PT (2014).Antimicrobial activity of *Leptogium burnetiae*, *Ramalina hossei, Roccella montagnei* and *Heterodermia diademata*. *International Journal of Pharmaceutical and Phytopharmacological Research*. 4(3):164-168.

Kaushal K and Upreti DK (2001).*Parmelia* spp.(lichens) in ancient medicinal plant lore of India. *Economic Botany*. 55(3):458-459.

Kosanić M and Ranković B (2010).Screening of antimicrobial activity of some lichen species in vitro. *Kragujevac J Sci*. 32(3):65-72.

Kosanić M, Ranković B and Vukojević J (2011) Antioxidant properties of some lichen species. *Journal of food science and technology*. 48(5):584-590.

Kosanić M, Ranković B and Stanojković T (2013) Investigation of selected serbian lichens for antioxidant, antimicrobial and anticancer properties. *The Journal of Animal & Plant Sciences.* 23(6):1628-1633.

Kuldeep S and Prodyut B (2015). Lichen as a Bio-Indicator Tool for Assessment of Climate and Air Pollution Vulnerability. *International Research Journal of Environment Sciences*. 4(12):107-117.

Kumar A, Gupta R, Mishra RK, Shukla AC and Dikshit A (2012).Pharmaco-phylogenetic investigation of Micromeriabiflora Benth and Citrus reticulata Blanco. *National Academy Science Letters*. 35(4), 253-257.

Kumar A, Singh M, Pandey A, Shukla AC and Dikshit A (2014).*Trachyspermum ammi* L.: the traditional medicinal plants and its bioefficacy against the human pathogenic fungi. 29 – 35.

Kunchandy E and Rao MNA (1990).Oxygen radical scavenging activity of curcumin. *International Journal of Pharmaceutics*. 58(3):237-240.

Lakshmi V, Ameta K, Mishra V, Srivastava A, Agarwal SK, Palit G and Mahdi AA (2013). Gastroprotective effect of ethanolic extract of *Parmelia perlata* in rats. *J Phytopharmacol*. 2(6):19-25.

Lalrinawmi H, Zothanzama J, Held BW, Vabeikhokhei JM and Blanchette R (2018). The gilled mushroom Amanita spissacea (Amanitaceae): a new report for India. *Journal of Threatened Taxa*. 10(10):12413-12417.

Lalzarzovi ST and Lalnuntluanga (2013). Floristic composition of shrub community of Reiek forest, Mamit district Mizoram. *NeBIO*, 4(5):55-56.

Lalzarzovi ST and Lalnuntluanga (2018).Plant Community Structure of Tropical Semi-Evergreen Forest of Reiek in Mamit District of Mizoram.Science and Technology Journal. 5(1):58-62.

Lawrey JD (1986). "Biological role of lichen substances. *Bryologist*. 89(2):111-122.

Lawrey JD (1989). Lichen secondary compounds: evidence for a correspondence between antiherbivore and antimicrobial function. *Bryologist*.326-328.

Linnaeus C (1753). Species Plantarum. (Stockholm). Pp 1200.

Logesh AR, Chinlampianga M, Shukla AC and Upreti DK (2015).Studies on Lichens of Mizoram, Northeast India. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*. 87(2):1-13.

Luangsuphabool T, Lumbsch HT, Piapukiew J and Sangvichien E (2018).*Architrypetheliummurisporum* (Ascomycota, Trypetheliaceae), a remarkable new lichen species from Thailand challenging ascospore septation as an indicator of phylogenetic relationships. *MycoKeys*. 34: 25-34.

Lücking R, Hodkinson BP and Leavitt SD (2017).The 2016 classification of lichenized fungi in the Ascomycota and Basidiomycota–Approaching one thousand genera. *The Bryologist*. 119(4):361-417.

Marshall NL (1910). Mosses and lichens: a popular guide to the identification and study of our commoner mosses and lichens, their uses, and methods of preserving (Vol. 14). Doubleday, Pp. 1907

McCune B, Rogers P, Ruchty A and Ryan B (1998). Lichen communities for forest health monitoring in Colorado, USA. A Report to the USDA Forest Service. Pp 1-28.

Millot M, Girardot M, Dutreix L, Mambu L and Imbert C (2017). Antifungal and anti-biofilm activities of acetone lichen extracts against *Candida albicans*. *Molecules*. 22(4):651.

Mishra GK, Upreti DK (2016). Diversity and distribution of macro-lichen in Kumaun Himalaya, Uttarakhand. *International Journal of Advanced Research*. 4(2):912-925.

Misra K, Dhillon GS, Brar SK and Verma M (2014).Antioxidants. In Biotransformation of Waste Biomass into High Value Biochemicals Springer, New York, NY. pp. 117-138

MoEF (2018) Ministry of Environment, Forest & Climate Change, Government of India. Http:// Www. Moef.Gov.In/Sites/Default/Files/Minutes, 30th meeting, 20th June.Pdf. Accessed 9 July 2018.

Molnár K and Farkas E (2010). Current results on biological activities of lichen secondary metabolites: a review. *Zeitschriftfür Naturforschung*. *65*(3-4):157-173.

Müller K (2001). Pharmaceutically relevant metabolites from lichens. *Applied Microbiology and Biotechnology*. 56(1-2):9-16.

Myers N., Mittermeier RA, Mittermeier CG, da Fonseca GAB and Kent J (2000). Biodiversity hotspots for conservation priorities. *Nature*. 403:853–858.

Nayaka S (2004) Revisionary studies on lichen genus *Lecanora* s*ensulato* in India. Ph. D. thesis, Dr. RML Avadh University Faizabad, India.

Nayaka S, Upreti DK and Khare R (2010). Medicinal lichens of India. Drugs from plants. Jaipur: Avishkar Publishers, Distributors, 1-54.

Nayaka S (2014). Methods and techniques in collection, preservation and identification of lichens. *PlantToxonomy and Biosystematics: Classical and Modern Methods*. Pp 101-105.

Negi HR and Kareem A (1996). Lichens: The unsung heroes. *Amrut*. 1: 3-6.

Nimis PL, Hafellner J, Roux C, Clerc P, Mayrhofer H, Martellos S and Bilovitz PO (2018). The lichens of the Alps–an annotated checklist. *MycoKeys*. 31:1-632.

No OT (2001). 423: Acute oral toxicity-acute toxic class method. OECD guidelines for the testing of chemicals (section 4: health effects), pp.1-14.

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):59-65.

Oksanen I (2006).Ecological and biotechnological aspects of lichens. *Applied Microbiology and Biotechnology*. 73(4):723-734.

Oyaizu M (1986). Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition*. 44:307–315.

Padhan DK and Pattnaik S (2014). In vivo antifungal activity of *Acmella* essential oil on a dermatomycotic strain *Trichophyton mentagrophytes* (MTCC-7687). *Der Pharmacia Sinica*. 5(1):40-44.

Padhi S and Tayung K (2015). In vitro antimicrobial potentials of endolichenic fungi isolated from thalli of *Parmelia* lichen against some human

pathogens. *Beni-Suef University Journal of Basic and Applied Sciences*. 4(4):299-306.

Pathak A, Mishra RK, Kant SS, Kumar R, Pandey M, Pandey M, Qidwai A and Dikshit A (2016).In vitro evaluation of antidermatophytic activity of five lichens. *Cogent Biology*. 2(1):2331-2025

Pavithra, GM, Vinayaka KS, Rakesh KN, Junaid S, Dileep N and Naik AS (2013).Antimicrobial and antioxidant activities of a macrolichen Usnea pictoides G. Awasthi (Parmeliaceae).*Journal of Applied Pharmaceutical Science*. 3(8):154-160.

Percival M (1997). Phytonutrients and detoxification. *Clinical nutrition insights*, 5(2):1-4.

Pinokiyo A, Singh KP and Borthakur SK (2004).Foliicolous species of *Porina*(Lichens) from Arunachal Pradesh, India. *Indian Journal Forestry*. 27(4):407-416.

Pinokiyo A, Singh KP and Singh JS (2008). Diversity and distribution of lichens in relation to altitude within a protected biodiversity hot spot, north-east India. *The lichenologist*. 40(1):47-62.

Radika S (2013). Development of treated bandage using lichen extract for wound healing. *International Journal of Latest Research in Science and Technology*, 2(2):163-66.

Rai H, Khare R, Baniya CB, Upreti DK and Gupta RK (2015) Elevational gradients of terricolous lichen species richness in the Western Himalaya. *Biodiversity and Conservation*. 24(5):1155-1174.

Rajan VP, Gunasekaran S, Ramanathan S, Murugaiyah V, Samsudin M, Din L (2016) Biological activities of four *Parmotrema* species of Malaysian origin and their chemical constituents. *Journal of Applied Pharmaceutical Science*. 6(8):36-43.

Raju D, Ilango K, Chitra V and Ashish K (2009). Evaluation of Anti-ulcer activity of methanolic extract of *Terminalia chebula* fruits in experimental rats. *Journal of Pharmaceutical Sciences and research*. 1(3):101.

Randhir R, Lin YT and Shetty K (2004). Stimulation of phenolics, antioxidant and antimicrobial activities in dark germinated mung bean sprouts in response to peptide and phytochemical elicitors. *Process Biochemistry*. 39(5), 637-646.

Ranković B, Mišić M and Sukdolak S (2007). Antimicrobial activity of extracts of the lichens *Cladonia furcata, Parmelia caperata, Parmelia pertusa, Hypogymnia physodes* and *Umbilicaria polyphylla*. *British journal of biomedical science*. 64(4):143-148.

Ranković B, Mišić M and Sukdolak S (2009). Antimicrobial activity of extracts of the lichens *Cladonia furcata, Parmelia caperata, Parmelia pertusa, Hypogymnia physodes* and *Umbilicaria polyphylla*. *Biologia*. 64(1):53-58.

Ranković B and Kosanić M (2012).Antimicrobial activities of different extracts of *Lecanora atra, Lecanora muralis, Parmelia saxatilis, Parmelia sulcata* and *Parmeliopsis ambigua*. *Pak. J. Bot*. 44(1):429-33.

Ranković B and Kosanić M (2015).Lichens as a potential source of bioactive secondary metabolites. In Lichen Secondary Metabolites, Springer, Cham, pp 1-26.

Ranković B and Kosanić M (2019).Lichens as a potential source of bioactive secondary metabolites.In Lichen secondary metabolites.Springer, Cham. pp. 1-29

Rashmi S and Rajkumar HG (2014). Preliminary phytochemical screening of different solvent extracts of lichens from Kodagu district, Karnataka. *Journal of pharmacognosy and phytochemistry*. 3(4):209-212.

Richardson DHS (1988).Medicinal and other economic aspects of lichens.CRC handbook of lichenology. 3:93-108.

Rout J, Das P and Upreti DK (2010). Epiphytic lichen diversity in a reserve forest in southern Assam, Northeast India. *Tropical ecology*. 51(2):281.

Rowe JG, Gimenez MG and Rodriguez MS (1999). Some lichen products have antimicrobial activity. *Zeitschriftfür Naturforschung C*. 54(7-8):605-609.

Sagun K, Roy VK, Kumar RS, Ibrahim KS, Parimelazhagan T, Kumar NS and Gurusubramanian G (2017).Antioxidant potential, anti-inflammatory activity and gastroprotective mechanisms of *Mallotus roxburghianus* (Muell.) against ethanol-induced gastric ulcers in Wistar albino rats. *Journal of Functional Foods*.36:448-458.

Şahin F, Karaman I, Güllüce M, Öğütçü H, Şengül M, Adıgüzel A and Kotan R (2003). Evaluation of antimicrobial activities of *Saturejahortensis* L. *Journal of ethnopharmacology*. 87(1):61-65

Saklani A and Upreti DK (1992). Folk use of lichens in Sikkim. *Journals of Ethnopharmacology*. 37: 229-233.

Saranya P, Geetha A and Selvamathy SN (2011). A biochemical study on the gastroprotective effect of andrographolide in rats induced with gastric ulcer. *Indian journal of pharmaceutical sciences*. 73(5):550-557.

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

Savic S and Tibell L (2006).Checklist of the lichens of Serbia. *Mycologia balcanica*. 3(2-3):187-215.

Schneider A (1898). A Guide to the Study of Lichens. B. Whidden.

Sepulveda B, Chamy MC, Piovano M and Areche C (2013). Lichens: might be considered as a source of gastroprotective molecules?.*Journal of the Chilean Chemical Society*. 58(2):1750-1752.

Shahi SK, Shukla AC, Bajaj AK, Banerjee U, Rimek D, Midgely G andDikshitA (2000). Broad spectrum herbal therapy against superficial fungal infections. *Skin Pharmacology and Physiology*. 13(1):60-64.

Shahi SK, Shukla AC, Dikshit A andUperti DK (2001).Broad spectrum antifungal properties of the lichen *Heterodermia leucomela*. *The Lichenologist*. 33(2): 177-179.

Shahi SK, Patra M, Dikshit A and Upreti DK (2003). *Parmelia cirrhatum*: a potential source of broad spectrum natural antifungal. *Phytotherapy Research*. 17(4): 399-400.

Shailajan S, Joshi M and Tiwari B (2014).Hepatoprotective activity of *Parmelia perlata* (Huds.)Ach.against CCl4 induced liver toxicity in Albino Wistar rats. *Journal of Applied Pharmaceutical Science*. 4(2):70-74.

Sharma B, Kumar P and Joshi SC (2011).Topical treatment of dermatophytic lesion on mice (Mus musculus) model. *Indian journal of microbiology*. 51(2):217-222.

Sharma BC and Kalikotay S (2012).Screening of antioxidant activity of lichens *Parmotrema reticulatum* and *Usnea* sp. from Darjeeling hills, India.*IOSR Journal of Pharmacy*. 2(6): 54-60.

Sharma BC, Kalikotay S and Rai B (2012).Assessment of antimicrobial activity of extracts of few common lichens of Darjeeling hills. *Ind J Fund Appl Life Sci*. 2:120-126.

Sharma J, Gupta SS, Kumar BP, Upreti DK, Khare R and Rao CV (2014). Effect of *usnic* acid and *Cladonia furcata* extract on gastroesophageal reflux disease in rat. *Intern J Exp Pharm*. 4(1):55-60.

Shendge AK, Basu T, Sinha GP and Mandal N (2017). Assessment of the antioxidant activity and phytochemical analysis of a lichen, *Everniastrum cirrhatum* (fr.) hale ex. sipman. *World journal of pharmacy and pharmaceutical sciences*. 6(9):1440-1464.

Shukla AC, Pandey KP, Dutta S, Shahi SK, Upreti DK and Dikshit A (2003).Antifungal activity of lichens *Ramalina* sp. and *Stereocaulon* sp. against some storage fungi.*Univ. Allahabad Studies* (New Millennium Series). 2(1): 7-9.

Shukla AC, Chinlampianga M, Archana V, Anupam D and Upreti DK (2011).Efficacy and potency of lichens of Mizoram as antimycotic agents. *Indian Phytopathology*. 64(4):367-370.

Shukla AC, Pandey KP, Mishra RK, Dikshit A and Shukla N (2011).Broad spectrum antimycotic plant as a potential source of therapeutic agent. *Journal of natural products*. 4:42-50.

Shukla AC, Lalsangluaii F, Kumar A and Dikshit A (2013).In vitro antidermatophytic activity of *Lantana camara* L. against *Trichophyton mentagrophytes* and *T. rubrum. Current Discovery* 2(1):86-91.

Shukla AC, Lalsangluaii F, Singh B, Kumar A, Lalramnghinglova H and Dikshit A (2015).*Homalomena aromatica*: an ethnomedicinal plant can be a potential source of antimicrobial drug development. *European Journal of Environmental Ecology*. 2(2):96-104.

Shukla P, Upreti DK, Tewari LM (2014). Lichen genus *Usnea* (Parmeliaceae, Ascomycota) in Uttarakhand. *India. Current Research in Environment and Applied Mycology*. 4(2):188-201.

Shukla V, Upreti DK and Bajpai R (2014).Lichens to biomonitor the environment. Springer India.

Singh A (1980). Lichenology in Indian subcontinent, pp.1966-1977.

Singh BN, Pandey GP, Jadaun V, Singh S, Bajpai R, Nayaka S, Naqvi AH, Rawat AKS, Upreti DK and Singh BR (2015). Development and characterization of a novel Swarna-basedherbo-metallic colloidal nano-formulation-inhibitor of *Streptococcus mutans* quarum sensing. *RSC Adv*. 5:5809-5822.

Singh KP (1981). Michrolichens from Manipur, India.*Geophytology*.11:242–256

Singh KP and Sinha GP (2010). Indian lichens: annotated checklist. Botanical Survey of India, Kolkata.

Singh KP, Sigh P and Sinha GP (2018). Lichen diversity in the Eastern Himalaya biodiversity hotspot region, India. *Cryptogam Biodiversity and Assessment*, 71-114.

Singh P and Singh K (2014). *Cyphelium inquinans* (Sm.) Trev.(lichenized: Ascomycota): a new record for India. Check List, 10(1): 178–179.

Singh P and Singh KP (2014). Two new species of *Graphis* (Ascomycota: Ostropales: Graphidaceae), from the Indo-Burma biodiversity hotspot. *Mycosphere*. 5(4):504-509.

Singh P and Singh KP (2015). Additional lichen records of Graphidaceae for Manipur, Meghalaya and Nagaland, North-East India. *Geophytology*. 45(2):181-194.

Singh P and Singh KP (2015). Additional lichen records of Graphidaceae for Manipur, Meghalaya and Nagaland, North-East India. *Geophytology*. 45(2):181-194.

Singh P and Singh KP (2016). New distribution records of lichens for the state of Meghalaya, North-East India. *Indian Journal of Forestry*. 39(3):245-254.

Singh P and Singh KP (2016). New distribution records of lichens for the state of Meghalaya, North-East India. *Indian Journal of Forestry*. 39(3):245-254.

Singh P, Singh KP and Bhatt AB (2013). New species and new records of thelotremoidGraphidaceae (lichenized Ascomycota) from Arunachal Pradesh (India). *Taiwania*. 58(4):246-250.

Sinha GP and Singh KP (1986).Three new records of foliose lichens from Nagaland (India). *Current Science*. 55(14):661-662.

Sinha GP and Singh KP (1987). Foliicolous lichens from Nagaland, India. *Geophytology*. 17(2):174-185.

Sinha GP, Nayaka S and Joseph S (2018).Additions to the checklist of Indian lichens after 2010. *Cryptogam biodiversity and assessment*. pp.197-206

Sinha GP, Ram TAM (2011). Lichen diversity in Sikkim. Biodiversity of Sikkim. Exploring and Conserving a Global Hotspot, pp 13-28.

Sinha SN (2011). Evaluation of antibacterial activity of some lichen from ravangla, sikkim, India. *International Journal of Pharma and Bio Sciences*. 2(4):23-28.

Sipman HJ and Aptroot A (2001). Where are the missing lichens?.*Mycological Research*. 105(12):1433-1439.

Sistani Karampour N, Arzi A, Rezaie A, Pashmforoosh M and Kordi F (2019).Gastroprotective Effect of *Zingerone* on Ethanol-Induced Gastric Ulcers in Rats. *Medicina*. 55(3):64.

Slinkard K and Singleton VL (1977). Total phenol analysis: automation and comparison with manual methods. *American journal of enology and viticulture*. 28(1):49-55.

Smitha KC and Garampalli RH (2016). Evaluation of phytochemicals and in vitro antioxidant activity *of Ramalina pacifica* and *Roccella montagnei*. *Journal of Pharmacognosy and Phytochemistry*. 5(2): 270.

Søchting U (1999). Lichens of Bhutan: biodiversity and use. University of Copenhagen, Botanical Institute, Department of Mycology.

Swathi D, Suchitha Y, Kekuda PTR, Venugopal TM, Vinayaka KS, Mallikarjun N, and Raghavendra HL (2010). Antimicrobial, anthelmintic and insecticidal activity of a macrolichen *Everniastrum cirrhatum* (Fr.) Hale. *International Journal of Drug Development and Research*. 2(4):780-789.

Thangjam NM, Kumar A, Shukla AC and Upreti DK (2019).Diversity and Distribution of Lichens in Murlen National Park of Mizoram, India. *Environment and Ecology*.37(3): 664–672.

Thangjam NM, Kumar A, Shukla AC, Shukla N and Singh CB (2019). Antifertility studies of *Phologacanthus Thyrsiflorus* nees: A potential ethno-medicinal plant of north east India. *The Journal of Indian Botanical Society*. 98(3and4):207-211.

Timbreza LP, Delos Reyes JL, Flores CH, Perez RJLA, Stockel MAS and Santiago, KAA (2017). Antibacterial activities of the lichen *Ramalina* and *Usnea* collected from Mt. Banoi, Batangas and Dahilayan, Bukidnon, against multi-drug resistant (MDR) bacteria. *Austrian Journal of Mycology*. 26:27-42.

Tiwari P, Rai H, Upreti DK, Trivedi S and Shukla P (2011).Assessment of antifungal activity of some Himalayan foliose lichens against plant pathogenic fungi. *American Journal of Plant Sciences*. 2(6):841.

Tomovic J, Kosanic M, Rankovic B, Vasiljevic P, Najman STEVO and Manojlovic, N (2019). Phytochemical analysis and biological activity of extracts of lichen *physcia semipinnata*: as a new source of pharmacologically active compounds. *Farmacia*. 67(2): 346-353.

Türk AÖ, Yılmaz M, Kıvanç M and Türk H (2003).The antimicrobial activity of extracts of the lichen *Cetraria aculeata* and its protolichesterinic acid constituent. *Zeitschriftfür Naturforschung C*. 58(11-12):850-854.

Upreti DK (1990). Lichen genus *Pyrenula* in India-1-*Pyrenula subducta* spore type. *Journal of the Hattori Botanical Laboratory*.68: 269-278.

Upreti DK (1991). Lichen genus *Pyrenula* from India: The species with spores of *Pyrenula brunnea* type. *Bulletin de la Société Botanique de France. Lettres Botaniques.* 138(3):241-247.

Upreti DK (1995). Loss of diversity in Indian lichen flora. *Envrionmental Conserv*.22:362- 363.

Upreti DK, Divakar PK and Nayaka S (2005).Commercial and ethnic use of lichens in India. *Economic Botany*. *59*(3):269.

Upreti DK and Chatterjee S (2007). Significance of lichens and their secondary metabolites: A review. In Fungi multifaceted microbes (Eds. Ganguli B.N. and Deshmukh, S.K.)Anamaya Publishers, New Delhi. pp. 169-188.

Upreti DK and Nayaka S (2008) Need for creation of lichen gardens and sanctuaries in India. *Current science.* 94(8):976-978.

Upreti DK, Debnath R, Uppadhyay V and Rout J (2015). Diversity and distribution of lichens in north and west districts of Tripura. *Phytotaxonomy.*14: 122–129.

Vattem DA, Lin YT, Labbe RG and Shetty K (2004).Phenolic antioxidant mobilization in cranberry pomace by solid-state bioprocessing using food grade fungus Lentinus edodes and effect on antimicrobial activity against select food borne pathogens. *Innovative food science & emerging technologies*. 5(1):81-91.

Walker FJ, James PW (1980). A revised guide to microchemical techniques for the identification of lichen products. British Lichen Society, London.

Wayne PA (2002).Reference method for broth dilution antifungal susceptibility testing of yeasts, approved standard. CLSI document M27-A2.

Web1:http://www.biologydiscussion.com/lichens-2/lichens-classification-nature and-physiology-plants/53722

Web2:http://www.biologydiscussion.com/notes/lichens/lichens-meaning characteristics-and-classfication/46549

Web3:http://courses.washington.edu/medch401/pdf_text/401_06_VI_Antifungal.pdf

Web4: https://mizoram.gov.in/page/reiek

Web5:https://shodhganga.inflibnet.ac.in/bitstream/10603/1207/11/11_chapter%203.pdf

White PA, Oliveira R, Oliveira AP, Serafini MR, Araújo AA, Gelain DP and Santos MR (2014). Antioxidant activity and mechanisms of action of natural compounds isolated from lichens: a systematic review. *Molecules*. 19(9):14496-14527.

Yau S, Abdulazeez MA, Anigo KM and Garba A (2017). Gastro-protective effect of Ziziphus abyssinica root extracts in ethanol-induced acute ulcer in Wistar rats. *Journal of Acute Disease*. *6*(2):62.

Yılmaz M, Türk AÖ, Tay T, Kıvanç M (2004). The Antimicrobial Activity of Extracts of the Lichen *Cladonia foliacea* and its (-)-Usnic Acid, Atranorin, and Fumarprotocetraric Acid Constituents. *Zeitschriftfür Naturforschung C*. 59(3-4):249-254.

Zhao WH, Hu ZQ, Okubo S, Hara Y and Shimamura T (2001).Mechanism of synergy between epigallocatechin gallate and β-lactams against methicillin-resistant *Staphylococcus aureus*. *Antimicrobial agents and chemotherapy*. 45(6):1737-1742.

PARTICULARS OF THE CANDIDATE

NAME OF THE CANDIDATE	:	NURPEN MEITEI THANGJAM
DEGREE	:	Ph.D.
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EXTENSION(IFANY)	:	NO

Head

Department of Horticulture Aromatic and Medicinal Plants

Head
बागवानी सुगन्धित एवं औषधीय पौधे विभाग
Dept. of Horticulture, Aromatic & Medicinal Plants
मिजोरम विश्वविद्यालय
Mizoram University

BRIEF BIODATA OF THE CANDIDATE

Name **Nurpen Meitei Thangjam**

Address Department of Horticulture Aromatics and Medicinal Plants
School of Earth Sciences & NRM Mizoram University,
Tanhril, Aizawl-796 004

Mobile **+919862702393**

E-mail takeama_2004@yahoo.com

Educational Qualifications

Degree	Institution	Year
Ph.D.	Mizoram University, Aizawl	2020
M.Pharm.	Dr.MGR Medical University,Chennai	2014
B.Pharm.	Dr.MGR Medical University,Chennai	2012

Area of Interest/Specialization

In-vitro and *In- vivo* Pharmacological studies, Microbial, Lichenologist, Ethnomedicine.

Publications Profile

Number of publications: **06**

ABSTRACT

PHARMACOLOGICAL STUDIES OF SELECTED LICHENS OF MIZORAM

By

NURPEN MEITEI THANGJAM

Department of Horticulture Aromatic and Medicinal Plants

Under the Supervisor

Dr. AWADHESH KUMAR

Submitted
In partial fulfillment of the requirement of the Degree of Doctor of Philosophy in Department of Horticulture Aromatic and Medicinal Plants of Mizoram University, Aizawl

ABSTRACT

Lichens are considered as a constant and self-sustaining relationship between fungi as mycobionts and the algal partners as photobionts. As the mycobiont is sole in the symbiotic relationship and it is typically controls the relationship (Ranković and Kosanić, 2015). Lichens are widely distributed in all the geographical region of the world. The total amount of lichens species was reported that number of accepted species is 19,387 in 995 genera, 115 families. (Lücking et al., 2017). Singha et al., (2018) reported the addition of 411 species to the list of Annotated Checklist 2010; therefore the total number of lichens recorded from India became 2714 species. In the context of eastern Himalayas; Singh et al., (2018) reported 1047 species Further, Logesh et al., (2017) reported 159 species of lichens in Mizoram including Thangjam et al., (2019).

Lichens are known for its unique secondary metabolites, it can synthesize a great variety of metabolites (Richardson, 1988; Lawrey, 1989; Elix, 1996). Lichens were believed that it is widely used in different traditional system of medicines (TSM) such as Ayurveda, Chinese medicine (TCM), Homeopathic and Unani for the treatment of various ailments (Saklani and Upreti, 1992). They play a very important role in the ancient and traditional system of medicinal practices. Lichens are also used in different other purpose *viz.* food, fodder, spices, dyes, cosmetics, perfumery. Lichens as whole and its identified metabolites are also having numerous biological activities as antimicrobial, antiprotozoal, antiviral, antiproliferative, anti-inflammatory, analgesic, antipyretic, antitermite, antioxidant, cytotoxic, enzyme inhibitory, insecticidal, wound healing, antitumor as well as enzyme inhibitory (Yılmaz et al., 2004, Kosanić et al., 2013, Rajan et al., 2016).

Despite of all this, the use of lichens and their products in medicine still hold a considerable interest as alternative medicine to cure various diseases in different parts of the world. More than 50% lichen species known from the world exhibited presence of peculiar antibiotic substances. Thus, there is a lot of scope for carrying out such studies in India and particularly in the state of Mizoram where lichen grows luxuriantly. Therefore, the main target of this research purpose is to find out the antifungal effects to

reinforce bioprospecting of lichens as herbal medicine. The first phase of the experiment was involved the collection of the lichen's species and their identification, preparation of the extracts and its phytochemical properties followed by determination of antioxidants, antifungal activity against the common pathogenic fungi; In addition to this, extract was also evaluated for its gastroprotective activity.

The present study documented a total of 78 species under 30 genus and 19 families from the 3 different studies sites of various districts of Mizoram. Genus *parmotrema* is one of the most dominated in the region with 15 species followed by *Heteroderimia* with 7 species. Among the families, Parmeliaceae was the most dominant and it was growing luxuriantly with 30 species followed by Graphidaceae and Physciaceae with 10 and 8 species respectively. In addition to previous findings, the investigation also enumerates 42 species from a small region of National Park, 20 species from a tourist spot and 16 species from a university campus, which clearly indicates the richness of lichens diversity in state. Moreover, the new record of *Pyrenula* species (*Pyrenula dissimulans*) also indicated the potential of this region; and it inviting the researchers to come forward for exploration of lichens.

Apart from taxonomic studies, the preliminary phytochemical screening on selected some lichen species in three different solvents was performed to known the presence of secondary metabolites by which lichens species were evaluated for its medicinal properties. Most of the lichen's species showed the presence of secondary metabolites. All previous cited findings observations were also supported the findings of present study. Henceforth, it can say that the diverse range of secondary metabolites makes Lichen more important in field of Pharmacology as well as for the drug discovery. For this purpose, selected lichens were evaluated for antioxidants properties. The DPPH scavenging activity of methanol extract of *Usnea baileyi*, *Everniastrum cirhhatum, Parmotrema reticulatum* and *Ramalina conduplicans* has shown higher percentage in Radical scavenging activity from the range of 64 – 85 % and the acetone extract were slight lower in its scavenging activities with the range from 63–69 %; whereas, aqueous extracts were reported for least activity (60–64 %) in comparison to

other extracts (Table 4.6–4.10). In case of reducing power activities (Table 4.11–4.15); the same lichens species have shown a very strong reducing power and the activities were in between 0.61–0.94, while standard ascorbic acid showed the activity as 1.08. Moreover, the Superoxide radical scavenging activity of *Parmotrema latissimum, Parmotrema reticulatum,* and *Everniastrum cirrhatum* were shown higher by methanol extract with 71–75 percent of inhibition, while *Parmotrema saccatilobum* acetone extract have shown higher percentage of inhibition i.e., 74.69%. Likewise, present finding also the total phenol and total flavanoids contents of some selected lichens viz. *Parmotrema latissimum, Parmotrema reticulatum, Parmotrema saccatilobum, Everniastrum cirrhatum* had shown a very high content. Furthermore, antifungal studies also carried out in carried out in extraction of 6 selected lichens species like *Everniastrum cirrhatum, Parmotrema saccatilobum, Parmotrema reticulatum, Usnea baileyi, Parmotrema latissimum* and *Ramalina conduplicans* with three different solvents like acetone, aqueous and methanol and tested against 4 dermatophytic fungi viz. *Epidermophyton floccosum, Trichophyton mentagrophytes, Aspergillus flavus* and *Aspergillus fumigates.* Accordingly, the minimum inhibitory concentration of the acetone and aqueous extracts was observed higher in *Ramalina conduplicans* at 100µg/ml and *Parmotrema reticulatum* at 150µg/ml while the rest at 200µg/ml. similarly; in the methanol extract it was observed that *everniastrum cirrhatum* and *Parmotrema reticulatum* was effective the MIC at 150µg/ml while the other methanolic extract of lichen species at 200µg/ml. Further; in the acetone extracts, the zone of inhibition (ZIC) was found maximum inhibition against *Aspergillus fumigates*. While in aqueous extract, (ZIC) was found maximum inhibition against *Trichophyton mentagrophytes.* In the methanol extracts; (ZIC) was observed the highest inhibition against *Epidermophyton floccosum.*

Along with the invitro studies, invivo studies was also carried out on the methanolic extracts of *Usnea baileyi* of family Parmeliaceae for its gastroprotective activity, the gastric lesions were reduced significantly at the dose dependent manner with the oral administration of methanolic extract of *Usnea baileyi* at dose 50, 100 and

200 mg/kg body weight. In contrast to extract, standard drug Omeprazole was used as proton pump inhibitor has shown high activity.

Altogether, it is summarized that lichens are the key primary producers with important linkage to nutrient cycling and forest food chain, moreover, their genetic richness is also more accountable in protected forest area with respect to biodiversity bioprospection (Jovan 2008, Rout et al. 2010). The results also clearly indicate that the state has rich diversity of lichens and there is need of further intensive and extensive survey and documentation in different geographical parts of Mizoram to get a clear picture of lichen diversity in the state. The future lichen exploration in the state will not only bring out some other new lichen taxa to the science but also provide a base for carrying out environmental biomonitoring and other bioprospecting studies filed of lichenology.

Moreover, it is an urgent need to studies on different others fungi to bring out the effective properties of antimicrobial in lichens and need to isolate each and every compound present in the extract to find out the novel compound in the future. Further, some of these observations may help in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings. Additionally, conservation strategies must be developed for its sustainable harvesting and future researches.