International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 4 July-Aug 2021, pp: 564-580 www.ijprajournal.com ISSN: 2249-7781

Lichens as potential Antimicrobial agents: A comprehensive review

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Date of Submission: 15-07-2021 Date of Acceptance: 30-07-2021

ABSTRACT: This paper presents a comprehensive account of research findings reporting antimicrobial potential of lichens including their anti-bacterial, anti-fungal, anti-viral and anti-protozoal activities. Lichen is an intimate long term symbiotic association between a fungal partner (mycobiont) and an alga or cyanobacterium (phycobiont). The biochemistry of the two individuals alone and when they are in symbiotic association are different. This validates the search for bioactive metabolites in lichen as well as in fungal counterparts separately. More than 800 secondary metabolites have been reported to be produced from lichens, majority of which are unique to those lichen species and only a small fraction of those secondary metabolites are found in higher plants or other fungal organisms. These unique secondary metabolites are screened for various exclusive biological activities such as, antimicrobial, anti-cancer, anti-oxidant, anti-inflammatory, and many others. Appearance of multi-drug resistant organisms, appearance of new diseases, re-emergence of threatening diseases thought to be eliminated before, and the rapid development of drug resistance, are the major problems existing in the health sector created an urge for the discovery of novel antimicrobial compounds. Lichens have been largely underexplored and hence new species are being recorded with an increasing frequency with current insurgence of research interest in biodiversity. Hence, probing for antimicrobials from lichens would be a wise approach to cope up with these arising health threats. At present, lichens remain as under-tapped organisms and thus, exploring lichens with further research and development would provide new bioprospecting opportunities and solutions to rising research problems.

Key words: lichens, symbiotic association, drug resistance, antimicrobials, bioprospecting

I. INTRODUCTION

Lichens are unique organisms resulted from a symbiotic association between a fungus, termed as the mycobiont, and a cyanobacterium or alga, termed as the phycobiont [1]. There are about 20,000 recorded lichen species worldwide, covering about 8% of the world's land surface [2]. The current estimation suggests 28,000 and interestingly half of that is entirely harbored in the tropics. The symbiotic relationship itself is an adaptation for lichens to thrive in a variety of terrestrial habitats, from the tropics to Polar Regions [3]. Lichens are ecologically important in many aspects. Acting as carbon sinks, food sources, bioindicators and maintaining soil ecology are a few to mention [4, 5]. Their unique secondary metabolites are screened for important biological activities such as anti-bacterial, anti-fungal, antiviral, analgesic, antipyretic, antitumor, allergenic, plant growth inhibitory, enzyme inhibitory, anti-inflammatory, antipyretic, anti-proliferative, cytotoxic effects etc. [5-8]. Despite their value, the therapeutic potential of lichens has not been fully exploited.

Major threats, currently prevailing in the health sector are the appearance of multi-drug resistant organisms, rapid development of drug resistance, appearance of new diseases, and resurfacing diseases thought to be eliminated long before [9]. According to the estimates, 300 million people would die prematurely as a result of drug resistance over the next 35 years, and global Gross Domestic Product (GDP) will decrease by 2 to 3.5% in 2050 when compared to the current situation [10]. If development of antimicrobial drug resistance is not properly addressed, it is predicted that the world can expect to lose between 60 and 100 trillion dollars in economic production between now and 2050 [10]. World Health Organization has prioritized the pathogens as (i) Priority 1: Critical (Acinetobacter baumannii,Pseudomonas aeruginosa, and Enterobacteriaceae), (ii) Priority 2: High (Enterococcus faecium, Staphylococcus aureus,

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 4 July-Aug 2021, pp: 564-580 www.ijprajournal.com ISSN: 2249-7781

Helicobacter pylori, Campylobacter, Salmonella spp., and Neisseria gonorrhoeae), and (iii) Priority 3: Medium (Streptococcus pneumoniae,Haemophilus influenza, and Shigella spp.) for the novel antibiotic development [11].

This looming global health crisis has escalated the research towards development of novel antimicrobial compounds. In search for those with low side effects, researchers have turned their interest into compounds of natural origin. Among many organisms, lichens are of great interest due to their unique combination with fungi. Anti-bacterial, anti-fungal, anti-viral and anti-protozoal activities of lichens have been well documented [12, 13]. Promising results obtained in those studies highlight the significance of lichen substances in high throughput screening programs for pinpointing new lead compounds with antimicrobial potential. The enthusiasm of lichenologists on lichen chemistry reflect the demand for secondary metabolites extracted from microorganisms with interesting biological activities, especially natural lead compounds with unique structural diversity, which aid drug discovery programs [14]. This review is an effort to summarize the research findings which point out the antimicrobial potential of lichens including their anti-bacterial, anti-fungal, anti-viral and anti-protozoal activities to emphasize the importance of lichen research and development in future to overcome the current threats of heath sector.

II. METHODOLOGY

Information on various biological activities of lichens was collected from various sources and anti-microbial potential including anti-bacterial, anti-fungal, anti-viral, and anti-protozoal activities demonstrated by lichens were comprehensively analyzed. Finally the summary of the findings, challenges in the lichen research and recommendations for the future are documented in this review paper.

III. RESULTS

3.1 Anti-bacterial activities

The antibacterial activity of crude lichen extracts has been reported in several studies. Burkholder initiated the research on anti-bacterial activity of lichens on 1940. A number of 42 lichens have been evaluated and 27 of them were found to suppress bacterial growth [115]. In another study where 69 New Zealand lichen species were tested, growth inhibition of Escherichia coli, Enterococcus, Pseudomonas, Bacillus, Streptococcus, and Mycobacterium [16] was reported. Anti-bacterial activity of salazinic acid isolated from the lichen extract of Parmelia sulcata was also documented against Streptococcus faecalis, Yersinia enterocolitica, Bacillus cereus, Proteus vulgaris, Aeromonas hydrophila, Listeria monocytogenes, and Staphylococcus aureus [17]. Protolichesterinic acid from the extract of the lichen Cetraria aculeata showed inhibition against 9 bacteria: both Gram-positive and Gram-negative [18]. Lichen species from Serbia:Umbilicaria polyphylla, Umbilicaria cylindrica, Parmelia sulcata, Lasallia pustulata, Parmelia caperata, Cladonia furcata, Parmelia pertusa, and Hypogimnia physodes showed anti-bacterial activity whereas the strongest inhibition was reported from the extracts of Parmelia sulcata and Parmelia pertusa [19].

Usnea ghattensis extracts also showed promising activity against Bacillus subtilis, Bacillus licheniformis, Staphylococcus aureus, and Bacillus megaterium [20]. Also, 11 lichens of Turkey including Ramalina farinacea and Peltigera polydactyla were evaluated and anti-bacterial activities were documented [21]. The lichens Flavoparmelia caperata, Hypogymnia physodes, Evernia prunastri, and Cladonia foliaceawere active against 15 bacterial strains whereas the strongest inhibition was observed against the Gram-positive species [22]. In vitro screening of the lichen extracts of Parmeliopsis hyperopta and Lecanora frustulosa also revealed their anti-bacterial potential against Bacillus mycoides, Enterobacter cloacae, Escherichia coli, and Staphylococcus aureus [23]. Paudel et al. [24] reported the anti-bacterial activity of some Antarctica lichens including Stereocaulon alpinum, Caloplaca regalis, Lecanora sp., Ramalina terebrata, and Caloplaca sp.

Saenz et al. [25] reported that, Lecanora muralis, Ramalina canariensis, Cladonia firma, and Ramalina subfarinacea lichen species inhibited the growth of some Gram-positive bacterial strains. Vibrio cholera, Shewanella sp., Klebsiella pneumonia, Salmonella sp., and Enterococci sp. were also inhibited by the lichen extracts of Roccella belangeriana whereas the strongest inhibition was recorded against Vibrio cholera [26]. In a past investigation, where 34 North American species were

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 4 July-Aug 2021, pp: 564-580 www.ijprajournal.com ISSN: 2249-7781

evaluated, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, and methicillin-resistant Staphylococcus aureus were inhibited by most of the lichens including Vulpicida canadensis, Letharia vulpina, and Letharia columbiana [27].Indian lichens Parmotrema tinctorum, Parmotrema praesorediosum, and Parmotrema grayanum showed anti-bacterial activity against 5 Gram-negative and 3 Gram-positive bacterial strains [28].

Ristić et al. [29] reported the anti-bacterial activities of lichens Melanelia fuliginosa and Melanelia subaurifera whereas the MIC values for the tested lichen extracts against bacterial strains fluctuated between 1.25 to 20 mg/mL. The lichen extract of Lasallia pustulata also inhibited Escherichia coli, Staphylococcus aureus, Proteus mirabilis, Bacillus cereus, and Bacillus subtilis [30]. Likewise, bacterial strains including Escherichia coli and Bacillus cereus were inhibited in a previous study, where the lichens Ramalina hossei, Ramalina conduplicans, and Ramalina pacifica were investigated [31].Similarly, Staphylococcus aureus, Staphylococcus pneumonia, and Bacillus subtilis were inhibited by the lichen extracts of Parmelia vagans [32]. Also, anti-bacterial activities of Cladonia fimbriata, Cladonia rangiferina, Cladonia furcata, and Cladonia foliacea were reported in a previous research study with the strongest inhibition from Cladonia fimbriata [33].

In a similar research, anti-bacterial activities of the lichen extracts of Heterodermia boryi, Parmotrema melanothrix, Usnea nilgirica, Parmotrema stuppeum, and Pyxine sp. were reported against Acinetobacter sp., Pseudomonas sp., Escherichia coli, Streptococcus viridans, Klebsiella sp., Staphylococcus aureus, and Coagulase-negative Staphylococcus aureus with the strongest inhibition from Parmotrema stuppeum [34]. Various other lichen research studies reported the antibacterial activities of the lichen extracts of Parmotrema pseudotinctorum [35], Parmotrema reticulatum [36], Parmotrema sanctiangelii [37], Parmotrema nilgherrensis [38], Usnea barbata [39], Pseudoevernia furfuracea [40], Stereocaulon paschale [41], and Bryoria capillaris [42]. These findings emphasizes that the anti-bacterial potential of lichens should be further explored for the wellbeing of the society.

3.2 Anti-fungal activities

Lichens have proven themselves as potential candidates for the screening of anti-fungal compounds in many past studies. The pathogenic fungi Trichophyton mentagrophytes, Trichophyton rubrum, and Microsporum gypseum were inhibited by the extracts of Usnea florida and Protousnea poeppigii [43]. Lichen species from Serbia including Umbilicaria cylindrica, Cladonia furcate, Hypogymnia physodes, Umbilicaria crustulosa, Parmelia pertusa, Lasallia pustulata, Parmelia caperata, Parmelia sulcata, and Umbilicaria polyphylla inhibited the growth of ten fungal species with the strongest inhibition from Parmelia sulcata and Parmelia pertusa [44]. Extracts of Roccella belangerianainhibited the growth of Rhizopus sp. and Aspergillus niger [26]. Antifungal activities of the lichens Cladonia foliacea, Evernia prunastri, Hypogymnia physodes, and Flavoparmelia caperata were also revealed in a previous study. In that study, Hypogymnia physodes and Evernia prunastri strongly inhibited filamentous fungal strains and yeasts, respectively [45].

Goel et al. [46] reported the anti-fungal activities of the lichen Parmelia reticulata against Pythium aphanidermatum, Sclerotium rolfsii, Rhizoctonia solani, Pythium debaryanum, and Rhizoctonia bataticola which are soil-borne pathogens. Moreover, Trichoderma harsianum, Candida albicans, Mucor mucedo, Aspergillus fumigatus, Aspergillus flavus, Penicillium verrucosum, Fusarium oxysporum, Penicillium purpurescens, and Paecilomyces variotii were inhibited by the extracts of lichens Parmeliopsis hyperopta and Lecanora frustulosa [23]. Extracts of Protousnea poeppigii inhibited the growth of several fungal strains including Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Saccharomyces cerevisiae, Candida tropicalis, Candida albicans, Microsporum gypseum, Trichophyton rubrum, and Trichophyton mentagrophytes as well [47]. Some plant pathogenic fungal strains such as, Ustilago maydis, Phytophthora infestans, Fusarium roseum, and Pythium ultimum were inhibited by the lichen extracts of Parmotrema tinctorum, Hypogymnia physodes, and Evernia prunastri [48]. In addition, the anti-fungal activities of the lichens Lasallia pustulata [30], Alectoria sarmentosa [19], Cladonia portentosa [48], and

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Cladonia rangiferina [19] were reported in many previous investigations.

Various bio active compounds from lichens have proven their anti-fungal potential in the past studies as well. Anthraquinones extracted from the lichens Caloplaca cerina, Xanthoria sp., and Caloplaca cerina showed anti-fungal activities [49-51]. Also, the researchers have found out the anti-fungal potential of the several other bioactive compounds including divaricatinic acid, fumarprotoacetraric acid, Usnic acid, salazinic acid, Parietin, 5-propylresorcinol, lecanoric acid, isodivaricatic acid, atranorin, stictic acid, and zeorin [17,23,47,52&53].Thus, the anti-fungal potential of lichens should be further explored in order to compete with the threatening fungal strains.

3.3 Anti-viral activities

Lichens have been found to be a good source of antiviral drugs. Results of numerous past studies showed the anti-viral activities of lichens. Anti-viral compounds against vesicular stomatitis virus (VSV), HSV-2, parainfluenza virus, and vaccinia virus were extracted from the lichen Hypericum perforatum. Those compounds were later identified as hypericin and emodin [54]. In a lichen study, where 69 New Zealand lichen species were evaluated, P. homoeophylla, Cladia retipora, and Pseudocyphellaria glabra lichens showed anti-viral activity [55]. Anti-viral activities of some other lichen extracts against Epstein-Barr virus, HIV and human cytomegalovirus were also documented [56-58]. Lichenan is a secondary metabolite of lichens which is widely known for its anti-viral activity against TMV (Tobacco Mosaic Virus). Along with lichenan, some other secondary metabolites of lichens such as, hypericin, parietin, bianthrones, and anthraquinones isolated from the lichens Heterodermia obscurata and Nephroma laevigatum also inhibited viruses including Herpes Simplex Virus type 1 (HSV-1) in previous investigations [59,60].

Anti-viral activity against Human parainfluenza virus type 2 (HPIV-2) were shown by the lichen extracts of Xanthoparmelia tinctina and Xanthoria parietina [61]. Also, the derivatives of secondary metabolites isolated from the lichen Ramalina farinacea inhibited adenoviruses and lentiviruses [62]. Also, 13 phenolic compounds isolated from the lichen R. farinacea showed inhibition against the respiratory syncytial virus (RSV) [63]. Researchers from West Africa found out anti-viral activity against infectious bursal disease virus, yellow fever virus and polio virus when evaluating the lichen extracts of Parmelia perlata [62].In a similar research, anti-viral activities of parietin extracted from the lichen Teloschistes chrysophthalmus against Junin and Tacaribe arenaviruses were recorded [64].

Usnic acid and the derivatives of it are known to be more promising in the treatment of influenza virus. They were investigated in many past studies as well [65]. Some other viruses including Herpes simplex type 1 virus, Epstein–Barr virus, Polio virus, respiratory syncytial virus, arenavirus, and human papillomavirus were also inhibited by usnic acid and its derivatives [66-68]. Lichens continue to yield new antiviral chemicals, making them an appealing potential source of novel antiviral compounds in the future. Given the variety of options provided by lichens, we anticipate that they will continue to be an intriguing focus of our research in the hunt for effective antiviral treatments for life-threatening viral infections and diseases.

3.4 Anti-protozoal activities

Similar to the anti-bacterial, anti-fungal, and anti-viral activities, anti-protozoal potential of lichens has also proven in some investigations. Usnic acid, which is one of the most common lichen substances, demonstrated anti-protozoal activities in the past investigations [69]. Along with usnic acid, divaricatic acid, 5-propylresorcinol and isodivaricatic acid extracted from the lichen Protousnea poeppigii also showed anti-protozoal activities [13]. In addition to these findings, anti-plasmodial activities of lichens have also been documented in many previous investigations. Plasmodium is a parasitc protozoan and extracts of various lichens showed inhibition against it [70]. Pamenta et al. [71] found out that the extracts of Usnea longissima inhibited Plasmodium falciparum, which is the causative agent of malaria. Also, a novel compound extracted from a Nigerian lichen Dirinaria picta also demonstrated its anti-plasmodial potential in a past research [72].These results indicate that lichen source can be exploited more to further explore the anti-protozoal potential.

Anti-microbial activities of various lichens and the bioactive compounds which are responsible for the antimicrobial activities are summarized in Table 1.

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Table 1: Antimicrobial activities of lichens

Lichens	Bioactive compound	Antimicrobial activity	References
Stereocaulon ramulosum	Methyl haematommate	Anti-fungal activity	[73]
Ochrolechia androgyna	Lecanoric acid	Anti-bacterial activity	[13]
Umbilicaria esculenta	Lichen polysaccharide sulfate	Anti-viral activity	[56]
Cetraria aculeata	Protolichesterinic acid	Anti-bacterial activity	[18]
Roccella belangeriana		Anti-bacterial activity	[26]
Cetraria islandica	Protolichesterinic acid	Anti-bacterial activity	[74]
Alectoria nigricans	Salazinic acid, alectorialic acid	Anti-viral activity	[75]
Cladonia arbuscula	Usnic acid	Anti-mycobacterial activity	[76]
Dirinaria picta	Poly phenolic depside	Anti-plasmodial activity	[72]
Ramalina farinacea	Sekikaic acid	Anti-viral activity	[63]
Evernia prunastri	Lichenic acid	Anti-fungal activity	[48]
Protousnea poeppigii	Isodivaricatic acid, 5-propylresorcinol, divaricatic acid, usnic acid	Anti-fungal and anti-protozoal activities	[47]
Cladonia portentosa	Lichenic acid	Anti-fungal activity	[13]
Nephroma laevigatum, Heterodermia obscurata	Emodin, 7-chloroemodin, 7-chloro-1- O-methylemodin, 5,7-dichloroemodin, 7,70 dichlorohypericin, and hypericin	Anti-viral activity	[60]
Hypogymnia physodes	Lichenic acid	Anti-fungal activity	[13]
Usnea campestris	Usnic acid	Anti-fungal and anti-bacterial activities	[77]
Everniastrum cirrhatum	Protolichesterinic acid, Atranorin, Salazinic acid	Anti-fungal and anti-bacterial activities	[78]
Umbilicaria proboscidea		Anti-fungal activity	[13]
Cladonia cristatella		Anti-viral activity	[58]
Ochrolechia androgyna	Lecanoric acid	Anti-fungal and anti-bacterial activities	[13]
Nephromopsis pallescens		Anti-fungal activity	[79]
Teloschistes chrysophthalmus	Parietin	Anti-viral activity	[64]
Cladonia foliacea	Usnic acid, Atranorin, Fumarprotocetraric acid	Anti-bacterial activity	[53]
Cladonia furcata	Fumarprotocetraric acid	Anti-fungal and anti-bacterial activities	[13]
Ramalina conduplicans		Anti-fungal activity	[79]
Parmelia saxatilis	Salazinic acid, alectorialic acid	Anti-viral activity	[75]
Parmotrema screminiae	Norlobaridone and protolichesterinic acid	Anti-bacterial activity	[80]
Lecanora frustulosa	Zeorin, Divaricatic acid	Anti-fungal and anti-bacterial activities	[81]

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Lasallia pustulata		Anti-bacterial activity	[44,45]
Melanelia sp.		Anti-fungal activity	[13]
Several lichens	Lichenan	Anti-viral activity	[82]
Cladia retipora	Usnic acid	Anti-fungal and anti-bacterial activities	[83]
Parmelia conspresa	Stictic acid	Anti-fungal and anti-bacterial activities	[13]
Parmeliopsis hyperopta	Zeorin, Divaricatic acid	Anti-bacterial and anti-fungal activities	[81]
Pseudocyphellaria homoeophylla	Usnic acid	Anti-viral activity	[16]
Usnea rigida	usnic acid,divaricatinic acid, 5-resorcinol, and isodivaricatinic acid	Anti-fungal activity	[47]
Parmotrema tinctorum	Phenolic substances	Anti-bacterial activity	[35]
Parmotrema tinctorum	Lecanoric acid	Anti-fungal activity	[84]
Usnea subfloridans		Anti-fungal activity	[85]
Cladia aggregata	Barbatic acid	Anti-bacterial activity	[86]
Pseudocyphellaria glabra	Usnic acid	Anti-viral activity	[16]
Stereocaulon vesuvianum	Atranol	Anti-bacterial activity	[74]
Parmelia caperata	Protocetraric acid	Anti-fungal and anti-bacterial activities	[13]
Pertusaria sp.		Anti-fungal activity	[79]
Caloplaca cerina	parietin	Anti-fungal activity	[87]
Peltigera polydactyla	evernic acid, vulpinic acid and hirtusneanoside	Anti-bacterial activity	[13]
Pseudoparmelia sphaerospora	Phenolic substances	Anti-bacterial activity	[88]

3.5 Probable mechanisms of antimicrobial action of lichens

Antimicrobial action of lichens is defined as their ability to kill or hinder the growth of bacteria. Antimicrobial activity can be attributed to a variety of pathways such as, Inhibition of cell wall synthesis, Inhibition of protein synthesis, Disruption of cell membrane, Inhibition of nucleic acid synthesis, anti-metabolite action, mitochondrial dysfunction, and inhibition of efflux pumps (Figure 1). Inhibition of cell wall synthesis can be resulted from various actions including inhibition of biosynthetic enzymes, combining with carrier molecules, combining with cell wall substances, and inhibition of polymerization and attachment of new peptidoglycan to cell wall [89].Protein synthesis inhibitors act at the ribosomal units, preventing the pathogen from synthesizing proteins by misreading the amino acid sequence, and therefore preventing the pathogenic cells from functioning [90].

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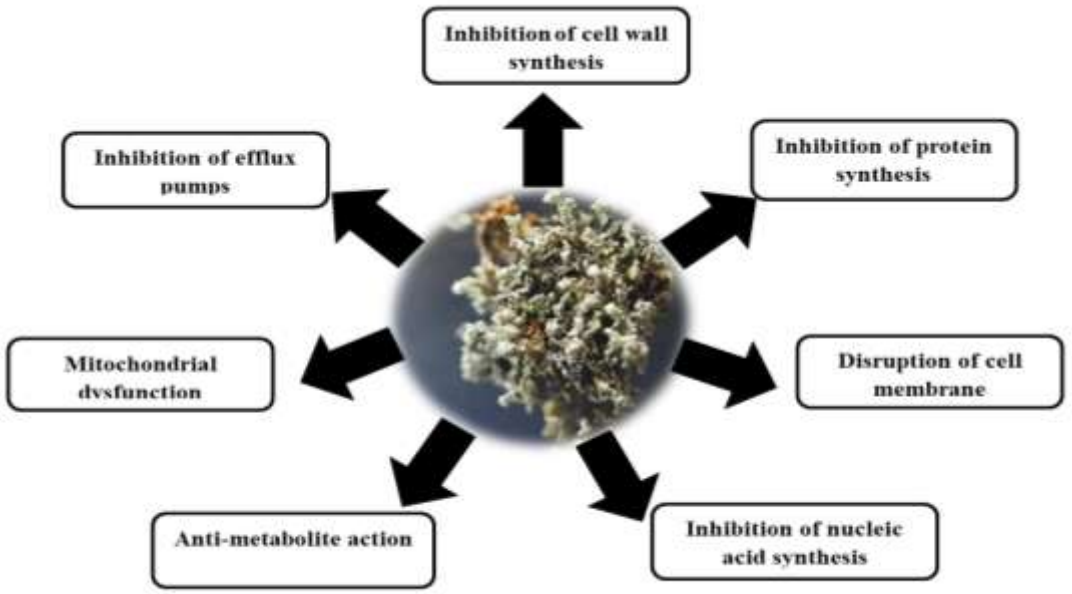

Figure 1: Various mechanisms of antimicrobial action of lichens

Disorganization of cytoplasmic membrane and production of pores in membrane can also trigger the alteration in cell membranes [91] which can lead to the growth inhibition of particular microorganism. Interference with nucleotide synthesis, impairing the template function of DNA, inhibition of DNA-Directed DNA polymerase, and inhibition of DNA replication may also lead to the inhibition of nucleic acid synthesis [92], which can be ultimately resulted in the inhibition of the growth of microorganism. Furthermore, antimetabolites can also block the ability of the cell to carry out a metabolic reaction. They work by inhibiting enzymes competitively and erroneously incorporating into nucleic acids. In both circumstances, the cells of microorganisms lose their ability to operate normally [37].

3.6 Commercialized products of lichen origin

Various commercialized products based on lichen extracts which can act as commercial pharmaceutical agents are offered by various companies world-wide. Lamprene®, Broncholind®, Isla-Moos®, Gessato™, Lichensed®, Isla® Ginger 30Pastilles, and Usnea Extract Dropper are some of those commercialized products of lichen origin (Figure 2) [93,94].

Figure 2: Lamprene® (Clofazimine) (A), Lichensed® capsules (B), Isla® Ginger 30 Pastilles (C), Usnea Extract Dropper (D), and Isla-Moos® capsules (E)

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In a previous study, a group of researchers found out a new series of agents called 'riminophenazines' which showed high anti-mycobacterial/anti-tuberculous activity in 1957. A pharmaceutical company named as 'Novartis' later developed B-663, which is the most effective among those novel agents, as a commercial pharmaceutical product with the brand name Lamprene® (Generic name: Clofazimine). Currently, Lamprene® capsules are available in the global market as antimycobacterial drugs[95]. Broncholind® and Isla-Moos® are the trade names of the commercially approved drugs used to treat some respiratory issues and they were made using the extracts of Icelandic lichens[94]. Isla® Ginger 30 Pastilles are the commercial products of Germany which were developed using Icelandic lichen extracts in order to treat sore throat and soothe symptoms of hoarseness as well[96].

Usnea Extract Dropper is also accessible in the global market in various commercial forms of different companies for the purpose of detoxification and cleansing[97]. Globally available Lichensed® capsules developed from the extracts of the lichens Cetraria islandica, are being used to treat some throat infections[93].Some of the German companies have incorporated usnic acid, which is a very common lichen substance in some of their antiseptic products including Camillen 60 Fudes spray, Gessato™ and nail oil[94]. In addition, lichen extract of Xanthoparmelia scabrosa is tone of the main ingredients in the aphrodisiac formulations which are available in the global market [93] and pharmaceutical companies of Nepal have used Parmelia nepalense in their pharmacological products for sore throat and toothache as well [98]. Extracts of lichens are used in several commercial products of various countries such as Japan, Italy, Germany, and United States in the forms of neutraceuticals, pharmaceuticals, and cosmetics[99].

3.7 Challenges and Future directions

Despite their wide range of biological activities, mycologists and agro-chemists have disregarded lichens, owing to their slow growth in nature and challenges in artificial culturing. As a result, lichen metabolites have yet to reach the stage of large-scale industrial manufacturing. In some previous investigations, despite the fact that lichen solvent extracts showed antimicrobial effects, none of the isolated chemical compounds had the same effects. This might be attributed to either very low amount of active ingredient in the lichen extracts or loss of synergism. Therefore, mass culturing of lichens is essential since spectroscopic techniques demand large volumes of afforded products for characterization; hence a way to culture vast quantities on a larger scale is needed [13]. This has prompted scientists to conduct additional research on the artificial culturing of lichens. To prospect lichens or their secondary metabolites, we must improve the traditional lichen tissue culture technique by developing more appropriate nutrient media and physiological parameters that can support symbiont growth and the production of secondary metabolites in greater quantities in a shorter period of time [100].In contrast, these differences between the natural environment and artificial medium can also turn as an advantage. The alterations in the culturing parameters including pH, salinity, composition, temperature, and others may trigger the metabolome of the species evaluated, which may lead to the amplification of little changes to enormous ones in a good way[101].

Bioreactor-based large-scale production of lichen culture biomass is also one of the promising technologies that could be widely used in the future for industrial-scale lichen secondary compound exploitation. Using OSMAC (One Strain Many Active Compounds) technique is also one of the most beneficial approaches in order to accomplish the artificial lichen culturing successfully as this technique is capable of covering the complete metabolome. The metabolic diversity of the lichen inhabiting fungi can also be increased by co-culturing bacterial species with those fungal strains. OSMAC approach involves co-culturing of the symbionts and natural conditions are given to the culturing medium by introducing the cell lines of the host tissue[102].However, to develop, optimize, and scale-up promising lichen-based technologies of great economic and global value, more research and development is necessary. Looking for novel lichens and exploiting them may also help to discover novel, unique chemical compounds with anti-microbial potential.

Secondary metabolite production of lichens can be triggered by various environmental conditions. Increase of global temperature, lengthy droughts, altered precipitation and evaporation rates, elevation

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Volume 6, Issue 4 July-Aug 2021, pp: 564-580 www.ijprajournal.com ISSN: 2249-7781

of the global CO_2 level, and rising of the sea level are some of the adverse effects of the current global climate change. The majority of these alterations will have an impact on the future adaptation and secondary metabolite synthesis of lichens. However, these secondary metabolites, on the other hand, guard against rising environmental challenges as the lichens can tolerate extreme environments. Lichens, especially the fungal mycobionts are robust and can create a variety of chemicals that have similar effects even in triggered environmental conditions. However, these interactions and the role of the colonization of other holobionts in these adaptations, can be further studied at the laboratory level in future using the advanced techniques such as, molecular Imaging, carbon isotope labeling, and 'Omics' approaches[103].

There are several factors associated with the low metabolite yield and poor separation. Sample degradation due to the various other chemicals in the metabolite extract, Interference of solvents used for quenching and extraction, quantitative errors in chromatography and spectrometry techniques, and peak misidentification are some of those factors which can lead to low metabolite yield and poor separation. Improving protocols using full scan approaches and multiple SRM(Selected Reaction Monitoring), Implementation of techniques like systems-level thermodynamic analysis for the identification and quantification of mis-measured metabolites, utilization of advanced including NMR (Nuclear Magnetic Resonance),Performing spiking experiments to check quenching and analyte stability, and building a well-functioning metabolite analysis pipeline can be done to improve the procedure downstream analysis in order to obtain high metabolite yield[104].

Synergistic activity of compounds is another obstacle in natural product science. The topic of synergy in natural product formulations has received much attention recently, and the importance of multi-target combination therapy has risen to prominence [105]. Many tools have been developed to make natural product formulations less difficult. In the better identification of combination effects, recent models using the explicit mean equation and zero interaction potency models, and various other robust reference models have been used. Furthermore, metabolomics and biochemometric techniques are also being used as attractive approaches for examining synergy, and they have just initiated to be used to uncover elements that participate in combination effects. Future study and development in this field will also benefit from the use of statistical techniques capable of detecting non-linear correlations [106]. Additionally, untargeted approaches in identifying molecular targets of synergistic or antagonistic modes of action have only recently begun to be investigated, and further research on this issue is crucial. Big Data advanced approaches also hold a lot of promise for identifying active mixture components, describing their interactions, and understanding their probable mechanisms of action.

Several investigations, on the other hand, have found that some lichen compounds can trigger allergies and are hazardous to organs. Several natural lichen chemicals, such as atranorin, stictic, fumarprotocetraric, and physodic acids, have been proven to be allergic [107]. Furthermore, usnic and other lichen acids have been linked to hepatotoxicity in various investigations. Despite the fact that usnic acid and its derivatives have been sold in the United States, there were multiple instances of liver toxicity linked to the intake of dietary supplements containing usnic acid [108,109]. Drug discovery from natural compounds and molecules can also be considered as a difficult multifaceted task, particularly in terms of safety. The use of lichen metabolites in medicine is a potential subject that necessitates interdisciplinary research from drug technologists, medicine chemists, nutritionists, and toxicologists in future to overcome these kinds of issues.Isolation and characterization of active compounds, as well as elucidation mechanism, may become easier in the future with the use of contemporary equipment and procedures. This method would provide a big volume of material while having no negative impact on the environment. Appropriate chemical alteration of natural products may improve their stability and minimize their toxicity, as well as boost their potency. In the near future, specific analytical methodologies must be carefully examined by in vitro and in vivo studies of problematic lichen compounds, as well as the evaluation of possible adverse human impacts [12].

Advancement of science and technology can also play a vital role in lichen research and development in future. To overcome the limited availability of biologically active, financially useful, and medicinally significant secondary metabolite

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 4 July-Aug 2021, pp: 564-580 www.ijprajournal.com ISSN: 2249-7781

substances, metabolic engineering and biotechnological techniques can be employed as an alternative production strategy [100].The increasing application of genetic techniques in the regulation of secondary metabolic pathways will lay the groundwork for economically feasible production of lichen substances. To minimize the slow growth rate of lichens, genetic engineering can be employed to locate the genes responsible for the generation of lichen secondary metabolites, as well as the expression of the gene in fast-growing fungus [110]. Researchers can also incorporate advanced techniques like multi-omics approach in lichen research to identify and further investigate the processes of certain substances found in natural lichens. Also, chemical synthesis of isolated and identified compounds is another aspect to be developed. Yet sometimes the steric effect may be lost and hence may not be successful. Furthermore, combining chemistry and computer-aided drug design may be able to produce novel synthetic analogues of lichen compounds, thereby expanding the access or pharmaceutical industry to new medication discovery [100].

Conservation of lichens is also main consideration when extracting potential bioactive compounds from the lichen species since they are very slow growers. Below listed are some proposed strategies which can be implemented to conserve lichen species[111].

- Inventorying and documenting the lichen rich environments.
- Conserving the permanent lichen habitats including grasslands, mountain ridges, gravel fields, and forests.
- Giving strict protection to the greater population of rare and endangered lichen species, as they are the propagule pools, which are essential for long-term persistence of the species.
- Conservation and reservation of areas located at least within 100m from such propogaule pools, as they are the future habitats of such lichen species.
- Identifying specific threats to different lichen communities such as soil lichens and implementing suitable remedies.
- Developing simplified illustrated identification manuals for lichens
- Creating awareness about lichens and the need to conserve them at appropriate levels.
- Establishing organizations or groups dedicated for the conservation of lichens worldwide.

IV. CONCLUSION

Lichens have remarkable antimicrobial potential, which should be extensively studied and used for the betterment of human health and society. More than 800 lichen metabolites have structures accessible, but many more to be defined in future [112].Because of their sluggish development and difficulty in artificial cultivation, lichens were usually overlooked by pharmaceutical companies. However, more research and development is needed, using enhanced modes of action based on next-generation sequencing and multi-omics methods including transcriptomics, metabolomics, and proteomics, etc. to explore the antimicrobial potential of lichens fully. Lichen tissue culture and gene transfer procedures may potentially aid in expanding access to lichen-derived drugs and pharmaceutical screening requirementsfor the discovery of novel antimicrobial compounds. However, conservation of lichens should be mainly considered when utilizing lichens for the purpose of extracting novel bioactive compounds.

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