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REVIEW ARTICLE

Secondary Metabolites from Lichen *Usnea longissima* and its Pharmacological Relevance

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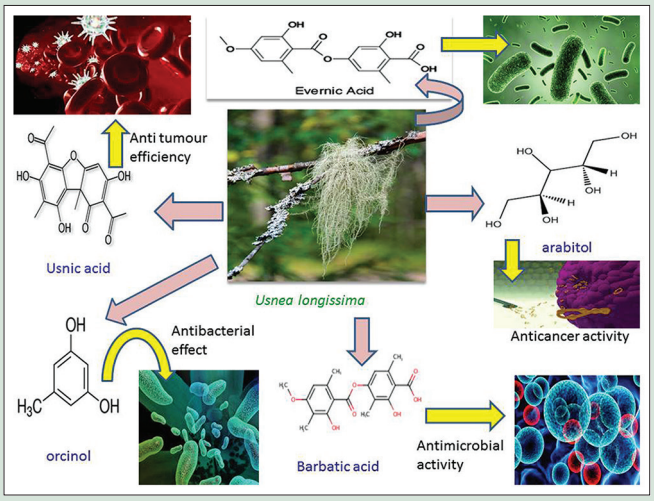
ABSTRACT

The objective of the study was to isolate chemical compounds from lichen *Usnea longissima* (UL) and to use lichen metabolites as antibiotics, antifungal, anti-HIV, anticancer, antiprotozoan, and antibacterial agents. Preparation of the lichen extracts: Fine powder of UL lichen was extracted using acetone, methanol, and water in a Soxhlet extractor. In a rotary evaporator, the extracts were filtered and then concentrated under reduced pressure. The dry extracts were stored at 18°C until they were used in the tests. The extracts were used for various assays. Sixteen compounds were isolated from lichen UL using various chromatographic techniques, including silica gel, Sephadex LH-20, operational data store, and semipreparative high-performance liquid chromatography. By spectroscopic data analyses, their structures were identified as (1) useanol, (2) lecanorin, (3) 3-hydroxy-5-methylphenyl 2-hydroxy-4-methoxy-6-methylbenzoate, (4) lecanorin E, (5) 3′-methylevernic acid, (6) evernic acid, (7) barbatinic acid, (8) 3,7-dihydroxy-1,9-dimethyldibenzofuran, (9) orcinol, (10) O-methyl orcinol, (11) methyl orsellinate, (12) methyl everninate, (13) 2,5-dimethyl-1,3-benzenediol, (14) 2-hydroxy-4-methoxy-3,6-dimethyl benzoic acid, (15) ethyl everninate, and (16) ethyl 2,4-dihydroxy-6-methylbenzoate. UL lichen is a great source of secondary metabolites, which can be used as antimicrobial, antiproliferative, and anticancer agents. This lichen can be used as a natural antioxidant or antitumor agent in clinical practice; commercially, it can be used as an anticancer drug.

Key words: Antifungal, antimicrobial, antioxidant, antitumor, lichen, *Usnea longissima*

SUMMARY

- *Usnea longissima* is a fruticose lichen mostly growing in the Himalayan region of West Bengal and the northern mountain region of India. It is growing in harsh cold conditions and also sometimes found in extreme northern pole. They evolve great metabolic systems, and secondary metabolites from them are very unique to resist the environmental states. In the present study, we enlisted different types of isolated chemicals such as usnic acid, useanol, evernic acid, barbatinic acid, and orcinol of lichenian origin. These chemicals have several bioactive properties such as antimicrobial, antibacterial, antifungal, antitumor, antiviral, and antioxidant. Usnic acid and its derivatives are more effective than normal medicines in recent reemerging mutant infectious stains. Hence, this medicinal herb may be used as a proven drug in the near future for clinical application.



Abbreviations Used: AFB: Acid-fast bacilli, AFB1: Acid-fast bacilli 1, ATCC: American Type Culture Collection, BA: Barbatinic acid, Bcap: B-cell adapter for PI3K, BEH: Ethylene bridged hybrid, DA: Diffractaic acid, DNA: Deoxyribonucleic acid, DPPH: 2,2-diphenyl-1-picrylhydrazyl, EA: Evernic acid, ERK1/2: Extracellular signal-regulated kinase ½, GPx: Glutathione peroxidase, HPLC: High-performance liquid chromatography, LPO: Lipid peroxidation, MDA: Malondialdehyde, MIC: Minimum inhibitory concentration, MN: Micronucleus, MRSA: Methicillin-resistant *Staphylococcus aureus*, NCCLS: National Committee for Clinical Laboratory Standards, ODS: Operational data store, ppm: Parts per million, ROS: Reactive oxygen species, SCE: Sister chromatid exchange, SOD: Superoxide dismutase, TB: Tuberculosis, UA: Usnic acid, UME: Methanolic extract of *Usnea longissima*, USN: Ultrasonic nebulizer, UPLC: Ultra-performance liquid chromatography, VEGFR: Vascular endothelial growth factor receptor, VRE: Vancomycin-resistant enterococci.

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INTRODUCTION

Usnea is a genus of lichen that has been classified under the family Parmeliaceae with around 1000 species worldwide. In modern days, the genus *Usnea* has been classified into a number of smaller genera according to thallus morphology. Some of the members of genus *Usnea* are *Usnea barbata*, *Usnea dasypoga*, *Usnea florida*, *Usnea hirta*, *Usnea longissima* (UL), *Usnea rubiginea*, and *Usnea subfloridana*.[1] Various acids and sterols have been isolated from UL.[2] Lichenic acids isolated from UL are growth inhibitors. UL is an epiphyte species

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known to produce one or more secondary metabolic products which have been characterized as weak phenolic acids.[3] UL was used as a dermatological aid for dressing wounds in the Pacific Northwest. The metabolic products that have antibiotic activity may have function of protecting the organisms from attack by other fungi.[4] This lichen extract retards the growth of lower phycomycetes and *Neurospora crassa*.[5] Reported in Finland, complex derivative of usnic acid of lichen has been reported with enhanced antibiotic activity.[6] Sodium usnate has been successfully used for the control of various plant diseases in greenhouse.[7] In the present study, the extracts of UL were investigated for their antimicrobial activity against the following pathogenic bacteria: *Staphylococcus aureus, Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Shigella dysenteriae, Escherichia coli*, and *Salmonella typhi* and some dermatophytic fungi such as *Candida albicans* and *Trichoderma viride*. The natural compounds are usually secondary metabolite produced by the metabolism process of plants.[8]

Natural ingredients are easily accepted by the body; it is because of the conformity among the reactions that occur in a series of metabolic cycles of human and plants.[9]

Lichens produce various unique chemicals that can be used for pharmaceutical purposes. Thirteen lichen samples are collected for screening of novel lichen secondary metabolites, showing inhibitory activity against lung cancer cell motility. In both the absence and the presence of epidermal growth factor stimulation, physciosporin also suppresses KAI1 C-terminal interacting tetraspanin-mediated AP-1 activity. Moreover, Cdc42 and Rac1 activities were decreased by physciosporin. The lichen secondary metabolite, physciosporin, inhibits lung cancer cell motility through novel mechanisms of action and is revealed by this demonstration.[10]

Some 700 substances with identified structures are currently known in lichens. Most of the isolated molecules belong to depsides which have ester groups linked 2-4-dihydroxybenzoic acid residues. Depsidones are major compounds which have an additional ether bond between aromatic rings. Many substances extracted from lichens have antimicrobial actions. The best known is usnic acid, an antibiotic with a phenol structure. Wounds, burns, fissures, etc., are treated by a sodium salt derivative of usnic acid. The search for synthetic analogs of the unique lichen antibiotics is relevant. This article presents the structures of a number of lichen compounds (41 structures), these compound extraction procedure, and their characteristics. Thus, lichens long used in folk medicine are now potential sources of pharmacologically active substances.[11]

Lichens are symbiotic organisms containing a mycobiont (fungal partner) and a photobiont (photoautotrophic partner), lichens are complex assamblages of algae and fungi and sometimes a third organism may contribute to the symbiosis which produce a large amount of secondary biomolecules. Dominant fungal partner produces the compounds which are isolated and these continue to be of great interest for their unique chemistry and biotechnological potential. In recent years, it has become apparent that many photobionts and lichen-associated bacteria also produce a range of potentially valuable molecules. Cyanobacterial photobionts produce compounds that differ from free-living cyanobacteria and are unique to symbiotic organisms.[12] Lichen secondary metabolites have a role in communication and maintaining is poorly understood still now. Metagenomic screening, re-engineering and re-construction of lichen derived bio-synthetic gene pool open a new era to invaluable benefits of natural products.[13]

The lichen secondary biomolecules have attracted the attention of scientists for over 100 years about structural uniqueness, evolutionary significant origin, and phylogenetic importance. Lichens synthesize unique secondary metabolites. About 1050 lichen substances are identified through analytical techniques and experimental methods.[14] Lichen secondary biomolecules such as usnic acid, polyphenols, anthraquinones, dibenzofurans, depsides, depsidones, depsones, triterpenes, gamma-lactones, and pulvinic acid derivatives show different biological activities such as antiviral, antibiotic, antiproliferative, antiallergenic, plant growth inhibitory, and enzyme inhibitory effect. The polyketide biosynthetic pathway appears to be responsible for most of the classes of lichen compounds, whereas pulvinic acids are shikimate derivatives, and the abundance of di- and triterpenoids found in lichens is formed through the mevalonate pathway. Large numbers of studies report the methods of isolation and characterization of individual components of lichen extracts. The ability of certain lichen metabolites to reduce the mutagenicity of chemical mutagens has attracted moderate attention. The depsidones and physodic and physodalic acid prevent the formation of reactive metabolites by blocking the oxidation systems present in the hepatic microsomal fraction. The para-depside gyrophoric and diffractaic acid (DA) also inhibit the proliferation of human keratinocyte cells. Human epidermoid carcinoma cells are inhibited by ursolic acid through affecting tyrosine kinase activity. The use of ursolic acid for the manufacture of an anticancer agent for suppressing metastasis has been patented.[15]

TECHNICAL DESCRIPTION

UL is greenish-white or grayish-green fruticose and pendant; main branches are cylindrical in shape, almost 3–4 m long, with many dense, short perpendicular side branches and fibrils of about equal length (3–40 mm) [Figure 1]. The cortex is smooth and not thoroughly joined on the main stems, forming uneven spot of whitish medulla on light brown central cord. The covered central cord under the cortex layer is whitish, but usually turns into reddish-brown decorticated central axis. Papillae are lacking. Soredia or isidia occasionally forms on the side branches. They are disk shaped, 1–3 mm across, terminal on the ends of side branches, with numerous fibrils extending from the thalline margin.[16]

CHEMISTRY

Usnea longissima found in different parts of India shows different colours in spot test or colour test which indicates the presence of different chemicals in different regions.

Central cord shows Iodine test positive

Figure 1: External morphology of *Usnea longissima* collected from: Sandakphu, Latitude: 27° 03′ 36.00″ N; Longitude: 88° 00′ 0.00″ E

Cortex and medulla shows K –ve (K test negative)

C-ve (C test negative)

KC–ve(KC test negative)

PD–ve (PD test negative)

I test=Iodine test

K test =potassium hydroxide test

C test=bleach test

PD test= para-phenylenediamine test

Cortex and medulla of *Usnea longissima* contains various combinations of evernic acid, diffractaic acid, barbatic and 4-o-demethylbarbatic acid and sometimes with usnic acid.

IDENTIFICATION TIPS

UL is a well-known lichen species. *Usnea* species have a central cord at the center of lichen thallus surrounded by medulla and outer cortex layer. Fruticose pendant lichen genera such as *Alectoria* and *Ramalina* do not have characteristic central axis like of *Usnea* species. UL is distinguished from other *Usnea* species by the extremely long, mostly unbranched main strands, which have perpendicular side branches and fibrils, and a patchy surface due to the eroded cortex. Short fragments of *Usnea* species may be confused with small fragments of other pendant specimens, but only one difference is that UL has a I + violet or dark bluish central cord and eroding cortex.[17]

Study population

Range, distribution, and abundance

Historically, this taxon is fairly common and nearly circumboreal in distribution, occurring in North India, Northeastern Himalaya, Himachal Pradesh, Arunachal Pradesh, Sikkim, and even in Kashmir. Although about 8% of the terrestrial ecosystem consists of lichens and more than 20,000 lichen species are distributed throughout the world, their biological activities and biologically active compounds remain unexplored in great extent. *Usnea* species is endemic fruticose lichen that grows on different trees and shrubs in the northern Western Ghats of India. It is a common lichen in temperate and alpine Himalayas.[18]

Chemical constituents of Usnea sp.

Secondary metabolite compounds from chloroform fraction of UL are isolated and identified, and antibacterial activity test has been performed. The chemical compounds are isolated using gravity column chromatography and thin-layered chromatography. The result showed the needle crystal-shaped isolated compound with translucent white color. The 1D-NMR (^{1}H and ^{13}C-NMR) data analysis and comparison of the similar data from the isolated compound literature was (5E, 6E) 5-ethylidene-7-formil-6,7-dihydroxy methyl hept-6-enoate reviewed.[19]

Various chromatographic techniques including silica gel, Sephadex LH-20, operational data store, and semipreparative high-performance liquid chromatography were used for isolation of 16 compounds from lichen UL. By spectroscopic data analyses, their structures were identified as (1) useanol, (2) lecanorin, (3) 3-hydroxy-5-methylphenyl 2-hydroxy-4-methoxy-6-methylbenzoate, (4) lecanorin E, (5) 3'-methylevernic acid, (6) evernic acid (EA), (7) barbatinic acid, (8) 3,7-dihydroxy-1,9-dimethyldibenzofuran, (9) orcinol, (10) omethylorcinol, (11) methyl orsellinate, (12) methyl everninate, (13) 2,5-dimethyl-1,3-benzenediol, (14) 2-hydroxy-4-methoxy-3,6-dimethyl benzoic acid, (15) ethyl everninate, and (16) ethyl 2,4-dihydroxy-6-methylbenzoate. Useanol is the natural product and 3, 4, 8, 10, 12, and 13 were isolated from Usneaceae family for the first time. Compound 1, 8, and 13 showed significant anti-inflammatory activity against nitric oxide production in RAW 267.4 cells with IC_{50} values of 6.8, 3.9, and 4.8 μmol/L, respectively, compared with the positive controls curcumin (IC_{50} 15.3 μmol/L) and indomethacin (IC_{50} 42.9 μmol/L).[20]

Densitometric determination of evernic (EV) and (+)-usnic acids (USN) in *For Usnea aciculifera no need of short form.*

UA = usnic acid

Usnea aciculifera ≠ *(UA)*, *U. ghattensis*, UL, and *U. stigmatoides* (US) was performed with a simple sensitive thin-layer chromatography. Comparative antioxidant activity revealed that US is a better free radical scavenger in comparison with other three *Usnea* species.[21]

Furthermore, these results indicated that USN and EV are not solely responsible for antioxidant potential, but it may be due to the synergistic effect UL (*Usnea longissima*) and it is used in pharmaceuticals, food, and cosmetics. UL possess different types of chemicals such as EA, barbatic acid (BA), DA, and usnic acid (UA) and shows a wide range of biological activity. All parameters, such as lower limit of quantitation, linearity, specificity, precision, accuracy, extraction recovery, matrix effect, and stability, were within appropriate limits and suitable for biological specimen analysis.[20]

Pharmacological activity of Usnea sp.

Lichens are complex symbiotic organisms consisting of multicellular fungal mycobiont and unicellular algal phycobiont. For very slow growth rates and often stressful living situations, lichens produce various protective secondary metabolites. Several lichen extracts and their compounds have been used in traditional medicine in Europe, Asia, and North America. Lichen metabolites shows different types of biological activities such as antibiotic, antimycotic, antiviral, anti-inflammatory, analgesic, antipyretic, antiproliferative, immunomodulatory, cytotoxic, antioxidant, antiherbivore, antibacterial, antigenotoxic and antitumor activity.[22]

Usnic acid from *Usnea* sp. is used as a traditional medicine to cure respiratory problems, skin infections, and external ulcers. It is still used today in Traditional Chinese Medicine in liquid extract and tincture to treat tuberculosis (TB) lymphadenitis.

ANTIMICROBIAL ACTIVITY

Usnic acid is a good medication for human papillomavirus treatment and as an oral hygiene agent with limited effectiveness. Antimicrobial activity of acetone, methanol, and ethanol extracts of *Usnea* species was investigated *in vitro* for testing micro-organisms where some of them cause diseases in human, animal, and plant and produce toxins and promote food deterioration. Lichens synthesize numerous metabolites called lichen substances, including aliphatic, cycloaliphatic, aromatic, and terpenic components. Mycobiont of lichen produces antimicrobial secondary metabolites that save many animals from disease-causing pathogens. Antibiotic properties of lichens are investigated a lot.[23] Vartia reported antimicrobial properties of several lichens against Gram-positive and Gram-negative bacteria which have been studied earlier by different scientists. For new drug discovery, the search for novel natural bioactive compounds is receiving attention. Nowdays new strains of multiple drug-resistant pathogens become resistant to previously prepared standard drugs.[11] Manojlovic (1951) earlier reported the medicinal properties of fruticose lichen *Usnea philippina*.[10] Manojlovic *et al.* tested the biological activities of these lichens and other fruticose lichens, e.g., *Usnea* sp., *Ramalina* sp., and *Stereocaulon* sp., and reported their inhibitory activities against Gram-positive bacteria such as *Micrococcus pyogenes*, *Bacillus subtilis*, and acid-fast bacilli. They are also reported to produce secondary metabolites with antimicrobial activity. Though lichens are the novel source of potential drugs, the biological potentiality of lichens is inadequately studied. Interestingly, the latter

is known to have acquired resistance against major anti-TB drugs due to incomplete or partial treatment and necessitates treatment with new antibiotics. The demand of bioactive secondary molecules becomes the primary concern because infectious pathogens are continuously emerging and reemerging. For example, *Mycobacterium tuberculosis* infects approximately 9 million new individuals every year, with 1.7 million deaths annually.[24]

ANTIBACTERIAL ACTIVITY

The antibacterial bioactivity test using the method of paper disc diffusion showed that the chloroform extract inhibited the bacterial growth at the concentration of 100 ppm, 250 ppm, 500 ppm, and 1000 ppm for *E. coli* ATCC 35218, *S. aureus* ATCC 25923, and *S. typhi* YCTC. (1) Ethyl hematommate, (2) friedelin, (3) beta-amyrin, (4) beta-sitosterol, (5) methyl-2,4-dihydroxy-3,6-dimethylbenzoate, (6) barbatinic acid, (7) zeorin, (8) ethyl orsellinate, (9) 3 beta-hydroxy-glutin-5-ene, (10) oleanolic acid, (11) (+)-usnic acid, (12) methylorsellinate, and (13) 4-methyl-2,6-dihydroxy-benzaldehyde are the thirteen compounds obtained and isolated.[20]

Lichens also show antibacterial activity against a wide range of pathogenic microbial species. UL is an epiphyte species of lichen belongs to the family *Parmeliaceae*. Lichenic acids isolated from UL are growth inhibitors. UL was used as a dermatological aid for wounds in the Pacific Northwest. The ethanol extract of UL showed potential antibacterial activity and antifungal activity using agar well diffusion method. UL was screened their level of antimicrobial potential.[25] *Usnea ghattensis* endemic fruticose lichen found growing luxuriantly in the northern Western Ghats of India. Usnic acid is a major chemical from UL and tested against some human pathogenic bacteria. Usnic acid was used for *in vitro* antimicrobial activity test and broth microdilution method was used for confirmation of minimum inhibitory concentration using according to the NCCLS guidelines. The present study demonstrates the relatively higher activity of this lichen against Gram-positive, but significantly also against Gram-negative bacteria. Following objectives were taken for screening of antibacterial activity of *Usnea* sp. Antibacterial activity of ethanolic and methanolic extracts of *Usnea* sp. was done.[26]

ANTITUMOR ACTIVITY

Tumor growth depends on angiogenesis and inducing angiogenesis is one of the most important hallmarks in the cancer development. Treatment with small molecules that inhibit angiogenesis has been an effective strategy for anticancer therapy. Some antiangiogenic factors are derived from traditional Chinese herbs. Usnic acid (UA), an active compound mainly found in lichens, has shown some biological and physiological activities. However, the role and mechanism of UA in tumor angiogenesis are still unknown. The aim of this study was to assess the effects of UA on tumor angiogenesis. In this study, we demonstrated that UA strongly inhibited *in vivo* angiogenesis in a chick embryo chorioallantoic membrane assay and vascular endothelial growth factor-induced mouse corneal angiogenesis model. In a mouse xenograft tumor model, UA suppressed Bcap-37 breast tumor growth and angiogenesis without affecting mice body weight. In an *in vitro* assay, UA not only significantly inhibited endothelial cell proliferation, migration, and tube formation, but also induced morphological changes and apoptosis in endothelial cells. In addition, UA inhibited Bcap-37 tumor cell proliferation. Moreover, Western blot analysis of cell signaling molecules indicated that UA blocked vascular endothelial growth factor receptor (VEGFR) 2-mediated extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) and AKT/P70S6K signaling pathways in endothelial cells. These results provided the first evidence of the biological function and molecular mechanism of UA in tumor angiogenesis.[27]

ANTIVIRAL ACTIVITY

A bacterium is a microscopic single-celled organism. A virus is a small infectious agent that cause infections to living cells. Bacteria can be relatively easy to kill, but viruses are a different matter. When a person has a virus infection, the virus exists within the person's cells. To kill the virus can mean killing your own cells. This may be a problem. Some herbs such as *Usnea* species are effective against viruses. It was investigated that common viral infections such as herpes simplex and Epstein–Barr are inhibited by metabolites of *Usnea* herb. The *Usnea* are their own delicate medicinal miracles. They have a special inhibitory effect for rebalancing bacteria and inhibit infection throughout gastrointestinal tract and urinary tract and on. This medicine has a precious value for people suffering from lung and bronchial infection and often shows symptoms such as yellow or green phlegm, chest pain, and difficulty in breathing.[28]

ANTIFUNGAL ACTIVITY

*Usne*a is an antimicrobial herb and has an antifungal activity. Different types of fungal infections, including *Candida* species, are killed by *Usnea* herb. Fungal infections such as athlete's foot, jock itch, dandruff, ringworm, and vaginal infections can be treated by *Usnea* herb. Fungal infections can be especially difficult to get rid of, so this herb is very beneficial. In general, diet and other lifestyle consideration will also have to be taken into effect and other herbs may be needed for extensive periods of time to fully resolve the issue. These metabolites exert a wide variety of biological action. India is a rich center of biodiversity contributing nearly 15% of the 13,500 species of lichens. Dyspepsia, bleeding piles, bronchitis, scabies, stomach disorders, and many disorders of blood and heart are cured by lichen species of Himalayan region. Even though manifold activities of lichen metabolites have now been recognized, their therapeutic potential has yet not been explored and thus remains pharmaceutically unexploited.[29] Lichens are good sources of biologically active secondary metabolites. In America and in Europe, lichens are used to treat wounds, stomach diseases, and whooping cough. Because lichens offer alternative sources, Usnic acid was identified as an antifungal agent.[30] *Cetraria islandica* (Parmeliaceae) and *Lobaria pulmonaria* are used for the treatment of pulmonary TB. The use of lichens in medicine is based on the fact that they contain unique and varied biologically active substances, mainly with antimicrobial actions. Lichens have antimicrobial activity and secondary metabolites of lichens and vascular plants attract great attention of scientists as new significant sources of bioactive substances. Many investigators have evaluated the bioactivity of lichen extracts and the isolated constituents against serious infectious organisms.[31] Modern science is given a foundation for exploration of lichen species and their chemical constituents by investigating traditional uses of these lichen herbs. UL is widely used as an expectorant, as a wound dressing, and to stanch nose bleeding, as well as in the treatment of ulcers in the folk medicine of different countries of the world. It has also been used,[32] in the treatment of injuries of the legs and loins, bone fractures, and skin eruptions. Usnic acid isolated from *Usnea* species has been widely used in the pharmaceutical and cosmetic industries because of its high antimicrobial, antitermite, and antioxidant activities.[33] In addition, preclinical studies have permitted researchers to hypothesize about its possible use as an antineoplastic agent.[34] Aflatoxin B1 (AFB1) is known to cause hepatotoxicity, teratogenicity, immunotoxicity, genotoxicity, and even death in animals and humans.[15] Although the mechanism of cellular damage caused by AFB1 has not been fully elucidated,[35] reactive oxygen species (ROS), lipid peroxidation (LPO), and direct binding to DNA have been considered to be main mechanisms in the toxicity of AFB.[36] LPO in membranes by attaching to unsaturated fatty acids is caused by ROS through damaging membrane proteins, which causes a degradation of membrane permeability, enzymes and receptor activities,

and activation of cells. Free radicals causes mutations on DNA that results cancer. Therefore, antioxidants have attracted much interest with respect to their protective effective against the free radical damage that may be a reason for many diseases, including cancer.[36]

In this way, antioxidant molecules prevent genotoxic damage. Sister chromatid exchange and micronucleus tests were performed for the study, the antigenotoxic effect of UME against AFB1, which supply sensitive and rapid monitoring of created genetic loss as primary DNA damage in human lymphocyte cell culture *in vitro*. The role of enzyme systems was determined by measuring the superoxide dismutase and glutathione peroxidase enzyme activities and the malondialdehyde levels in the human blood culture on the basis of antigenotoxic effects. Antimicrobial agent inhibits the growth of micro-organisms such as bacteria, fungi, and protozoan. Antimicrobial drugs both kill or prevent the growth of microbes. Infectious diseases cause deaths of one-third of all deaths worldwide.[37]

The spread of multidrug-resistant strains of microbes makes it necessary to discover new classes of antimicrobial and compounds that inhibit these resistance mechanisms. Antibiotic resistance has become a global concern. The efficiency of different existing antibiotics is being threatened by the upgrowing multidrug-resistant pathogens. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic micro-organisms has led to the screening of several medicinal plants for their antimicrobial activity.[38]

Whole world is frantically in search of new antibiotics due to their in appropriate and indiscriminate use. In search of new antibiotics, herbs and plants are being used. One of the plants contained of natural medicinal compounds are made from lichen. Lichen is one of the oldest plants that possess approximately 100,000 species in the world. In many countries, lichen is used as a mixture of herbs and natural herbs because it can cure with various diseases, such as anticancer, antifungi, antioxidant, and antimalaria.[35] There are approximately 350 chemical compounds having biological activity isolated from lichen, and more than 200 compounds have been characterized.[36] The study of chemo-taxonomy shows that the secondary metabolite of lichen plants is from the group of depsida, depsidon, dibenzofuran, and xanthone.[39] Some of xanthone compounds have toxic activities against some types of cancer cells and antimalarial activity against *Plasmodium falciparum*.[40] Medicine ingredients are prepared from lichen herbs according to their substance composition. Its substances are used for antibiotics, antifungi, antivirus, anti-inflammation, analgesic, antipyretics, antiproliferative, and cytotoxic effects.[26] Lichen is an indicator species for air pollution.It is sensitive to toxic air pollutants so that it is useful as an early warning indicator to monitor the environmental pollution.[18] In general, most of the secondary metabolites of lichen has ability of inhibiting the activities of bacteria and fungi.[28] Atranorin isolated from *Cladonia foliacea* and *Usnea* sp. shows the activity of antibacterial either the type of Gram-positive or Gram-negative bacteria.[41] Chloroatranorin from *Pseudevernia furfuracea* can inhibit the activities of bacteria and yeast.[42] Lecanoric acid shows the activity of inhibiting bacteria and fungi.[43] Protolichesterinic acid from *Cetraria aculeate* shows the activity of antibacterial.[44] Hence, isolation and identification of the secondary metabolic content structure of lichen from chloroform fraction of UL and its antibacterial activity against *E. coli* ATCC35218, *S. aureus* ATCC25923, and *S. typhi* YCTC is very important.

ANTIOXIDANT PROPERTIES

Some lichen extracts and metabolites have already been reported for antioxidant properties due to their phenolic content; for instance, the antioxidant activities of some depsides and depsidones isolated from several lichen species have been demonstrated as well as the *in vitro* properties of some lichen extracts. In general, antioxidant activity has been mainly evaluated based on some chemical assays, such as DPPH free radical scavenging activity, superoxide anion radical scavenging activity, and LPO inhibition. Methanol arises as one of the most used solvents for an efficient extraction of lichens bioactive compounds with antioxidant activities, and therefore, many antioxidant activity assays have been performed on methanol extracts.[34] The present review reports the biological activity of more than 75 different lichen species, as well as more than 65 purified metabolites, isolated from these or other species. The study of their antioxidant activities has recently been started and they have been determined by various chemical *in vitro* assays as first approach, with some of them showing interesting results. Further knowledge of this potential implies deeper research on their activities to understand the implied mechanisms.

Concerning antioxidation, the most interesting compounds are polyphenols. Many phenolic hydroxyl groups are present in lichens, which causes antioxidant properties of polyphenols, which are very good free radical scavengers. For instance, phenolic compounds can donate hydrogen to reactive radicals and break the chain reaction of lipid oxidation at the initiation step.[45]

Then, the strong antioxidant activity shown by some lichen extracts or metabolites, and assessed by different systems, can be attributed to their high total polyphenolic contents (specially depsides, depsidones, dibenzofurans, etc.), because a positive correlation between phenolic composition and antioxidant activity has been proved for most of them.[46] At least, it suggests that polyphenols might be the major antioxidant compounds in studied lichens. Some studies also reported that some lichens show no positive correlation between antioxidant activity and total phenolic content. Therefore, different other minor compounds should not be neglected. Antioxidant activity may be correlated to nonphenolic compounds in lichen.

Based on molecular data mainly, they are leading to a more complex classification of lichen families and species.[6] Moreover, phylogenetic analysis of biosynthetic genes can facilitate the discovery of novel compounds and novel genes. Phylogenetic analysis of biosynthetic gene clusters found in a lichen genome may invent unknown pharmacological relevant compounds. Considering the difficulties still found for the *in vitro* culture of lichens and different culture conditions result in different antioxidant activities of lichen extracts (due to the production of different amount and type of secondary metabolites depending on culture characteristics),[47] a global approach to the lichen metabolomic features seems to be crucial for the development of new and viable biotechnological processes. In this way, unique isolated antioxidant compounds production will be increased.

CLINICAL APPLICATION

Most serious clinical problems are vancomycin-resistant enterococci (VRE) and methicillin-resistant Staphylococci, most notably methicillin-resistant *S. aureus* (MRSA). Medicines such as arsenal have limited effect, and new mutants make the situation worse. We studied the activity of (+)-usnic acid, an old lichen-derived drug, and its sodium salt against clinical isolates of VRE and MRSA using the agar diffusion and minimal inhibitory concentration (MIC) methods. The acid and especially the sodium salt had potent antimicrobial activity against all clinical isolates of VRE and MRSA studied.[48] The MIC values of the sodium salt against VRE strains ranged between 4 and 16 μg/ml (1-day test) and between 4 and 31 μg/ml (2-day test), being below 8 μg/ml for most strains. The salt had potent activity even against those strains that were not inhibited by ampicillin (125 μg/ml), and it never lost its activity after 24 h, in contrast to ampicillin. Thus, except that, usnic acid may cause a toxic reaction in some cases, and its salt derivatives may be used

in clinical practices where other therapies have failed to resist mutant stains.

CONCLUSION

Usnic acid, a dibenzofuran derivative, is a common lichen metabolite and is biosynthesized in large amounts by many lichen species. It contains a chiral carbon atom in its chemical molecular structure and therefore it exists as the D- or L-form or as a racemic mixture in nature. It is widely used in many cosmetic and medical products such as medical creams, deodorants, toothpaste, dental mouthwash, and sunscreens.[49] In alternative medicine, it has also been used as an analgesic, antipyretic, antiseptic, wound healing agent, and expectorant.[50] Its analgesic, antibacterial, antiprotozoal, anti-inflammatory, antiulcer, hepatotoxic, antioxidant, cytotoxic, and antiproliferative properties have been reported.[51-53] According to P. Pramyothin (2004) and L. O. Hanug natrium salt of usnic acid is used to prepare medicines in United States and some European countries. It has also been found to be an inhibitor of breast, vulva, and lung cancers. In Europe, usnic acid is still used in the treatment of superficial infections either alone or together with other antibiotics.[54-56]

In particular, the *Usnea* species are rich species in terms of usnic acid content and these species synthesize up to approximately 6% usnic acid of their own weight. UL contain a high content of usnic acid. Other important aromatic metabolites in UL are diffractaic acid, BA, EA, fizot acid, salazinic acid, fumarprotocetraric acid, ramalinolic acid, fizodal acid, squamatic acid, orsinol, and atronorin. Recently, there have been some reports on the use in nanotechnology of lichen and lichen metabolites. For instance, nanoparticles of usnic acid which have an antifungal activity against some human pathogenic fungi were produced from mycobionts of UL in the culture medium.[57]

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Conflicts of interest

There are no conflicts of interest.

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