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Lichens: A Resource Chest of Herbal Antimicrobial Compounds

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ABSTRACT: Lichens are the best suitable example of a self-supporting, ecologically obligate in nature and a composite organism consisting of a mycobiont and a phycobionts. The lichens can be broadly classified into Macrolichens and microlichens. Pharmacological and biochemical research on the lichen compounds have found special consideration by scientist over a period of time because of their high antimicrobial potential. Lichens synthesize numerous metabolites, the lichen substances, and these compounds are classified in following groups; *viz.*, aliphatic lichen substances, aromatic lichen substances and carbohydrates. Bioprospection, meaning the possible role in the biological world, has been the main focus of the researchers dealing with medicinal and aromatic plants and their biological evaluation. Lichens have thus proved to be a excellent source of antibacterial and antifungal compounds.

Keywords: Mycobiont, Macrolichens, Pharmacological, Antimicrobial, Bioprospection, etc.

I. INTRODUCTION

A. Lichens: A Composite Plant Group

Lichens are the best suitable example of a self-supporting, ecologically obligate in nature and a composite organism. They are a stable symbiotic association of distinct and dissimilar group of organism i.e. fungi and algae. In this association, the former is dominant in organization, while the photoautotrophic algal partner, either unicellular or filamentous is present extracellularly, and hence, is classified under Kingdom Fungi. Since 1983, the name of a lichen refers to its mycobiont (Voss *et al.,* 1983). About 21% of the total fungi present in nature, have ability to lichenize and establish symbiosis, and particularly, act as a mycobiont (Honegger, 1991). Hence, they form the largest mutualistic group among the fungal group, while only a few, about 40 genera have been known to be involved as photobiont in lichenization (Kirk *et al.,* 2008).

The lichens can be broadly classified into two types: **Macrolichens** (relatively larger forms and easily visible morphologically) consisting of the foliose, fruticose and squamulose forms; and **Microlichens** (crust like, attached to substratum, with microscopic morphology) consists of crustose disco-lichens, lirrilate, leprose forms, terricolous and folicolous types of lichens come under this category. The thalloid form of compact light-exposed vegetative bodies of lichens, constitute the most complex and aesthetically pleasing morphologies evolved by fungi during the past 600 million years (Yuan *et al.,* 2005).

Being the pioneering species in any ecological system, lichen help to create a suitable habitat and niche for other plants (such as bryophytes, pteridophytes etc.) in early successional stages to render growth in that proximity. Here, lichens occupy an epiphytic habitat on substrate trees and other plant parts, lead to excessive biomass about, several hundred kg/ha (Coxson & Nadkarni, 1995). From microscopic observations, lichens differentiate varieties of tissues including cortex (a tissue covering and protecting thallus and consisting of gathered and adhered hyphae), algal layer (a tissue in which algae in thallus is surrounded and supported by hyphae), medulla (a basic tissue of thallus consisting of loosely entangled hyphae) and rhizine (a tissue projecting on the under-side surface and sticking the thallus on a carrier), which are the structural characteristics of lichens (Yamamato *et al.,* 1990). Many lichens are highly extreme-tolerant which allows them to live as pioneers in the alpine zone and other cold environments. Life under these conditions correlates with the production of a variety of compound classes.

Pharmacological and biochemical research on the lichen compounds went through a phase of extraordinary development as stated in a review of the Culberson and Culberson (2001).

These giants are well known for establishing novel methods of chemical analysis of the lichen secondary compounds, documentation of the known secondary metabolites, their chemical profile, structure, formula and even their application (Culberson, 1972, 1974; Culberson and Kristinsson, 1970; Culberson 1963a, b; Culberson and Culberson, 1956; Culberson and Culberson, 1958; Brodo *et al.,* 2008). However, scope for advancement is present in science; as such the field of lichenology is also not aloof; as considerable changes have come over years and lots of molecular investigation have come apart to bring this field a long way.

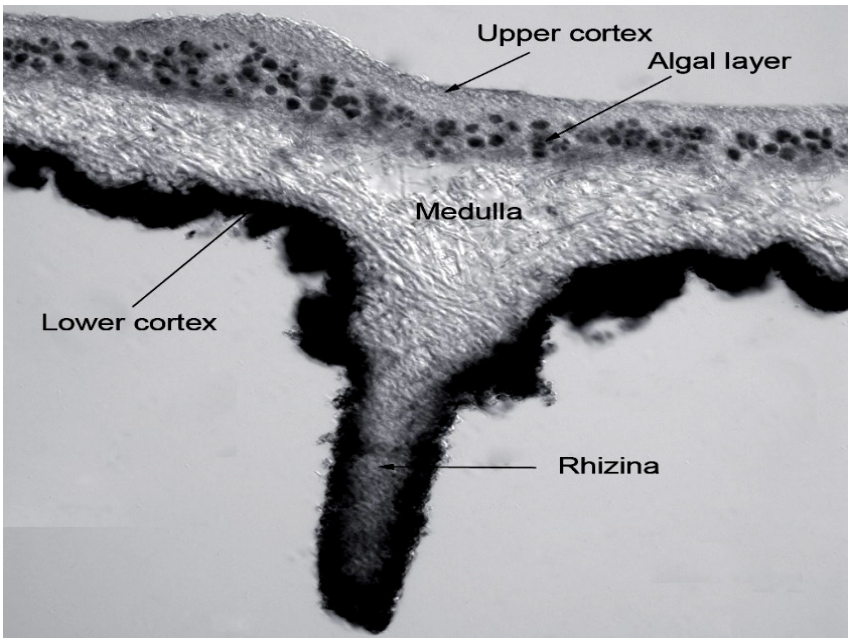

Fig. 1. Transverse section of a lichen thallus showing the hyphal upper and lower cortex and the medullary algal layer (Source: Zambare and Christopher (2012)).

A. Lichens secondary metabolites: Production and distribution

Lichens synthesize numerous metabolites, the "lichen substances," which comprise amino acid derivatives, sugar alcohols, aliphatic acids, macrocyclic lactones, mono-cyclic aromatic compounds, quinones, chromones, xanthhones, dibenzofuranes, depsides, depsidones, depsones, terpenoids, steroids, carotenoids and diphenyl ethers (Clix *et al.,* 1984; Fiedler *et al.,* 1986). Lichen compounds are classified in following groups by Asahina and Shibata (1971) and Dayan & Romagni (2001): (1) aliphatic lichen substances (including acids, zeorin compounds, polyhydric alcohols); (2) aromatic lichen substances (including pulvic acid derivatives, depsides, depsidones, quinones, xanthone derivatives, diphenyleneoxide derivatives, nitrogen containing compounds, triterpenes, tetronic acids); and (3) carbohydrates (polysaccharides).

The more complex secondary compounds mainly which are of diverse class of terpenoids, alkaloids, depsides, depsidones, anthraquinones, etc are produced by a different mechanism and are produced as a result of the various biochemical changes in the intermediates of the metabolic cycles of the lichen which serve as a starting fuel. There are three major cycles, which are followed for the production of these secondary metabolites:

(a) Sikhimic acid pathway

(b) Mevalonic acid pathway

(c) Acetate- polymalonate pathway.

The acetate- polymalonate pathway is the major pathway, through which majority of the secondary metabolites are produced (Culberson and Elix, 1989; Huneck, 2001).These include primarily the lichen acids, mainly the primary β- orcinol derivatives, depsides, tridepsides, tetradepsides, depsidones, benzofurans, aryls, usnic acid and derivatives, poylphenolics, xanthones, anthraquinones etc. The malonyl CoA and acetyl CoA serve as the precursor compounds. The Sikhimic acid is responsible for the production of pulvinic acid derivatives, variety of disaccharides, polysaccharides, etc from the precursors such as amino acids specially phenylalanine, polyols, which are produced as an intermediates in the primary metabolism.

The mevalonic acid pathway gives rise to the production of terpenoids including diterpenoids, triterpenoids, sesquiterpenoids, carotenoids, sterols, etc from the precursor compounds including mainly acetyl CoA. These secondary compounds give these lichens a very characteristic smell, taste and colour; which strengthen the base of chemotaxonomy. These compounds in lichen serve many purposes including self- protection through anti-herbivory (Lawry, 1989), anti-larval action (Emmerich *et al.,* 1993) or nematicidal action (Ahad *et al.,* 1991) and insecticidal action (Hesbacher *et al.,* 1995).

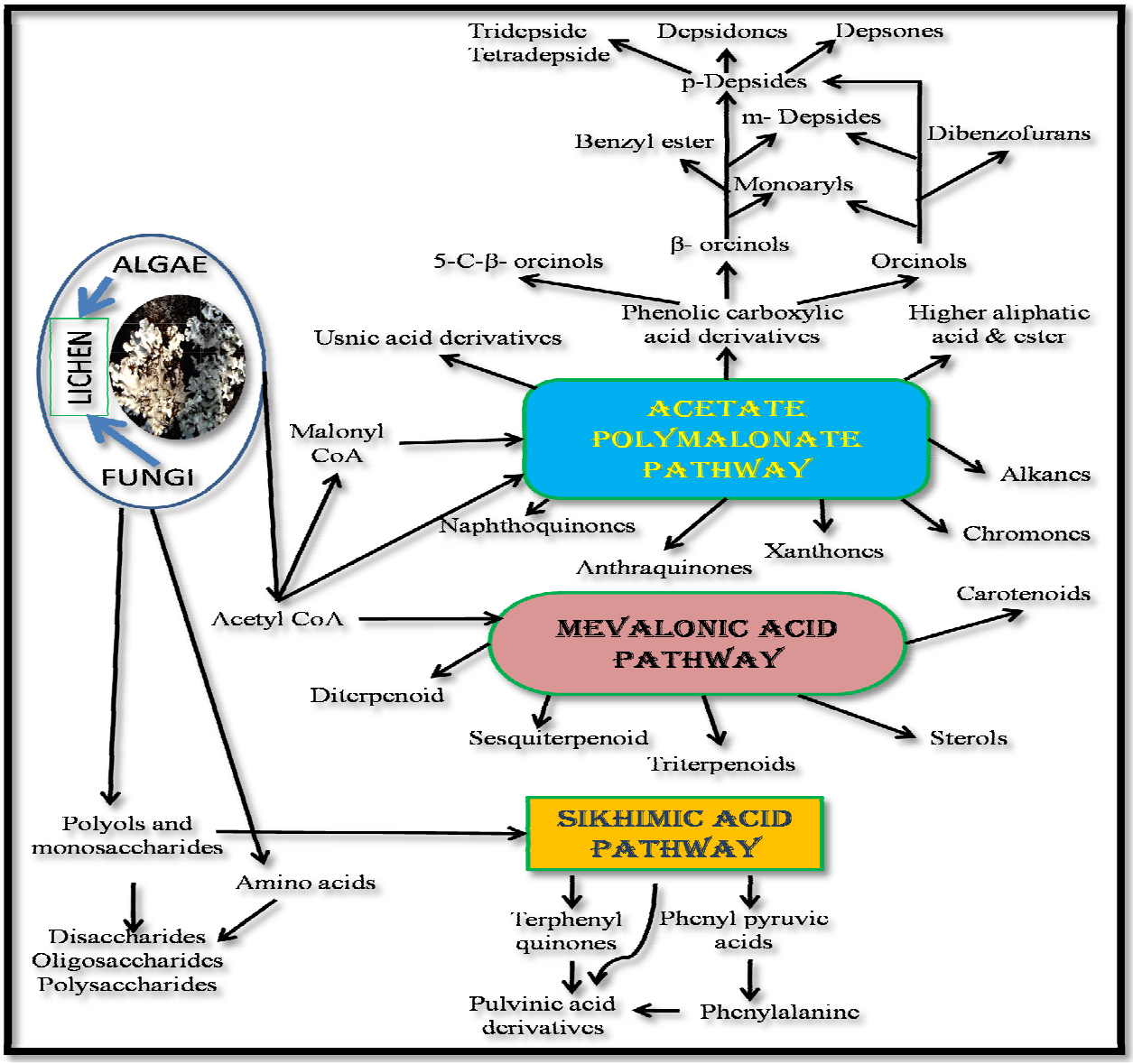

Fig. 2. Modified Secondary metabolite pathway and the resultant lichen compounds(Source: Nash, 2008; Stöcker-Wörgötter, 2008).

B. Bioprospective values of lichens with special reference to their Antimicrobial potential

Bioprospection, meaning the possible role in the biological world can be defined as harvesting of novel compounds for the various medicinal and pharmacological uses. The term is more commonly used by the commercial sector. The treasure chest of active secondary compounds has compelled the scientists to find and explore the lichens. The day to day increase in the resistivity of the pathogens towards the synthetic antibiotics and other important drugs has led to the emergence of herbal therapy; which may by costly at a time but is reliable and not at all harmful.

But the slow growing nature of lichens has been the reason for the major setback in exploring lichens which is known for more than 1000 secondary metabolites of which 80% are unique to lichens only and are not reported yet from any other natural source.

The lichens in the tropical and the sub- tropical regions of the country are exposed to some of the very challenging climatic conditions along with the various biotic and abiotic factors, which determine the growth of the thallus. These mainly include the rainfall, the bark chemistry, the temperature and the presence of the various other competing microorganisms in their niche; which may be sometimes pathogenic. Hence, it is much healthier chance that a unique array of secondary metabolites, which may play a role in their own defense mechanism, may be present in these lichens (Galloway, 1992). Lichens, especially the foliose and fruticose types have been well known for their lichen acids, which exhibit exceptionally good antibacterial and antifungal efficacy (Oksanen, 2006). Traditionally, *Cetraria islandica* (L.) was used to treat mild inflammation of the oral and pharyngeal mucosa, dyspepsia, and loss of appetite. In the European folk medicine, *Cetraria islandica* (L.) was used in cancer treatment (Chevallier, 1996). The lichen substances are a unique array of secondary metabolites including the lichen acids such as sekikaic acid, protocetraric acid, lecanoric acid, usnic acid etc. and their various anomeric, optical or isomeric derivatives and forms (Fiedler *et al.,* 1986). Various studies have limited their interest in lichen chemistry and focused on the identification and characterization of lichen compounds of various class and types from the natural thallus as well as the *in vitro* metabolized lichen compounds (Nash, 2008; Stöcker- Wörgötter, 2008; Culberson and Elix, 1989). These compounds are unique in their origin because of the composite nature of the thalli and the intermediates utilized in their synthesis. The lichen acids also are of great importance as they form the basis of the micro crystallization test and colour test for identification of lichens and thus are of taxonomic relevance in chemotaxonomy of lichens (Culberson, 1969; Fehrer *et al.,* 2008; Nelsen and Gargas, 2008; Nordin *et al.,* 2007).

Burkholder reported for the first time the presence of antibiotic substances in lichens (Burkholder *et al.,* 1944). Several lichen metabolites were found to be active against Gram-positive organisms (Lauterwein *et al.,* 1995). The anti-mycobacterial activity of lichen compounds was reported against non-tubercular species of *Mycobacterium* (Ingolfsdottir *et al.,* 1998).The well-known antibacterial topical drug in the market sold under the names of "USNO" and "EVOSIN" throughout the European countries has usnic acid as one of the major constituent (Dayan and Romagni, 2001).Usnic acid, a very active lichen compound is used in pharmaceutical preparations. Usnic acid and vulpinic acid (produced by mycobiont) are cell division regulators of autotrophic partner of lichen symbiosis-the photobiont (Backor *et al.,* 1998). Lichen extracts have cytotoxic activity in different degrees. The aqueous extract of *P. polydactyla* and the ethanol extract of the *R. farinacea* exhibited potent antibacterial activities (Karagöz *et al.,* 2009). Anthraquinones also constitute a major part of the secondary metabolites in lichens (Cohen and Towers, 1995; Cohen *et al.,* 1996); exhibiting antiviral activity against HIV. Hypericin, in particular is of great pharmaceutical importance because of its dramatic antiretroviral activity (Lavie *et al.,* 1995).

Medicinal values of Indian lichen have a long historical backdrop. A component of spices as well as folklore drug *charilla*; *Parmelia* sp. and *Parmelia nepalense* have their usage as aphrodisiac treatment of toothache and sore throat (Lal & Upreti, 1995; Kumar & Upreti, 2001; Kumar *et al.,* 1996). While *Thamnolia vermicularis* (Schwartz) Ach. (Icmadophilaceae) from Western Himalayas, is used as antiseptic (Negi & Kareem, 1996); and *Heterodermia diademata* (Talyor) D.D. Awas., (Physciaceae) was used for cuts and wounds (Saklani & Upreti, 1992). Many reviews have discussed the pharmaceutical potential and biological activities of lichen substances (Huneck, 1999; Muller, 2001; Yamamoto, 2000; Boustie & Grube, 2005). The medicinal use of lichens can be traced back to the 18th dynasty (1700–1800 BC) when *Evernia furfuracea* (L.) Mann or (Parmeliaceae) was first used as a drug (Launert, 1981). Some lichens were claimed to be good for coughs, jaundice, rabies and restoring lost hair. On an overall basis, lichens can be regarded source of multifold utility herbal compound (Fig. 3).

C. Lichen acids as a potential bactericidal compound

Pathogenic microbes are major threatening cause to human health and have currently been the most prevalent cause for institutional health care settings. The growing antibiotic resistance is an associated concern (Babita *et al.,* 2008). The rapidly increasing population has also increased the pollution at an alarming rate; thus several fatal bacterial, viral, protozoal, nematodal and fungal diseases have emerged which are somehow related to water, either for their dispersal or their growth.

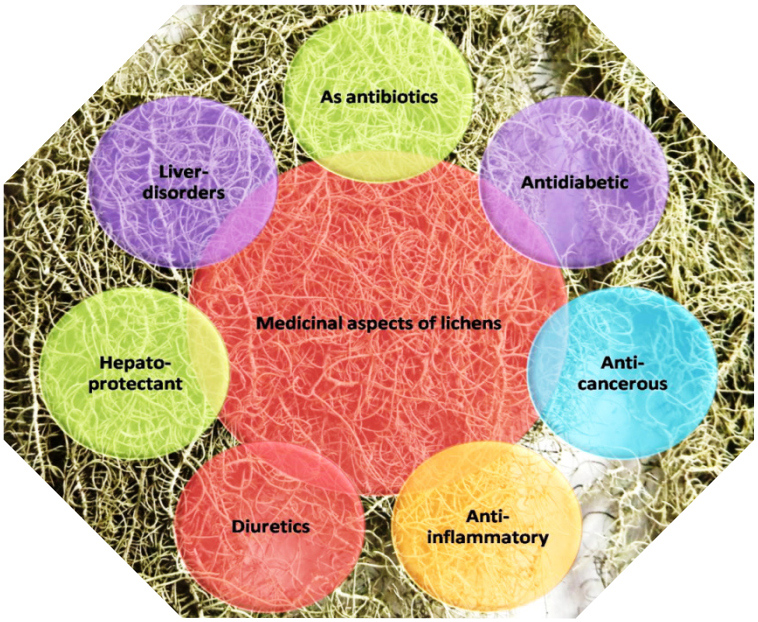

Fig. 3. Various aspects of medicinal properties of lichens.

Water borne diseases, in particular, diarrhoeal diseases alone cause 2.2 millions death per year of the total water related deaths counting for 3.4 millions (WHO, 2003). This death toll has only increased in the coming years and will move on to increase least effective measures for its prevention are taken on a global scale. Even our Indian Constitution gives us the Right to clean drinking water under the Article 21 and Article 39 (a) and 39 (b).The tropics and subtropical regions have the most favorable climatic conditions which suit for the spread of epidemics caused due to such diseases. Water borne bacterial diseases are amongst the most favorable cause in the tropical countries for disease epidemics.

The main physico-chemical properties of water which determine its purity are turbidity (clarity), taste, odour, colour and pH. Water being a universal solvent and a key component of human body is the most suitable for disease dissemination. Biologically the water quality is determined by its BOD, COD, coliform count etc. Presence of the large amount of organic pollutant in the local vicinity has led to the emergence of water- borne bacterial diseases. These include some of the most fatal diseases such as fecal dysentery, diarrhoea, cholera, typhoid fever etc. caused by mainly *Escherichia coli, Salmonella typhimurium, Shigella dysenteriae, Vibrio cholera*e, *Klebsiella pneumoniae, Bacillus subtilis, Streptococcus aureus.* The current disturbing element is the multi-drug resistant and biofilm forming bacterial population, which is day by emerging as big problem for the medical industry as well as serving as death cause for the patients.

Regarding the bactericidal effects of lichen metabolites, they have mostly tested upon *Bacillus, Pseudomonas, E. coli, Staphylococcus aureus, Kleibsiella, Candida, Salmonella*, *Yersinia* and *Proteus* sp. (Yilmaz *et al.,* 2004; Karagöz *et al.,* 2009; Martins *et al.,* 2010; Manojlovic *et al.,* 2010; Ranković *et al.,* 2010; Santiago *et al.,* 2010; Swathi *et al.,* 2010; Zambare *et al.,* 2010). Rankovic *et al.,* 2008; studied the antibacterial action of the lichen substances *viz.,* physodic acid, usnic acid, atranorin and gyrophoric acid derived from the natural thalli of lichens *Hypogymnia physodes, Parmelia caperata, Physcia aipolia* and *Umbilicaria polyphylla* against gram positive and gram negative bacteria. *Parmelia caperata* derived usnic acid and atranorin isolated from *Physcia aipolia* showed excellent activity comparably better than the synthetic standards i.e., Streptomycin and Ketoconazole; 0.0037 mg/ml and 0.031 mg/ml against *Klebsiella pneumoniae*; a water borne bacterial pathogen, 0.0075 mg/ml and 0.031 mg/ml against *Bacillus mycoides* respectively. Antibacterial potential of parmelloid lichens is evident from studies on acetone extract of *Hypotrachyna cirrhata* and *Flavoparmelia caperata*, which was found to be effective against *Vibrio cholerae, Klebsiella pneumoniae*and *Salmonella typhimurium* (Pathak *et al.,* 2015). In a study on bactericidal activity of some lichen extracts along with its active secondary compounds concluded, high bactericidal activity of lichens in order; *Parmelia nilgherrensis* >*Parmelia sancti-angelii* > *Cladonia ochrochlora* (Verma*et al.,* 2011).

Of the entire active secondary compound; atranorin, α-collatolicacid and hypoprotocetraric acid was found to be most active for all the bacterial strains tested whereas alectoronic acid, fumaroprotocetraric acid and protocetraric acid were found active but with high MIC range (Yilmaz *et al.,* 2004).

D. Lichen acids as a potential fungicidal compound

In day to day life, not only enterobacterial diseases are prevalent, but a variety of fungal infections come way. Skin being, one of the five senses of our body, and the foremost protective covering, is exposed to a variety of pathogenic microflora, including both bacteria and fungi. The chances of infection aggravate when there is any wound or skin is damaged. Fungal cutaneous generally produce boggy nodular swelling called kerion (Dias *et al.,* 2013). A group of filamentous keratinophilic fungi, *viz*., *Trichophyton*, *Microsporum*, and *Epidermophyton*, commonly known as dermatophytes, cause prevalent skin infection called as cutaneous mycoses (Peres, *et al.,* 2010). Dermatophytes produce specific enzymes which catalyze the fungal penetration into the host keratin rich tissues (O'Sullivan *et al.,* 1971; Padhye *et al.,* 1999). These pathogenic fungi associated with dermatophytoses, are a class of anamorphic fungus, globally are responsible for diseases such as tinea capitis, tinea corporis, tinea inguinalis, tinea manuum, tinea unguium, tinea faciei and tinea pedis (Wang*et al.,*2006). Reports coming from different parts of the world, have time to time ascertained the occurrence of dermatophytes. Individuals from 16 European countries, involved in a study, shown 35%–40% effected from infection of the foot (tinea pedis) caused by dermatophytes (Burzykowski, *et al.,* 2003).The World Health Organization estimated that dermatophytes affect about 25% of the world population (Peres, *et al.,* 2010). Apart from wide prevalence, the dermatophytes have exhibited resistance against griseofulvin, terbinafine, and fluconazole (Peres, *et al.,* 2010; Stephenson, *et al.,* 1997; Wingfield, *et al.,* 2004; Smith, *et al.,* 1986; Orozco, *et al.,* 1998).

Lichen acids have been found to be a good alternative for synthetic class of fungicides, especially the azoles, which are associated with high reoccurrence rate of the disease, even after the completion of the course medication. Report on extract of *Usnea orientalis* exhibited favorable antidermatophytic activity against tested pathogens, *E. floccosum*, whereas *T. mentagrophytes*, was found least susceptible (Pathak *et al.,* 2016). Other report on the acetone and methanol extracts of *Lasallia pustulata* (Umbilicariaceae), *Parmelia sulcata* and *Umbilicaria crustulosa* manifested a very selective antifungal activity (Ranković *et al.,* 2007). Lichen *Cladia aggregata* (Swartz) Nyl. has been found active against commensal yeast, *Malassezia globosa, M. furfur* and *M. restricta*; which are responsible for causing PV (Ptyriasis Versicolor) and dandruff (Pandey *et al.,* 2013). A number of higher plants have been reported effective against *Malassezia* (Dikshit *et al.,* 2012); but none have comparable potentiality with lichens against *Malassezia*. Established results on the antifungal activity of *Everniastrum cirrhatum* with minimum fungicidal concentration (MFC) of as low as 60 µL/ ml against human pathogenic fungi (dermatophytes) *viz*., *Epidermophyton floccosum, Microsporum gypseum, M. canis, M. audounii, Trichophyton rubrum, T. mentagrophytes, T. violaceum* and *T. tonsurance* have also been reported in the past (Shahi *et al.,* 2000). *Heterodermia leucomelos* was also found effective against human as well as plant pathogenic fungi (Shahi *et al.,* 2001). Broad spectrum antifungal properties at 80 µL/ ml were evident in the aqueous extract of *Parmelia cirrhatum* against some human and plant pathogens (Shahi *et al.,* 2003).

The phenolic compounds and their derivatives in lichen have been proved to be detrimental for pathogenic microbial fauna. These substances generally acidify the microbial cell wall and consequently, cause cytoplasm membrane rupture, inactivate or immobilize the enzymes, and interfere with physiological functions such as electrons transport, oxidative phosphorylation etc. (Randhir *et al.,* 2004; Vattem *et al.,* 2004 and Mueller *et al.,* 2001). In a study focusing on a large number of lichens and their cultured symbionts for their antibacterial and antioxidant activity, came to a conclusion that mycobiont and photobiont culture of *U. ghattensis* and *A. awasthii* have high antioxidative and antibacterial potential. The antioxidant activity was found to be 87% (ILP), 73% (FRS) for and 56% (ILP), 58% (FRS) for Both *U. ghattensis* and *A. awasthii* showed excellent antibacterial activity against *S. aureus, B. licheniformis, B. subtilis* and *B. megaterium* (Behera *et al.,* 2008). Dharmadhikari *et al.,* 2010; in their work on the foliose lichen *Parmelinella simplicolor* isolated the mycobiont and confirmed the cultured thalli by SEM and also confirmed the presence of atranorin and salzinic acid by HPLC. They also worked upon the antibacterial activity of the natural thalli as well as cultured mycobiont and were found active against all the tested pathogens except cultured mycobiont extract was found inactive against *Streptococcus faecalis*.

Table 1: Summarized data reflecting various dimensions of antimicrobial potential of lichens.

S. no.	Lichen	Antimicrobial (Antibacterial- AB and Antifungal- AF) activity	Reference
1.	*Hypotrachyna cirrhatum*, (AB)	*Escherichia coli, Klebsiella pneumoniae*	Pathak *et al.,* 2015
2.	*U. ghattensis* and *A. awasthii* (AB)	*S. aureus, B. licheniformis, B. subtilis* and *B. megaterium*	Behera *et al.,* 2008
3.	*Parmelinella simplicolor* (AB)	*Streptococcus faecalis*	Dharmadhikari *et al.,* 2010
4.	*Protousnea poeppigii* (AB)	*Leishmania amazonensis, L. brasiliensis, L. infantum*	Schmeda-Hirschmann *et al.,* 2008
5.	*Flavocetraria nivalis Flavocetraria cucculata* (AB)	*Staphyloccocus aureus* and *Pseudomonas aeruginosa* biofilms	Francolini *et al.,* 2004
6.	Antarctic lichen species (AB)	*Staphyloccocus aureus* and *Bacillus subtilis*	Paudel *et al*. 2008
7.	*Xanthoria parietina* (AB)	*Bacillus subtilis*	Ivanova *et al.,* 2000
8.	*Parmelia cirrhatum* (AF)	Plant pathogenic fungi	Shahi *et al.,* 2003
9.	*Ramalina conduplicans, Ramalina sinensis, Ramalina sp., Umbilicaria proboscidea* (AF)	*Botrytis cinerae, Sclerotium cepivorum, Rhizoctonia solani, Phythium sp., Botryosphaeria dothidea*	Oh *et al.,* 2006
10.	*Heterodermia* sp. (AF)	*Botrytis cinerea, Cercospora kikuchii,Collectotricum coccodes, Collectotricum gloeosporioides*	Hur *et al*., 2003
11.	*Cladia aggregata* (AF)	*Malassezia globosa* and *M. furfur*	Pandey *et al.,* 2013
12.	*Everniastrum cirrhatum* (AF)	*Epidermophyton floccosum*	Shahi *et al.,* 2000
13.	*Heterodermia leucomelos (AF)*	*Trichophyton rubrum, Microsporum gypseum, Aspergillus* sp.	Shahi *et al.,* 2001
14.	*Usnea orientalis* (AF)	*Epidermophyton floccosum*	Pathak *et al.,* 2016
15.	*Lasallia pustulata*, *Parmelia sulcata* and *Umbilicaria crustulosa* (AF)	Plant pathogenic fungi	Ranković *et al.,* 2007
16.	*Cladonia furcata, Cladonia pyxidata* (AF)	*Collectotrichum acutatum, C. coccodes, C. gloeosporioides*	Jeon *et al.,* 2009
17.	*Usnea subfloridans* NS (AF)	*Aspergillus niger*	Ekong *et al.,* 2008
18.	*Cladonia furcata*(AF)	*Candida albicans*	Ranković & Mišić, 2008
19.	*Parmotrema tinctorum* (AF)	*Cladosporium sphaerospermum*	Gomes *et al.,* 2002
20.	*Parmelia furfuracea* (AF)	*Fusarium moniliforme*	*Khanuja et al., 2007*

II. CONCLUSION

From the above examination and analysis of the facts and figures enumerated about the antimicrobial activity of the lichens with special reference to their antibacterial and antifungal activity; it can be concluded that lichens, though being a rare plant enigma, present an excellent example of herbal antimicrobials. They have vast potential vested in them, on which the pharmacy industry need to focus and work upon. The dual nature of the organism gives it a uniqueness in its physiological characters. The secondary metabolites, thus produced in them, are found only in lichens, and not any other group of plant. Thorough examination, in vitro screening, in vivo trials on animal models, double blind topical testing as well as organoleptic analysis can definitely lead to promising results for the pharmacological world.

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REFERENCES

[1]. Asahina, Y, Shibata, S. (1971). *Chemistry of Lichen Substances*. Tokyo, Japan: Japan Society for the Promotion of Science.

[2]. Babita, P, Hari, DB, Jin, SL, Soon, GH, Hyun, WS, Joung, HY (2008). Antibacterial potential of Antarctic lichens against human pathogenic Gram-positive bacteria. *Phytother Res*; **22**: 1269–1271.

[3]. Backor M, Hudák J, Repcák M, Ziegler M, and Backorová M (1998). The biological role of secondary metabolites from lichens. *Lichenologist;* **30**: 577-582.
[4]. Behera BC, Verma N, Sonone A, and Makhija U (2008). Antioxidant and antibacterial properties of some cultured lichens. *Bio-resourceTechnology*; **99**: 776–784.
[5]. Boustie, J, Grube, M. (2005). Lichens—a promising source of bioactive secondary metabolites. *Plant Genet Resour*, **3**, 273–287.
[6]. Brodo, IM, Culberson, WL, and Culberson, CF (2008). *Haematomma* (Lecanoraceae) in North and Central America, including the West Indies. *Bryologist*; **111:** 363- 423.
[7]. Burkholder PR, Evans AW, McVeigh I, and Thornton HK (1944). Antibiotic Activity of Lichens. *Proceedings on National Academic Science*, USA; **30**(9): 250-255.
[8]. Burzykowski, T, Molenberghs, G, Abeck, D, Haneke, E, Hay, R, Katsambas, A, Roseeuw, D, van de Kerkhof, P, van Aelst, R, Marynissen, G (2003). High prevalence of foot diseases in Europe: Results of the Achilles Project. *Mycoses;* **46**, 496–505.
[9]. Chevallier A. (1996). The Encyclopedia of Medicinal Plants. Dorling, Kindersley. London.
[10]. Clix JA, Whitton AA, and Sargent MV (1984). Recent progress in the chemistry of lichen substances. *Prog. Chem. Org. Nat. Prod.;* **45**: 207-211.
[11]. Cohen PA, and Towers GHN (1995). Anthraquinones and phenanthroperylenequinones from *Nephroma laevigatum. Journal of Natural Products*; **58**(4): 520- 526.
[12]. Cohen PA, Hudson JB, Towers GH. (1996). Antiviral activities of anthraquinones, bianthrones and hypericin derivatives from lichens. *Experientia*, **52**, 180–183.
[13]. Coxson DS, Nadkarni NM. (1995). Ecological roles of epiphytes in nutrient cycles of forest ecosystems. In: Lowman MD, Nadkarni NM, ed. *Forest Canopies*. London, Academic Press, pp. 495–543.
[14]. Culberson C. F. and Culberson W. L. (2001), Future directions in lichen chemistry. *Bryologist* **104**, 230–234.
[15]. Culberson CF (1963a). Sensitivities of some microchemical tests for usnic acid and atranorin. *Microchem. J.*; **7**: 153–159.
[16]. Culberson CF (1963b). The lichen substances of the genus *Evernia*. *Phytochemistry*; **2**: 335 – 340.
[17]. Culberson CF (1972). High-speed liquid chromatography of lichen extracts. *Bryologist*, **75**, 54- 62.
[18]. Culberson CF (1974). Conditions for the use of Merck silica gel 60 F254 plates in the standardized thin-layer chromatographic technique for lichen products. *J. Chromatogr.*; **97**: 107-108.
[19]. Culberson CF, and Elix JA (1989). Lichen substances. In: Methods in Plant Biochemistry, Vol. **1**, Plant Phenolics (Dey P. M. and Harborne J. B., eds.). Academic Press, London, pp. 509 – 535.
[20]. Culberson CF. and Kristinsson HD (1970). A standardized method for the identification of lichen products. *J. Chromatogr.*; **46**: 85 – 93.
[21]. Culberson WL (1969). The use of chemistry in the systematics of the lichens. *Taxon*; **18**: 152- 166.
[22]. Culberson WL, and Culberson CF (1956). The systematics of the *Parmelia dubia* group in North America. *Am. J. Bot*;. **43**: 678 – 687.
[23]. Culberson, WL (1958). The chemical strains of the lichen *Parmelia cetrarioides* Del. in North America. *Phyton*; **11**, 85–92.
[24]. Dayan JG and Romagni FE (2001). Structural diversity of lichen metabolites and their potential for use. In: Upadhyay, R.K (Ed.) *Advances in microbial toxin research and its biotechnological exploration.* Academic Publisher, New York; Pp: 151-169.
[25]. Dharmadhikari M, Jite PK, and Chettiar S (2010). Antimicrobial activity of extracts of the lichen *Parmelinella simplicolor* and its isolated mycobiont. *Asian J. Exp. Biol. Sci. Spl.*; Vol. : 54-58.
[26]. Dias, MFRG, Quaresma-Santos, MVP, Bernardes-Filho, F, Amorim, AGF, Schechtman, RC, Azulay, DR (2013). Update on therapy for superficial mycoses. *An. Bras. Dermatol.*; **88**, 764–774.
[27]. Dikshit A, Tiwari AK, Mishra RK, Kamran A, Pandey A, Kumar A, and Bajaj AK (2012). Botanicals for the management of dandruff. *Medicinal Plants*; **4**(2): 55-64.
[28]. Ekong US, Mgbii AI, Adikwu MU. (2008). Evaluation of the in vitro combination effect of colloidal silver concentrate on the antifungal activity of ethanolic extract of the lichen *Usnea subfloridans*. *Nigerian Annals Nat Sci*; **8**: 1–5.
[29]. Emmerich R, Geiz I, Lang OL, and Proksch P (1993). Toxicity and anti- feedant activity of lichen compounds against the polyphagous herbivorous insect *Spodoptera lilloralis*. *Phytochemistry*; 33: 1389- 1394.
[30]. Fehrer J, Slavikova-Bayerova S, and Orange A (2008). Large genetic divergence of new, morphologically similar species of sterile lichens from Europe (*Lepraria*, Stereocaulaceae, Ascomycota): concordance of DNA sequence data with secondary metabolites. *Cladistics*; **24**, 443 – 458.
[31]. Fiedler P, Gambaro V, Garbarino JA, and Quihot W (1986). Epiphorellic acids 1 and 2, two diaryl ethers from the lichen *Cornicularia epiphorella*. *Phytochemistry*; **25**: 461-465.
[32]. Francolini I, Norris P, Piozzi A et al (2004). Usnic acid, a natural antimicrobial agent able to inhibit bacterial biofilm and formation on polymer surfaces. *Antimicrob Agents Chemother*; **48**: 4360–4365.
[33]. Gargas A and Taylor JW (1992). Polymerase chain reaction (PCR) primers for amplifying and sequencing nuclear 18S rDNA from lichenized fungi. *Mycologia;* **84**: 589–92.
[34]. Gomes AT, Honda NK, Roese FM, Muzzi RM, Marques MR. (2002). Bioactive derivatives obtained from lecanoric acid, a constituent of the lichen *Parmotrema tinctorum* (Nyl.). Hale (Parmeliaceae). *Rev Bras Farmacogn*; **12**: 74–75.
[35]. Hesbacher S, Giez I, Embacher G, Fiedler K, Max W, Trawöger A, Türk R, Lange OL, and Proksch P (1995). Sequestration of lichen compounds by lichen- feeding members of the Arctidae (*Lepidoptera*). *Journal of Chemical Ecology*; **20**: 2079-2089.

[36]. Honegger R. (1991). Functional aspects of the lichen symbioses. Annu. Rev. Plant Physiol. *Plant Mol. Biol.*; **42**, 553–578.
[37]. Huneck S (1999). The signifi cance of lichens and their metabolites. *Naturwissenschaften* **86**, 559–570.
[38]. Huneck S (2001). New results on the chemistry of lichen substances. Fortschritte Chemie organischer Naturstoffe. *Progress in the Chemistry of Organic Natural Products*; **81**: Springer- Verlag, Wein, pp. 313.
[39]. Hur JS, Kim HJ, Lim KM, Koh YJ. (2003). Isolation, cultivation, and antifungal activity of a lichen-forming fungus. *Plant Pathol J*; **19**: 75–78.
[40]. Ingolfsdottir K, Chung GA, Skulason VG, Gissurarson SR, and Vilhelmsdottir M (1998). Antimycobacterial activity of lichen metabolites *in vitro*. *Europ J Pharm Sci;* 1998; **6**: 141-144.
[41]. Ivanova V, Schlegel R, Grafe U (2000) 2-Methoxy-4, 5, 7- trihydroxy-anthraquinone, a new lichen metabolite produced by *Xanthoria parietina*. *Pharmazie*; **55**: 785–786.
[42]. Jeon HS, Lökös L, Han KS, Ryu AA, Kim JA, Koh YJ, Hur JS. (2009). Isolation of lichen-forming fungi from Hungarian lichens and their antifungal activity against fungal pathogens of hot pepper anthracnose. *Plant Pathol J*; **25**: 38–46.
[43]. Karagöz A, Doğruöz N, Zeybek Z, Aslan A. (2009). Antibacterial activityof some lichen extracts. *J Med Plants Res*, **3**, 1034–1039.
[44]. Khanuja SPS, Tiruppadiripuliyur RSK, Gupta VK, Srivastava SK, Verma SC, Saikia D, Darokar MP, Shasany AK, Pal A. (2007). Antimicrobial and anticancer properties of methyl-betaorcinolcarboxylate from lichen (*Everniastrum cirrhatum*). *US Patent* No. 0099993A1.
[45]. Kirk, PM, Cannon, PF, Minter, DW, and Stalpers, JA (eds.) (2008). Dictionary of the Fungi, 10th ed. CAB, International, Wallingford, Oxon, UK.
[46]. Kumar K, Upreti DK. (2001). *Parmelia* sp. (lichens) in ancient medicinal plant lore of India. *Econ Bot*, **55**, 458–459.
[47]. Kumar SK, Banskota AH, Manandhar MD. (1996). Isolation and identification of some chemical constituents of *Parmelia nepalensis*. *Planta Med*, **62**, 93–94.
[48]. Lal B and Upreti DK (1995). Ethnobotanical notes on three Indian lichens. *Lichenologist*; **27**: 77–79.
[49]. Launert E. (1981). Edible and Medicinal Plants. London: Hamlyn Publisher.
[50]. Lauterwein M, Oethinger M, Belsner K, Peters T, and Marre R (1995). *In vitro* activities of the lichen secondary metabolites vulpinic acid, (+)-usnic acid and (–)-usnic acid against aerobic and anaerobic microorganisms. *Antimicrob Agents Chemother;* **39**: 2541–2543.
[51]. Lavie G, Mazur Y, Lavie D, and Meruelo D (1995). The chemical and the biological properties of the hypericin – a compound with broad spectrum of biological activities. *Medical Research Reviews*; **15**(2): 11-19.
[52]. Lawry JD (1989). Lichen secondary compounds: evidence for a correspondence between antiherbivore and antimicrobial function. *The Bryologist*; **89**: 111- 122.
[53]. Manojlović N, Vasiljević P, Gritsanapan W, Supabphol R, and Manojlović I (2010). Phytochemical and antioxidant studies of *Laurerabenguelensis* growing in Thailand. *Biol Res*; **43**: 169-176.
[54]. Martins MCB, Lima MJG, Silva FP, Ximenes EA, Silva NH, and Pereira EC (2010). *Cladiaaggregata* (lichen) from Brazilian Northeast: Chemical characterization and antimicrobial activity. *Brazilian Archives of Biology and Technology*; **53**: 115-122.
[55]. Mueller K (2001). Pharmaceutically relevant metabolites from lichens. *Applied Microbiology and Biotechnology*; **56**: 9-16
[56]. Nash T. H. III (ed.) (2008), Lichen Biology, 2nd ed. Cambridge University Press, Cambridge.
[57]. Negi HR, Kareem A. (1996). Lichens, the unsung heroes. *Amruth*; **1**: 3–6.
[58]. Nelsen MP and Gargas A (2008). Phylogenetic distribution and evolution of secondary metabolites in the lichenized fungal genus *Lepraria* (Lecanorales: Stereocaulaceae). *Nova Hedwigia*; **86**, 115–131.
[59]. Nordin, A, Tibell, L, and Owe-Larsson, B (2007). A preliminary phylogeny of *Aspicilia* in relation to morphological and secondary product variation. *Bibl. Lichenol.*; **96**, 247 – 266.
[60]. Oh SO, Jeon HS, Lim KM, Koh YJ, Hur JS. (2006). Antifungal activity of lichen-forming fungi isolated from Korean and Chinese lichen species against plant pathogenic fungi. *Plant Pathol J*; **22**: 381–385.
[61]. Oksanen I (2006). Ecological and biotechnological aspects of lichens. *Appl Microbiol Biotechnol;* **73**: 723–734.
[62]. Orozco A, Higginbotham L, Hitchcock C, Parkinson, T, Falconer, D, Ibrahim, A, Ghannoum, MA, Filler, SG (1998). Mechanism of fluconazole resistance in *Candida krusei*. *Antimicrob. Agents Chemother.*; **42**: 2645–2649.
[63]. O'Sullivan J and Mathison GE (1971). The localization and secretion of a propteolytic enzyme complex by the dermatophytic fungus *Microsporum canis*. *J Gen Microbiol.*; **68**: 319-326.
[64]. Padhye AA and Weitzman I (1999). The dermatophytes. Ajello L, Hay RJ (eds.) *Microbiology and microbial infection*, Vol 4. New York: Topley and Wilson; 215-293.
[65]. Pandey A, Mishra RK, Tiwari AK, Kumar A, Bajaj AK, and Dikshit A (2013). Management of Cosmetic Embarrassment Caused by *Malassezia* spp. with Fruticose Lichen *Cladia* Using Phylogenetic Approach. *BioMed Research International;* 2013: **8**. doi. 10.1155/2013/169794.
[66]. Pathak, A, Shukla, SK, Pandey, A, Mishra, RK, Kumar, R, and Dikshit, A (2015). *In vitro* antibacterial activity of ethno medicinally used lichens against three wound infecting genera of Enterobacteriaceae. *Proc. Natl. Acad. Sci., INDIA, Sect. B Biol. Sci.;* 2015:1-8.
[67]. Pathak, A, Upreti, DK, and Dikshit, A (2016). Antidermatophytic Activity of the Fruticose Lichen *Usnea orientalis*. *Medicines*; **3**(3): 24.
[68]. Paudel B, Bhattarai HD, Lee HG et al (2008) Antibacterial potential of Antarctic lichens against human pathogenic gram-positive bacteria. *Phytother Res*; **22**: 1269–1271
[69]. Peres, NTA, Maranhão, FCA, Rossi, A, Martinez-Rossi, NM (2010). Dermatophytes: Host-pathogen interaction and antifungal resistance. *An. Bras. Dermatol.;* **85**, 657–667.

[70]. Randhir R, Lin YT, and Shetty K (2004). Stimulation of phenolic, antioxidant and antimicrobial activities in dark germinated mung bean sprouts in response to peptide and phytochemical elicitors. *Process Biochemistry*; **39**: 637-646.
[71]. Ranković B, Kosanić M, Sukdolak S. (2010). Antimicrobial activity of some lichens and their components. In: Anninos P, Rossi M, Pham TD, Falugi C, Bussing A, Koukkou M, eds. *Recent Advances in Clinical Medicine*. Cambridge, UK: World Scientific and Engineering Academy and Society Press, pp. 279–286.
[72]. Rankovic B, Misic M, and Sukdolak S (2008). The antimicrobial activity of substances derived from the lichens *Physcia aipolia, Umbilicaria polyphylla, Parmelia caperata* and *Hypogymnia physodes*. *World J Microbiol Biotechnol*; 24:1239–1242.
[73]. Rankovic B, Misic M, Sukdolak S. (2007). Evaluation of antimicrobial activity of the lichens *Lasallia pustulata, Parmelia sulcata, Umbilicaria crustulosa*, and *Umbilicaria cylindrica*. *Mikrobiologiia*, **76**, 817–821.
[74]. Ranković B, Mišić M. (2008). The antimicrobial activity of the lichen substances of the lichens *Cladonia furcata, Ochrolechia androgyna, Parmelia caperata* and *Parmelia conspresa*. *Biotechnol Biotechnol Eq*; **22**: 1013–1016.
[75]. Saklani A, Upreti DK. (1992). Folk uses of some lichens in Sikkim. *J Ethnopharmacol*, **37**, 229–233.
[76]. Santiago KAA, Borricano JNC, Canal JN, Marcelo DMA, Perez MCP, dela Cruz TEE (2010). Antibacterial activities of fruticose lichens collected from selected sites in Luzon Island, Philippines. *Philip Sci Lett.,* **3**, 18–29.
[77]. Schmeda-Hirschmann G, Tapia A, Lima B et al (2008). A new antifungal and antiprotozoal depside from the Andean Lichen *Protousnea poeppigii*. *Phytother Res*; **22**: 349–355.
[78]. Shahi SK, Patra M, Dikshit A, and Upreti DK (2003). *Parmelia cirrhatum*: A potential source of broad spectrum natural antifungal. *Phytother. Res.*; **17**: 399–400.
[79]. Shahi SK, Shukla AC, Dikshit A, and Upreti DK (2001). Broad-spectrum antifungal properties of the lichen, *Heterodermialeucomelos*. *Lichenologist*; **33**(1): 177-179.
[80]. Shahi SK, Shukla AC, Upreti DK, and Dikshit A (2000). Use of lichens as antifungal drugs against superficial fungal infections. *J. Med. Aromatic Plant Sciences*; **22** (4A) and **23**(1A): 169-172.
[81]. Smith, KJ, Warnock, DW, Kennedy, CT, Johnson, EM, Hopwood, V, van Cutsem, J, Vanden Bossche, H (1986). Azole resistance in *Candida albicans*. *Med. Mycol.*; **24**: 133–144.
[82]. Stephenson, J (1997) Investigators seeking new ways to stem rising tide of resistant fungi. *J. Am. Med. Assoc.*; **277**: 5–6.
[83]. Stöcker Wörgötter E (2008). Metabolic diversity of lichen forming ascomycetous fungi: culturing, polyketide and sikhimate metabolite production, and PKS genes. *Natural Product Reports*; **25**: 188-200.
[84]. Swathi D, Suchitha Y, Prashith Kekuda TR, Venugopal TM, Vinayaka KS, Mallikarjun N, Raghavendra HL. (2010). Antimicrobial, antihlmintic and insecticidal activity of a macrolichen *Everniastrum cirrhatum* (FR.). Hale. *Int J Drug Develop Res*, **2**, 780–789.
[85]. Vattem DA, Lin YT, Lable RG, and Shetty K (2004). Phenolic antioxidant mobilization in cranberry pomace by solid-state bioprocessing using food fungus *Lentinusedodes* and effect on antimicrobial activity select food borne pathogens. *Innovative Food Science Emerging Technology*; **5**: 81-91.
[86]. Verma N, Behera BC, and Makhija U (2011). Studies on cytochromes of lichenized fungi under optimized culture conditions. *Mycoscience*; **52**: 65–68.
[87]. Voss, EG, Burdet, HM, Chaloner, WG, Demoulin, V, Hiepko, P, Mcneill, J, Meikle, RD, Nicolson, DH, Rollins, RC, Silva, PC, and Greuter, W (1983), International code of botanical nomenclature (Sydney Code). *Regnum Veg.* **111**, 1 – 472.
[88]. Wang L, Ma L, Leng W, Liu T, Yu L, Yang J, Yang L, Zhang W, Zhang Q, Dong J, Xue Y, Zhu Y, Xu X, Wan Z, Ding G, Yu F, Tu K, Li Y, Li R, Shen Y, and Jin Q (2006). Analysis of the dermatophyte *Trichophyton rubrum* expressed sequence tags. *BMC Genomics;* 7: 255 doi: 10.1186/1471-2164-7-255.
[89]. WHO (2003). Emerging Issues in Water and Infectious Disease, World Health Organization, Geneva.
[90]. Wingfield, AB, Fernandez-Obregon, AC, Wignall, FS, Greer, DL (2004). Treatment of tinea imbricata: A randomized clinical trial using griseofulvin, terbinafine, itraconazole and fluconazole. *Br. J. Dermatol.*; **150**: 119–126.
[91]. Yamamoto Y (1990). Studies of cell aggregates and the production of natural pigments in plant cell culture. *Nippon Paint Publications*, Osaka.
[92]. Yamamoto, Y (2000). Screening of biological activities and isolation of biological-active compounds from lichens. *Chem Regulation of plants (Shokubutsu no kagaku chosetu)*, **35**, 169–179.
[93]. Yilmaz M, Türk AO, Tay T, and Kivanc M (2004). The antimicrobial activity of extracts of the lichen *Cladonia foliacea* and its (-)-usnic acid, atranorin and fumaroprotectraric acid constituents. *Z Naturforsch*; **59c**: 249-254.
[94]. Yuan X, Xiao S, and Taylor TN (2005). Lichen-like symbiosis 600 million years ago. *Science*; **308**: 1017–1020.
[95]. Zambare VP, Zambare AV, Christopher LP. (2010). Antioxidant and antibacterial activity of extracts from lichen *Xanthoparmelia somloensis*, native to the black hills, South Dakota, USA. *Int J Med Sci Technol*, **3**, 46–51.
[96]. Zambare, VP and Christopher, LP (2012). Biopharmaceutical potential of lichens. *Pharmaceutical Biology*; 2012; **50**(6): 778–798.