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REVIEW

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The genus *Usnea*: a potent phytomedicine with multifarious ethnobotany, phytochemistry and pharmacology

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The genus *Usnea* Adans. (Parmeliaceae; lichenized Ascomycetes) is a typical group of mostly pale grayish-green fruticoselichens that grow as leafless mini-shrubs. More than 360 species of *Usnea* are known in the world. *Usnea* has long been thought to have treat various illnesses in addition to its historical use as dyes, cosmetics, preservatives, and deodorants, particularly in eastern countries such as China, Japan, Taiwan, India and Europe. The current review focuses on the traditional uses and phytochemistry aspects of different *Usnea* species, and discusses the pharmacological findings and toxicology of their extracts and isolated compounds. The available compilation of data will provide a new base for future perspectives and highlight the need for further studies of this potent herbal source to harvest more beneficial therapeutic drugs. Nineteen species of the genus *Usnea* are found to be important folk medicines all over the world. It is evident from the comparative analysis of the searched literature that the genus *Usnea* has been used for various purposes for centuries and its long and traditional medicinal history was well documented in the past. As per ancient records and recent scientific literature, the species of genus *Usnea* have been used as promising traditional medicines, exerting an array of therapeutic properties to relieve sore throats, bronchitis, cold, flu, infection, and indigestion. Phytochemical analysis confirms the general presence of a wide range of metabolites, polysaccharides, fatty acids, phenolic acids, flavonoids, terpenes, sterols, depsides, depsidones, and benzofurans. As specific constituents, usnic acid, polyphenols, and depsides have been considered as main efficacy component for antibacterial and antifungal activities. In addition, pharmacological analysis also revealed that other pure compounds and crude extracts of *Usnea* species prove to be significant anti-cancer, anti-proliferative, anti-oxidant, anti-viral, anti-inflammatory, anti-ulcer, hepatoprotective, and anti-genotoxic agents. However, there is a need for more precise investigations to examine the clinical value of both isolated pure compounds and crude extracts and to elucidate their mechanisms of action. Apart from clinical validation and elucidation of their mechanism of action, biosafety studies of the compounds are also important to legitimately use the potential bioactive compounds for the further development of future lead drugs.

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1. Introduction

Lichens are an obligate mutualism between a fungus (mycobiont) and one or more photosynthetic organisms, an alga or cyanobacterium (photobiont).[1,2] Typically the fungal partner delivers most of the composite organism's structure and mass, hence trading physical protection for carbohydrates manufactured by the photosynthetic partner. These organisms represent a unique division in the plant kingdom and are the most successful symbiotic organisms in nature, dominating 8% or more of the earth's terrestrial area.[2–4]

Lichens have been shown to produce a number of primary and secondary metabolites that may protect them against physical stresses or biological attack.[5,6] Some lichen species and their metabolites have been used for medicinal and industrial purposes.[7–10] Among the medicinal lichens, the genus *Usnea* Adans. (Parmeliaceae; lichenized Ascomycetes) is edible and is utilized in the preparation of traditional foods and medicines in both Eastern and Western countries.[11,12] This genus is regarded as one of the taxonomically most difficult genera of macrolichens.[13] Most of the species are globally distributed with more

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than 350 species and highly variable in morphology. Many species are also very variable in chemistry, and may include several chemotypes.[14] In India, 57 species of *Usnea* are known which grow luxuriantly in higher regions of Western Ghats and Himalaya.[15,16] Many species are also very variable in chemistry, and may include several chemotypes. The species of *Usnea* are known to be used in traditional medicines, in dyeing and in spices in various parts of the country.[15,17] The first recorded use of the *Usnea* species in traditional Chinese medicine dates to 101 B.C., when it was used as an antimicrobial agent under the Chinese name of Song Lo. Song Lo tea or its decoction has also been recorded for internal and external detoxification of the liver, treatment of malaria, wounds, snake bite, and cough.[18] In *Unani* literature, the medicinal uses of *Usnea* species are mentioned as astringent, antidote, analgesic, cardiotonic, resolvent and stomachic.[17,19–21]

Along with its emerging position in the herbal market, primary as well as secondary metabolites (extrolites), the chemical constituents of *Usnea* species have been broadly investigated. Usnic acid, protocetraric acid, barbatic acid, norstictic acid, salazinic acid, and stictic acid were characterized as the main bioactive chemical constituents in Indian species.[17,22,23] All the major secondary metabolites in *Usnea* belong to aromatic products formed from β-orcinol units, while ceparatic acid and protolichesterinic acid belong to higher aliphatic acids. Most of these metabolites are unique to *Usnea* lichens, being of great significance for systematics and phylogeny, and are employed at different taxonomic levels from species and subspecific to generic and higher ranks.[24,25] Furthermore, these bioactive metabolites play important ecological roles in nature such as UV protection and defense against predators and pathogenic microorganisms.[1,26]

Significant research has been done on *Usnea* and its metabolites which confirm various biological activities including anti-microbial, anti-oxidant, anti-tumor, anti-viral, anti-inflammatory, cardiovascular protective, and hepatoprotective properties.[17,20,22,27–33] These are closely correlated with the ethno-medicinal uses. Recent pharmacological studies have revealed significant anti-cancer, anti-genotoxic, anti-proliferative, and anti-neoplastic activities and these potentials have further put *Usnea* under the spotlight.[29,34–36] The aim of this review is to summarize the recent advances in phytochemistry and pharmacology of the genus *Usnea*. Phylogenetic and toxicological aspects are also given in brief.

2. Botanical characterization and distribution

The genus *Usnea* is highly diverse, with more than 350 estimated species, distributed in polar, temperate and tropical regions. This genus is characterized by fruticose habit and especially by the presence of a cartilaginous central axis. Dillenius (1742) first proposed the name *Usnea* in *Historia muscorum*.[37] The genus was placed in family Usneaceae until studies on apothecial ontogeny and ascus apical structures proved that *Usnea* belongs to the family Parmeliaceae.[38] One can easily recognize *Usnea* in fields by its shrubby to pendent greenish yellow thallus, radial symmetry and presence of central cartilaginous axis.[15] There are three forms of fruticose thallus in *Usnea*, erect/bushy when the thallus is small (*U. orientalis*), a long pendulous thallus hanging from tree branches (*U. angulate* and *U. longissima*) or a sub-pendent thallus of intermediate length (*U. aciculifera* and *U. rubicunda*). Dominant branching patterns including dichotomous, sub-dichotomous and sympodial are observed. The basic characteristics used for identification of *Usnea* up to species level include morphological features like the habit of the thallus, branching pattern, pigmentation of basal part, presence or absence of sorelia together with its morphology, isidia, pseudocyphellae, papillae, tubercles, fibrils, faveolae and shape of branches; anatomical features like the ratio of thickness of the cortex (C), medulla (M) and central axis (A), the compactness of fungal hyphae in medulla and the presence or absence of specific secondary metabolites. A combination of morphological, anatomical and chemical characters can be used to delimit species.

Usneasensu lato comprises of an assemblage of approximate 350 species worldwide.[15] The species belonging to the genus *Usnea* contain usnic acid, a bioactive compound, which imparts a yellow colour to the thalli. Using modern concepts, the taxonomist divided *Usnea* into three genera, *i.e.*, genus *Dolichousnea*, genus *Eumitria* and genus *Usnea sensu stricto*. The genus *Dolichousnea* is characterized by an annular pseudocyphellae between the segments, solid central axis and positive iodine reaction of central axis. The genus *Eumitria* is characterized by a fistulose central axis whereas genus *Usnea sensu stricto* is characterized by the absence of annular pseudocyphellae and a solid, I – central axis.

Usnea is a cosmopolitan genus occurring on all continents. Species diversity, however, is low in arid and arctic areas and is highest in humid regions of temperate latitudes. *U. aciculifera* is found in Eastern Asia and *U. angulate* is distributed in Australia, America and West Africa. *U. baileyi* is found in pantropical countries in world. *U. compressa* is widely distributed in India and Nepal. *U. fragilis* is known in the South-East Asian region. *U. himalayana* is from the Himalayas, Western Ghats and Africa. *U. indica* is endemic to North-West Himalayas and found in Uttarakhand in India. *U. ghattensis* is endemic to Western Ghats. *U. luridorufa* is found in North and South Asia. *U. nepalensis* is found in Himalayas and Western Ghats in India. Several species including *U. orientalis*, *U. pangiana*, *U. sinensis*, and *U. perplexans* are widely distributed in North-East Asia. *U. pseudosinensis* and *U. robusta* are restricted only to the Himalayas. *U. subflorida* is distributed in East Africa and North Asia, while *U. subfloridana* is found in Europe and North East Asia. *U. undulata* is found in South and East Africa.[39–41]

U. longissima is distributed throughout the Northern temperate zones, such as the sub-arctic and the coastal rainforests of Europe, Asia and North America.[41,42] In India, the species is distributed in North-Eastern Himalayan regions between 1500–4000 m altitudes in moist old mixed forests of *Quercus* and *Pinus*. Seven chemosyndromes of *U. longissima* are reported from India which include barbatic acid, squamatic acid, diffractaic acid, evernic acid, fumaroprotocetraric acid and usnic

acid strains. *U. longissima* is characterized by fruticose, a pendulous thallus of 60 cm long or more, of pale yellow-greyish green to light brown, a 0.5–1.0 mm diameter main branch, a 2–5 cm long, dense perpendicular usually decorticated or pulverulent to powdery lateral branches, sorediate or isidiate, with a colorless central lattice. Apothecia range from being rare to up to 5 mm in diameter, with ciliate margin. The species has more than seven chemotypes containing barbatic, squamatic, diffractiatic, evernic, fumarprotocetraric, and usnic acids.[41]

3. Phylogeny and classification

The phylogeny and classification of *Usnea* have been a matter of debate, given the lack of phenotypic characters to describe phylogenetic clades and the low degree of resolution of phylogenetic trees.[37] Motyka (1936–38) proposed a classification of *Usnea*, in which all fruticose lichens with an inner, cartilaginous tissue are included. He identified six subgenera: *Euusnea*, *Protousnea*, *Lethariella*, *Chlorea*, *Neuropogon*, and *Eumitria*.[43] Later *Protousnea* and *Lethariella* (including *Chlorea*) were elevated to generic rank by Krog (1976).[44] The position of *Neuropogon* as a subgenus to *Usnea* was accepted by several authors.[43] Krog (1982) suggested a classification of genera (usneoid *e.g. Neuropogon*, *Protousnea*, *Evernia*, *Letharia*, *Lethariella*).[45] In this hypothesis *Neuropogon* and *Usnea* are sister groups. *Protousnea* and *Evernia* form together the sister to the clade, comprising of *Neuropogon* and *Usnea*. Finally, *Letharia* and *Lethariella* form the sister group to the other usneoid genera. But gradually many diverse classifications have been proposed due to a lack of phenotypic characters.

A study based on the ITS sequence data supported the subgenera *Eumitria* and *Usnea*, and revealed a new subgenus, *Dolichousnea*.[46] The authors also concluded that *Usnea* contains at least three taxa at subgeneric level, *Usnea*, *Eumitria*, and *Dolichousnea*. *Neuropogon* was not included in this study and the position remained unclear. Molecular phylogenies based on the ITS-LSU nrDNA and part of the β-tubulin region have been used to examine the position of *Neuropogon* in *Usnea* s. lat.[43] Bayesian inference and maximum parsimony strongly supported the monophyly of *Neuropogon*. Subgenus *Usnea* and *Neuropogon* form a strongly supported group with subg. *Eumitria* and subg. *Dolichousnea* is a consecutive monophyletic sister group. The following generic classification was proposed: *Usnea* (subgenus *Usnea* only), *Neuropogon*, *Eumitria*, and *Dolichousnea*. *Dolichousnea* is elevated to generic rank. The following new combinations are made: *Dolichousnea* Articus, *D. longissima* (Ach.) Articus, *D. trichodeoides* (Vain.) Articus, *D. diffracta* (Vain.) Articus, and *Eumitria pectinata* (Taylor) Articus.[43] Recently, the phylogenetic relationships of 52 *Usnea* species from across the genus, based on ITS rDNA, nuLSU, and two protein-coding genes RPB1 and MCM7 have been investigated. The phylogeny based on the concatenated dataset revealed that the genus *Usnea* is subdivided into four highly-supported clades, corresponding to the traditionally circumscribed subgenera *Eumitria*, *Dolichousnea*, *Neuropogon* and *Usnea*.[47] However, characteristics that have been used to describe these clades are often homoplasious within the phylogeny and their parallel evolution is suggested. The study has suggested that combinations of phenotypic characters are suitable discriminators for delimitating species, but are inadequate to describe generic subdivisions.

4. Traditional uses and ethnopharmacology

The species of lichen genus *Usnea* is used for the treatment of various diseases such as diarrhea, ulcer, urinary infection, tuberculosis, pneumonia, stomachache, anti-fungal, human pathogens, and cattle fungal diseases.[48–51] Some other uses of the species are for strengthening, hair growth, sterility cure, flavoring agent, pulmonary disease, antiseptic, anti-tuberculosis and anti-viral diseases are summarized in Table 1.[52–60] The *Usnea* species are the most common source of antibiotic and antifungal lichen acids, particularly usnic acid. The species have widespread potential for medicinal applications. *Usnea* is used for weight loss, pain relief, fever control, and wound healing; and to make phlegm easier to cough up.[61,62] It was recorded that *Usnea* had been used directly on the skin for sore mouth and throat.

U. longissima grows commonly on bark, mostly on the twigs of trees, bushes and over soil and rock in temperate and alpine regions of India, it is known locally as "Syara" by the Bhotia and Garhwalis of remote areas of Uttarkashi district of Uttarakhand, India and is used for making pillows. The Baiga tribes of Madhya Pradesh, India used the species along with other ingredients for treating bone fractures.[62,63] Likewise the ancient Greeks used lichen as medicines. Hippocrates recommended a lichen, perhaps *U. barbata*, for uterine complaints. The Chinese used *U. longissima* as an expectorant and as a powder application to heal external ulcers in the name "Sun-Lo".[1] It is also a major ingredient of Chinese medicine.[57] In China, this species of *Usnea* is also called as "Lao-tzu's" beard, "Pine gauze" and used for stopping sweating dizziness cold, pain and phlegm. *U. longissima* is still utilizing today as a tincture to treat tuberculosis lymphadenitis. In the Bolivian Andes, *U. longissima* is commercially sold as a folk medicine for cough and hoarseness.[7,12] The Nitinaht Indians of Vancouver also used the species for wound dressing in Turkey.[63] *U. longissima* is used in the treatment of gastric ulcers by the Anatolians as a folk medicine. This species is also used as to strain impurities from hot pitch before the pitch was used as medicine. In Unani medicine, it has been described to stimulate menstruation or induce abortion, taken orally and inserted into the vagina.[63] However, it was used for treating cancer, tuberculosis, and ulcers in Turkey.[64] It has also been used as a decongestant and for the local treatment of ulcers and tuberculosis by Chinese people.[57]

U. barbata is used to treat mammary infections in cattle. The udder is washed several times with a decoction of lichen and used for indigestion in humans, where the tincture or decoction taken orally several times daily.[65] In the Philippines, it has been used for wounds, chopped, and mixed with coconut oil, spread over the wound and for abdominal pain where the decoction is used as a drink.[66] However, in Europe it was used for internal

Table 1 Traditional uses of *Usnea* spp.

Species/folk name	Country	Uses	References
Usnea spp. Dill. ex Adans. *ushna*	Unani medicine of India	Used for heart troubles, for reducing inflammation, for promoting digestion and improving appetite, as an antidote, as an astringent, and as an analgesic. It also helps wounds heal and lactation in women if applied as a paste on breast	157
U. aciculifera Vain.	China	Used for bladder infection, painful urination, urinary retention, swelling, and edema in heart and kidneys	76
U. articulata (L.) Hoffm. *hewas*	Tanzania	Used to treat stomachache. A handful of hewasis chewed fresh and the juice swallowed, it is bitter but relieves the pain	158
U. atlantica Vain. *barbas*	Canary Islands	Used as a disinfectant	159
U. baileyi (Stirt.) Zahlbr.	India	Mixed with other aromatic herbs, such as *Valeriana jatamansi* for favoring and curing tobacco	160
U. barbata (L.) Weber ex F.H. Wigg.	USA	Used to treat fungal infections of the mouth, stomach, intestines, anus, vagina, nose, ear, skin as well as "systematic fungal infection"	11
	South Africa	Applied to treat mammary infections in cattle, the udder is washed several times with decoction of lichen. Also used for indigestion in humans	65
	Nepal	Endangered medicinal lichen banned from raw export	161
	Philippines	Used for wounds, chopped and mixed with coconut oil, spread over wound. Also utilized for abdominal pain, it used as drink decoction	162
tagahumok puti	West Malaysia	Used for colds and strengthening after confinement	163
	Europe	Used to treat insomnia, nausea, and the uterus, also used for internal and external bleeding, whooping cough, jaundice, and growing hair	82
memby rakúíja	Spain	Utilized as drying agent and antiseptic for cracks and irritations of the feet	164
	Brazil	Liquid made from it is given to women to cure sterility	165
U. campestris R. Sant *barba de piedra*	Argentina	Unspecified medicine	166
U. ceratina Ach.	China	Adopted to treat coughs, inflamed lungs, pulmonary tuberculosis, hepatitis, and headache due to heat, infection due to injury, inflamed lymph channels, mastitis, and snakebites	100
U. densirostra Taylor, *U. durietzii* Mot. *yerba de la piedra; barba de piedra*	Argentina	Tea applied externally as astringent, antiseptic, and anti-inflammatory	167
U. diffracta Vain. *lao-jun-xu*, *Lao Tzu's beard*, *pine gauze*, *or female gauze*	China	Utilized to cure cough, tuberculosis of neck or lungs, headache, dizziness, sweating, dim vision, swelling, pus oozing from breasts or sores, burns and scalds, snakebite, traumatic injuries, bone fracture, bleeding from external injuries,	100

Table 1 (*Contd.*)

Species/folk name	Country	Uses	References
		vomiting, blood in feces, bleeding from uterus, menstrual disorders, vaginal discharge, swelling of female genitalia, urinary tract afflictions, parasitic infections, when it used as a drink decoction; or apply decoction or powdered lichen to affected area	
song-nag	Korea	Used to induce menstruation and treated tuberculosis of the neck	11
gser.skud	Tibet	Cured fevers of the lungs, liver, and channels and fever caused by poisoning	74
U.durietzii Mot. [syn. *Neuropogon durietzii*]	Argentina	Same as Argentine use of *U. densirostra*	168
Usnea filipendula Stirt. [syn. *Usnea dasypoga*]	Russia	Powdered form used to treat wounds and some infections	48
U. florida (L.) F. H. Wigg.	China	Used for aching in sinews and bones, stopping bleeding or infection from external injuries, skin diseases, painful urination, coughs, tuberculosis of lungs or neck, heart palpitations, and edema. Drink decoction; or apply decoction or powdered lichen to affected area	57 and 169
	South central Chile	Infusion taken for management of diarrhea	49
	Europe	Decoction used for colds and coughs	51
	Chile	Infusion used for diarrhea	49
U. himalayana C. Bab. *nayonayo saruogase*	Japan	Burned as a "lichen cigarette"	3
U. hirta (L.) F. H. Wigg.	Europe	Used for heal wounds and to prevent hair loss	51
U. laevis (Eschw.) Nyl. *barba de piedra or tusinya*	USA	Utilized to treat dermatosis, fungal infections, tuberculosis, and pneumonia	170
U. longissima Ach.	India	Used as a simple drug to stimulate menstruation or induce abortion, taken orally and inserted into the vagina	171
	China	Used in Chinese medicine especially as an expectorant and in the treatment of ulcers, stop sweating, dizziness cold, pain, phlegm, and stop swelling in female genitalia. Also applied as a decongestant for treatment of ulcers and tuberculosis	57,99 and 102
sun-lo	Mongolia	Used medicinally	7
	Madhya Pradesh, India	Used to treat bone fractures, along with other ingredients	63
	Turkey	Applied for treating cancer, tuberculosis, and ulcers	52 and 53
	Indo-Tibetan Himalayas	Used to heal bone fractures. Washed, air-dried, soaked overnight in salted water, and placed over affected part	172
urmil	Canada	Used to strain impurities out of hot pitch when making medicine, and for other unspecified medicines	173
U. nidifica Taylor *uru nū*	Rarotongan		174

Table 1 (*Contd.*)

Species/folk name	Country	Uses	References
		Thallus chewed and applied to cuts (to stop bleeding) and stings	
U. pectinata Taylor	China	Used for stopping bleeding from external injuries, relieving pain, bloody feces, and swelling	100
U. plicata (L.) Weber	Libya	Used as an ingredient in medicinal decoction called scıba	54 and 55
scíba	Europe	An astringent for internal and external use for whooping cough, jaundice, strengthening stomach and abdominal cavity, and restraining abortion	56
U. strigosa (Ach.) Eaton	Papua New Guinea	Concoction taken orally for headaches	58
U. sikkimensis Biswas sp. nov. *darimataghosa*	India	Used for lung troubles, hemorrhages, and asthma; powdered and used to strengthen hair. Also used to bandage surface wounds, skin eruptions, and boils, when it inserted into nostril to stop nose bleeds; put in shoes to prevent or treat blisters	83 and 84
U. strigosa (Ach.) Eaton *oleazu*	Kimi	Concoction taken orally for curing headaches	58
U. subfloridana Stirt.	Ireland	Applied for treating sore eyes, mixed with tobacco and butter, boiled, cooled, and applied as lotion to eyes	59
	China	Used for painful and reddened eyes, bleeding from external injuries, and swelling	57
U.subsordida Stirt. ayurvedic medicine	India	Same as ayurvedic use of *U. baileyi*	160
U. trichodeoides Vain.	China	Used for coughs, pulmonary tuberculosis, headaches, blurred vision, inflamed cornea, swellings, sores, uterine bleeding, menstrual disorders, and vaginal discharge	57
	Africa, Mt. Kilmanjaro	Used as an ingredient in herbal tea given by African guide to relieve altitude sickness	60

and external bleeding, whooping cough, jaundice, and growing hair.[67] Spanish people used it as drying agent and an antiseptic for cracks and irritations of the feet.[68] In China *U. aciculifera* was used to treat bladder infection, painful urination, urinary retention, swelling, and edema in the heart and kidneys.[57] In Tanzania, *U. articulate* was used for the treatment of stomach-ache.[69] A handful of *U. articulate* and *U. gigas* are chewed fresh and the bitter juice swallowed, relieving pain after a time. In China, *U. ceratina* was used for coughs, inflamed lungs, pulmonary tuberculosis, hepatitis, heat related headaches, infection due to injury, inflamed lymph channels, mastitis, and snakebites.[57]

In traditional Argentinian medicine, teas of *U. densirostra* and *U. durietzii* were used externally as astringents, antiseptics, and anti-inflammatory agents.[70,71] In China, *U. diffracta* has been applied to treat a range of problems such as cough, tuberculosis of neck or lungs, headache, dizziness, sweating, dim vision, swelling, pus oozing from breasts or sores, burns and scalds, snakebite, traumatic injuries, bone fracture, bleeding from external injuries, vomiting blood, blood in feces, bleeding from uterus, menstrual disorders, vaginal discharge, swelling of female genitalia, urinary tract afflictions, and ascarid or schistosoma parasitic infections.[72,73] The same species of *Usnea* were used to cure fevers of the lungs, liver, and heart and fever caused by poisoning in Tibet[74] while in Korea, the species was used to induce menstruation (Pusan) and treat tuberculosis of the neck.[75]

The traditional Chinese herbal medicine, *U. florida* has been used for aching in sinews and bones, stopping bleeding or infection from external injuries, skin diseases, painful urination, coughs, tuberculosis of lungs or neck, heart palpitations, and edema.[76] The decoction of *U. florida* was also used for colds

and coughs in Europe,[77] while in Chile, its infusion is used for diarrhea.[49] *U. himalayana* is burned as a "lichen cigarette" in Japan.[78] *U. hirta* has been used by European people to heal wounds and to prevent hair loss.[77] *U. laevis* has been widely used to treat different kind of microbial infections including dermatosis, fungal infections, tuberculosis, and pneumonia.[50] In Canada, most of the *Usnea* species were used for wound dressing, but *U. longissima* is preferred by wrapping around the wound.[79] It was recorded that *U. pectinata* had been used in China for stopping bleeding from external injuries, relieving pain, bloody feces, and swelling.[76]

U. plicata used as an astringent for internal and external use,[80] also for whooping cough,[81] jaundice, strengthening stomach and abdominal cavity, and restraining abortion in Europe.[82] As the traditional Indian herbal medicine, *U. sikkimensis* has been used for treating lung troubles, hemorrhages and asthma.[83] It has also been used to bandage surface wounds, skin eruptions, and boils.[84] The concoction of *U. strigosa* was taken orally for the treatment of headaches.[58] Moreover, in Ireland *U. subfloridana* was used to treat sore eyes. In China, it was used to treat painful and reddened eyes, bleeding from external injuries, and swelling.[76] The traditional Chinese herbal medicine, *U. trichodeoides* has been used to treat coughs, pulmonary tuberculosis, headaches, blurred vision, inflamed cornea, swellings, sores, and pus discharge, bleeding from external injuries, bloody feces, uterine bleeding, menstrual disorders, and vaginal discharge.

5. Phytochemistry

Recent investigations have revealed that lichens are slow-growing organisms that produce a wide array of secondary metabolites with different pharmacological activities.[1] Lichen secondary metabolites are mostly synthesized from the fungal metabolism. These extrolites are usually deposited as crystals on the surface of cortical and medullary hyphal cell walls, which poorly dilute in water and can usually be isolated from lichen by organic diluents.[85] The chemistry of *Usnea* is cynosure for all applied field researchers because of its wide range of medicinally important primary and secondary metabolites, only known in lichens, with significant variety in biological and biomedical properties. Until now, more than 60 compounds have been identified from *Usnea* species which belong to various classes such as depsidones, depsides, depsones, lactones, quinines, polyphenolics, polysaccharides, fatty acids, and dibenzofurans. Fig. 1–7 show the chemical structure of active compounds collected in Table 2.

5.1. Primary metabolites

To date, few primary metabolites of *Usnea* have been analyzed, however these metabolites have valuable standing compared to other classes of plants due to its medicinal properties which are described below.

5.1.1. Polysaccharides. Polysaccharides, present in the thallus of *Usnea* are categorized into glucan type [β-(1→3)(1→4)], lichenan homoglucan with β-(1→3)(1→4) linkage and pustulan [β-(1→6)]. Shahiba and colleagues described that *U. barbata*, *U. longissima*, and *U. bayleyi* contain lichenan (**1**) homoglucan with β-(1→3) and (1→4) linkages,[86] while *U. fasciata* produces isoliichenan (**2**).[87] Sumanarathna worked on the extraction and isolation of polysaccharides from *Usnea* species and identified the polymers of glucose, galactose and mannose having various linkages such as glucans [(1,3)-β-glucopyranosyl/(1,3)-β-glucopyranosyl] and galactomananns [(*O*-2)-α-D-galactopyranosyl (*O*-4)-α-D-galactopyranosyl (16)-α-D-manopyranosyl].[188]

5.1.2. Fatty acids. A new fatty acid, methyl 3,4-dicarboxy-3-hydroxy-19-oxoeicosanoate (**3**) has been isolated from *U. meridensis*.[88] Additionally, a few more fatty acids isolated from *Usnea* species are bourgeanic acid (**4**) from *U. esperatiana*,[21] and *U. florida*,[89] caperatic acid (**5**) from *U. lapponica*, *U. nipparensis*, *U. orientalis*, and *U. florida* and murolic acid complex from *U. hirta*.[90] Recently, isomuronic acid (**6**), murotic acid, lichesterinic acid (**7**), neuropogolic acid (**8**), protolichesteric acid (**9**) and 18*R*-hydroxydihydroalloprotolichensterinic acid have been isolated and identified from *U. longissima*.[7] so the complete fatty acid profiling of *Usnea* species is not yet available.

5.2. Secondary metabolites

Lichens had to evolve diverse biosynthetic pathways to produce such complex arrays of extrolites. 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 are formed *via* the mevalonate pathway. There are large numbers of studies reporting the isolation and characterization of individual components of lichen extracts.[91] Lichens are a rich source of unique secondary metabolites which are synthesized by the acetyl-polymalonate pathway (APP), shikimic acid pathway (SAP), and mevalonic acid pathway (MAP). All pathways are initiated by a central precursor molecule, acetyl-co A which is the main product of glucose catabolism but the most important pathway for lichen is APP which exerts unique metabolites *i.e.* depsides, depsidones *etc.* MAP and SAP derive more commonly occurring metabolites such as terpenes, terpenoids, steroids and pulvinic acid derivatives (Fig. 8).[92]

5.2.1. Depsides. Depsides, the major components of *Usnea* are polyphenolic compounds with 2 or more aromatic cyclic rings joined by ester linkage. Aciculiferin A, atranorin (**10**), baeomycesic acid (**11**), barbatic acid (**12**), diffractaic acid (**13**), squamatic acid (**14**), evernic acid (**15**), 4-*O*-demethylbarbatic (**16**), methyl beta-orsellinate (**17**), ethyl orsellinate (**18**), thamnolic acid (**19**), barbatolic acid (**20**), barbatinic acid, ethyl hematommate (**21**), methyl hematommate (**22**), alectorialic acid (**23**), 7-hydroxy-5-methoxy-6-methylphthalide (**24**), methyl-2,4-dihydroxy-3,6-dimethylbenzoate (**25**), and methyl beta orcinol carboxylate (**26**) have been found in various species of *Usnea*, presented in Table 2.[93–97]

5.2.2. Depsidones. The depsidones are not only composed of two or more aromatic cyclic rings but also bonded by ether linkage. To date, various species of *Usnea* were explored for the

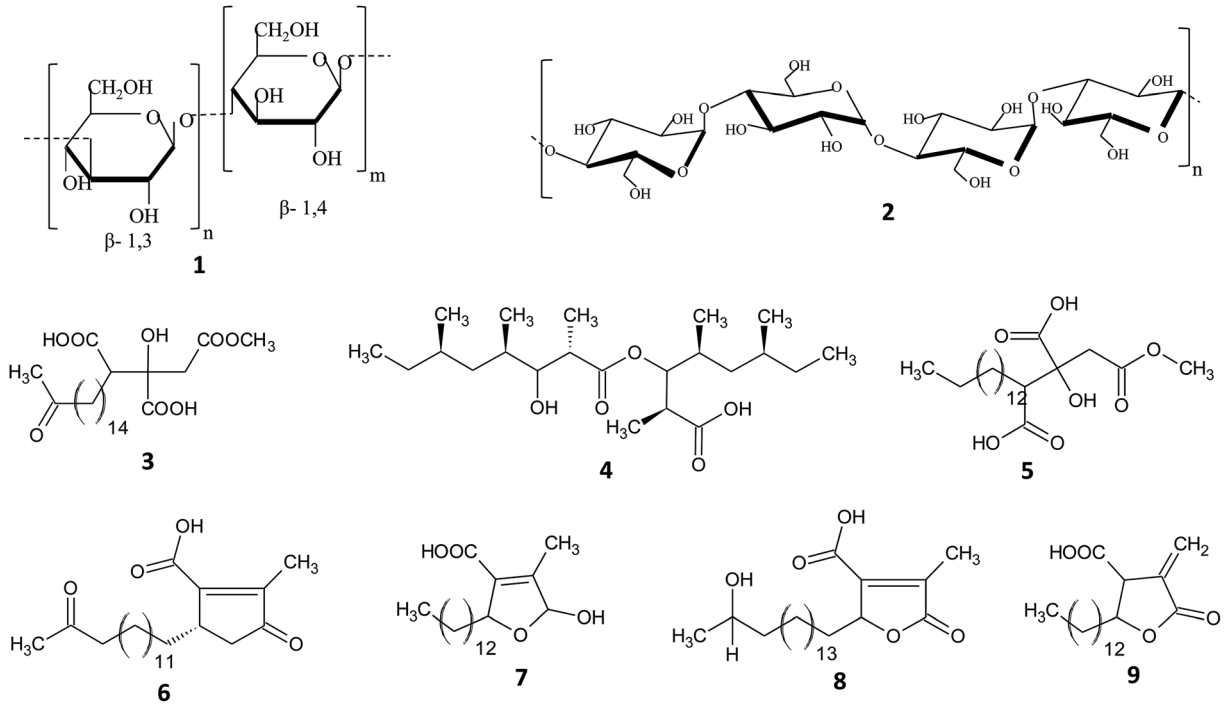

Fig. 1 Chemical structures of compounds **1–9** from *Usnea* spp.

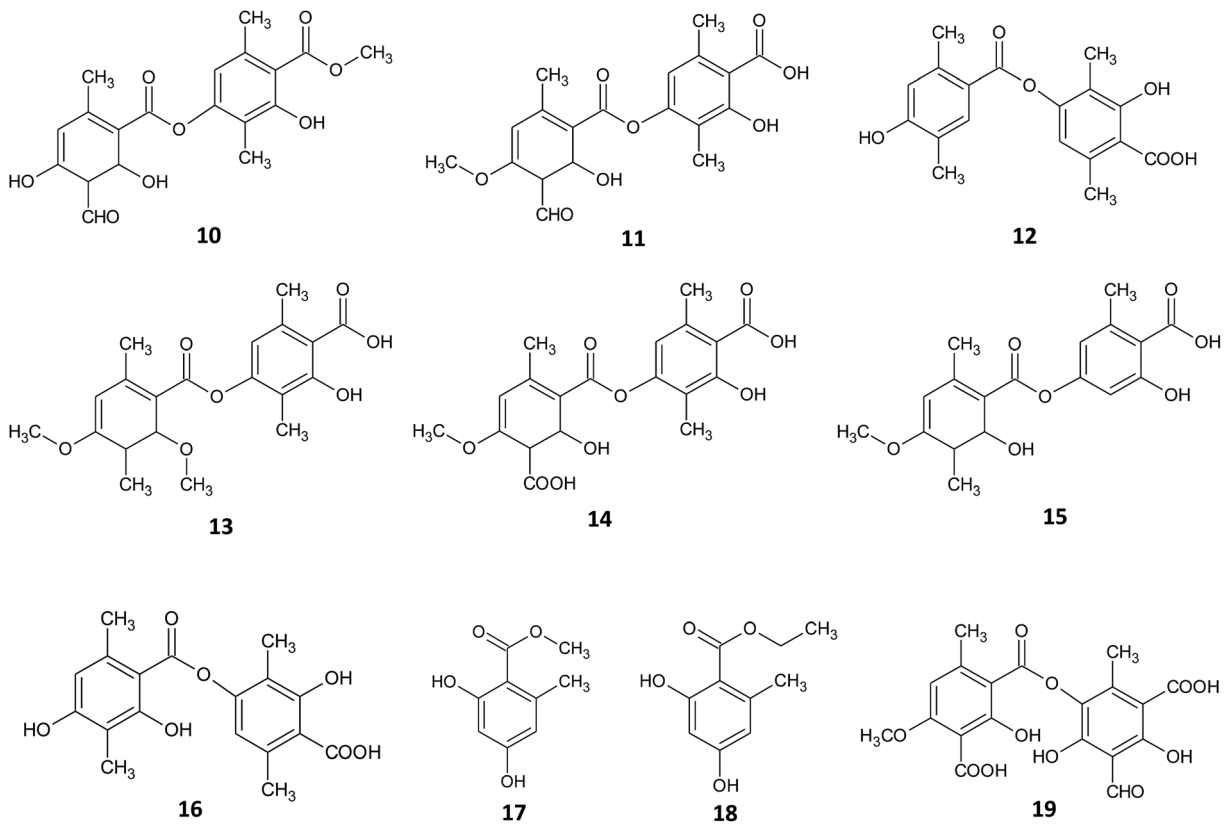

Fig. 2 Chemical structures of compounds **10–19** from *Usnea* spp.

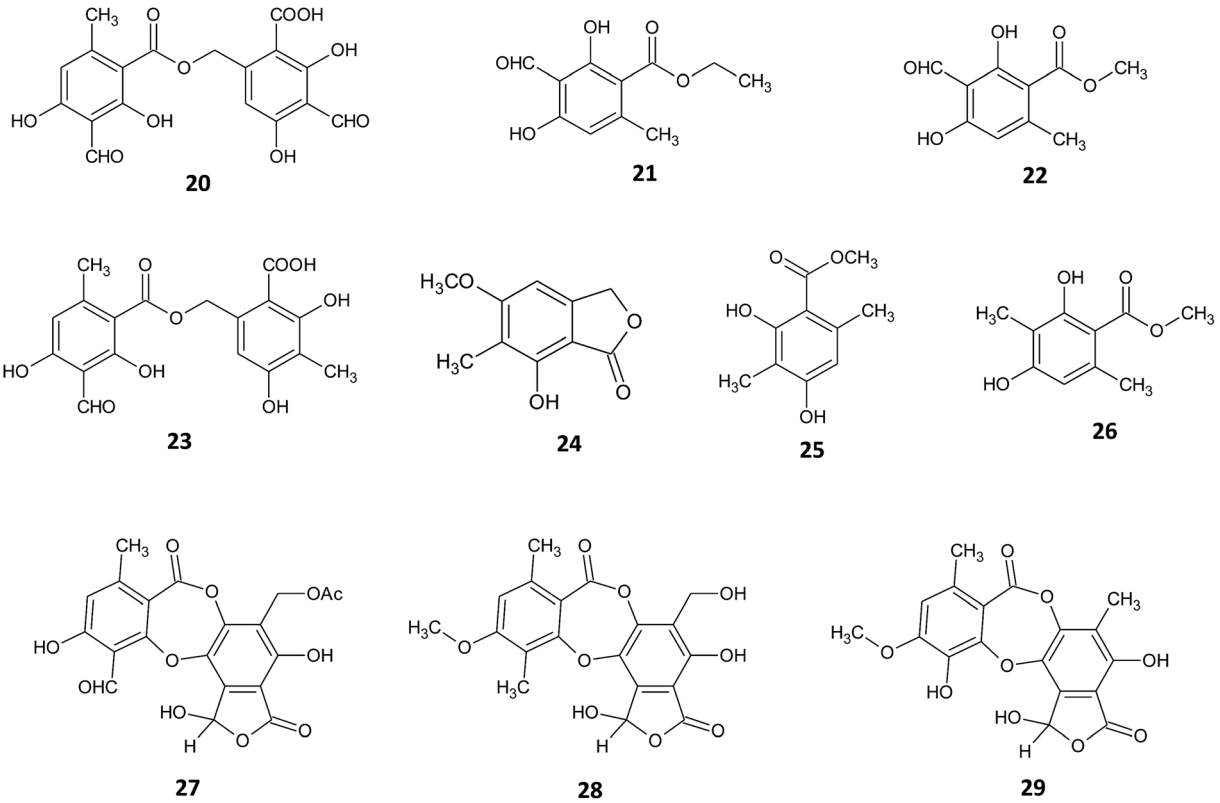

Fig. 3 Chemical structures of compounds 20–29 from *Usnea* spp.

identification of depsidone compounds and observed to synthesize galbinic acid (**27**), hypoconstictic acid (**28**), menegazziaic acid (**29**), norstictic acid (**30**), constictic acid (**31**), virensic acid, salazinic acid (**32**), lobaric acid (**33**), protocetraric acid (**34**), psoromic acid (**35**), and 2-*O*-methylhypostictic acid (**36**), stictic acid (**37**), fumaprotocetraric acid (**38**), and cryptostitic acid (**39**) (Table 2).[52,93,98]

5.2.3. Terpenes and terpenoids. Two terpenes, (glutinol (**40**) and beta-amyrin (**41**)) and three terpenoids, (friedelin (**42**), oleanolic acid (**43**), and zeorin (**44**)) were extracted and characterized from *U. longissima*.[99,100]

5.2.4. Sterols. Laxinamujila and colleagues extracted two sterols, 5,8-epidioxy-5alpha, 8alpha-ergosta-6,22*E*-dien-3beta-ol (**45**) and ergosterol (**46**) from *U. longissima*.[17] Moreover, beta-sitosterol (**47**) has also been isolated from the same lichen, the most common phytosterol.[101]

5.2.5. Benzofurans. A most significant benzofuran, usnic acid (**48**) is found in all species of *Usnea*. Some new benzofurans such as ethyl 2-(3,3-bis(7-acetyl-4,6-dihydroxy-3,5-dimethylbenzofuran-2-yl)acryloyl),[102] ethyl 4-(7-acetyl-4,6-dihydroxy-3,5-dimethyl-2-oxo-2,3-dihydrobenzofuran-3-yl)-4-(7-acetyl-4,6-dihydroxy-3,5-dimethylbenzofuran-2-yl)-3 oxo-butanoate (**49**), (*Z*)-2-acetyl-5,5-bis(7-acetyl-4,6-dihydroxy-3,5-dimeth-ylbenzofuran-2-yl)-4-hydroxy-penta-2,4-dienal, (7-acetyl-*C*(7-acetyl-2,3-dihydro-4,6-dihydro-3,5-dimethyl-2-oxo)-3-benzofuranyl)-4,6-dihydroxy-3,5-dimethyl-β-oxo-ethyl ester, (4*aR*,9*bS*)-2,6-diactyl-3,4*a*,7,9-tetrahydroxy-8,9*b*-dimethyl-1-oxo-1,4,4*a*,9*b*-tetrahydrodibenzo[*b*,*d*] furan diethanone, longiusnine,[17] and 2-benzofuranbutanoic acid[102] have recently been isolated from *U. longissima*.

5.3. Miscellaneous

Two new phenolic compounds, longissiminone A (**50**) and longissiminone B (**51**) have been extracted from *U. longissima*.[99] A new *O*-deoxyglycoside of dimeric tetrahydroxanthane and hirtusneanoside (**52**) identified in *U. hirta*[103] and anthraquinone and longissimausnone (**53**) have been isolated from *U. longissima*.[104] Yellow pigments such as eumitrins A1, A2, and B were also isolated from *U. bayleyi*.[105] A new flavanoid glycoside, apigenin 7-*O*′-D-glucuronide (**54**) and primary phenolic compounds such as atranol (**55**) and orcinol (**56**) have been characterized in the extract of *U. longissima*,[17] which plays a key role in the synthesis of depsides and depsidones. Recently, our group has isolated and identified an anti-candidal flavonoid, quercetin (**57**) from *U. longissima*.[28] Two alcoholic compounds, arabitol (**58**) and octanol (**59**) were also isolated from *U. longissima*.[17] Two new phenylalanine diketopiperazines have also been found, ambewelamide A (**60**) and B (**61**) from the chloroform extract of *Usnea* species.[106]

6. Pharmacological properties

Usnea species have been used as anti-microbial agents in different regions of the world and a number of formulations were developed as modern pharmaceuticals just prior to the advent of the penicillin antibiotics. Numerous investigations on

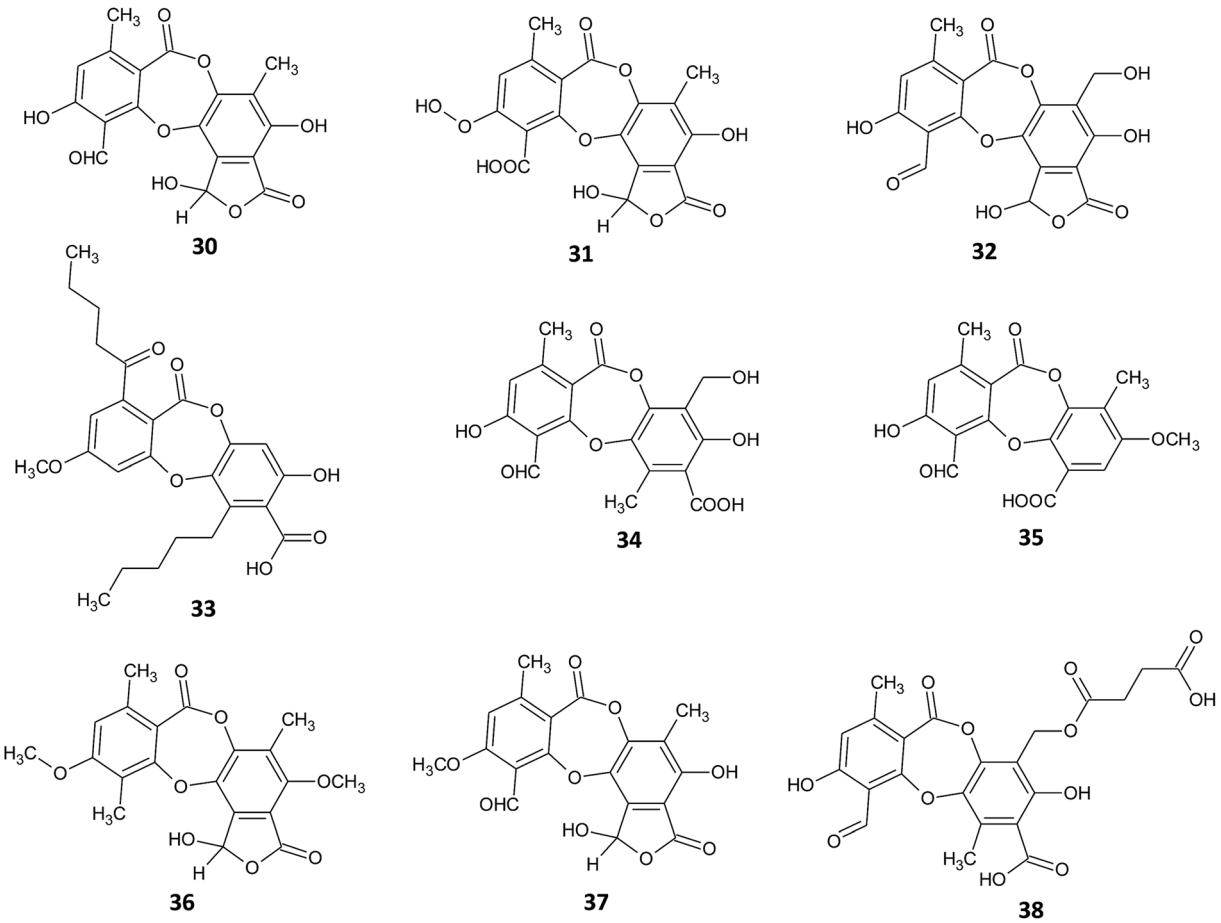

Fig. 4 Chemical structures of compounds 30–38 from *Usnea* spp.

the pharmacological properties of the *Usnea* species have enlightened their efficacious remedy for various illnesses. Tables 3 and 4, respectively, report the pharmacological activities of extracts and bioactive constituents obtained from different species of *Usnea*.[107–110]

6.1. Antimicrobial activity

6.1.1. Anti-fungal. Our group has isolated a dietary flavonoid quercetin (QC) from *U. longissima*, which sensitizes fluconazole (FCZ)-resistant *C. albicans* to induce FCZ-mediated cell death by modulating the quorum sensing (QS) system. QC (200 μg mL^{-1}) inhibited the secretion of *C. albicans* virulence factors, namely biofilm formation, hyphal development, phospholipase, proteinase, esterase, and hemolysin. It has also demonstrated that the sensitizing effect of QC was associated with the production of farnesol, a QS molecule that acts as a regulator of virulence factors of *C. albicans*.[28] Protocetaric acid (PA) was characterized from ethyl acetate extract of *U. albopunctata* using spectroscopic methods. PA was found to be a broad spectrum antimicrobial agent against medically important human pathogenic microbes. At 1 μg mL^{-1} of concentration, ethyl acetate extract showed significant antifungal activity against *Trichophyton rubrum*, compared to reference antifungal agents such as PA and amphotericin B.[22] The results suggested that *U. albopunctata* may contain also other antifungal compounds which show synergistic action.

Two new metabolites, depside and isodivaricatic acid and three known metabolites, 5-propylresorcinol, divaricatinic acid and usnic acid were isolated from *U. florida*. These metabolites displayed antimicrobial activity against human pathogenic fungi *Microsporum gypseum*, *Trichophyton mentagrophytes*, and *T. rubrum*. Among them, isodivaricatic and divaricatinic acids exhibited antifungal effect towards *M. gypseum*, *T. mentagrophytes*, and *T. rubrum* with minimum inhibitory concentration (MIC) values of 50, 50, and 100 μg mL^{-1}, respectively. However, isodivaricatic acid was found to be effective against *Leishmania amazonensis*, *L. brasiliensis*, and *L. infantumpromastigotes* by inducing 100% lysis at 100 μg mL^{-1}.[111]

6.1.2. Antibacterial. The novel multifunctional hydroxyphenylimino ligands such as L1, L2, and L3 were synthesized through the condensation of 2-aminophenol, 3-aminophenol, and 4-aminophenol with usnic acid, respectively.[112] The synthesized ligands and their complexes, Cu(II), Co(II), Ni(II) and Mn(II) were characterized using FT-IR, UV-Vis, $^{(1)}$H-NMR, $^{(13)}$C-NMR, 1D- and 2D NMR (DEPT, COSY, HMQC and HMBC), LC-MS, and TGA. The ligands and their complexes were tested against ten important pathogenic microorganisms, such as *Enterobacter aerogenes*, *Brevibacillus brevis*, *Micrococcus luteus*,

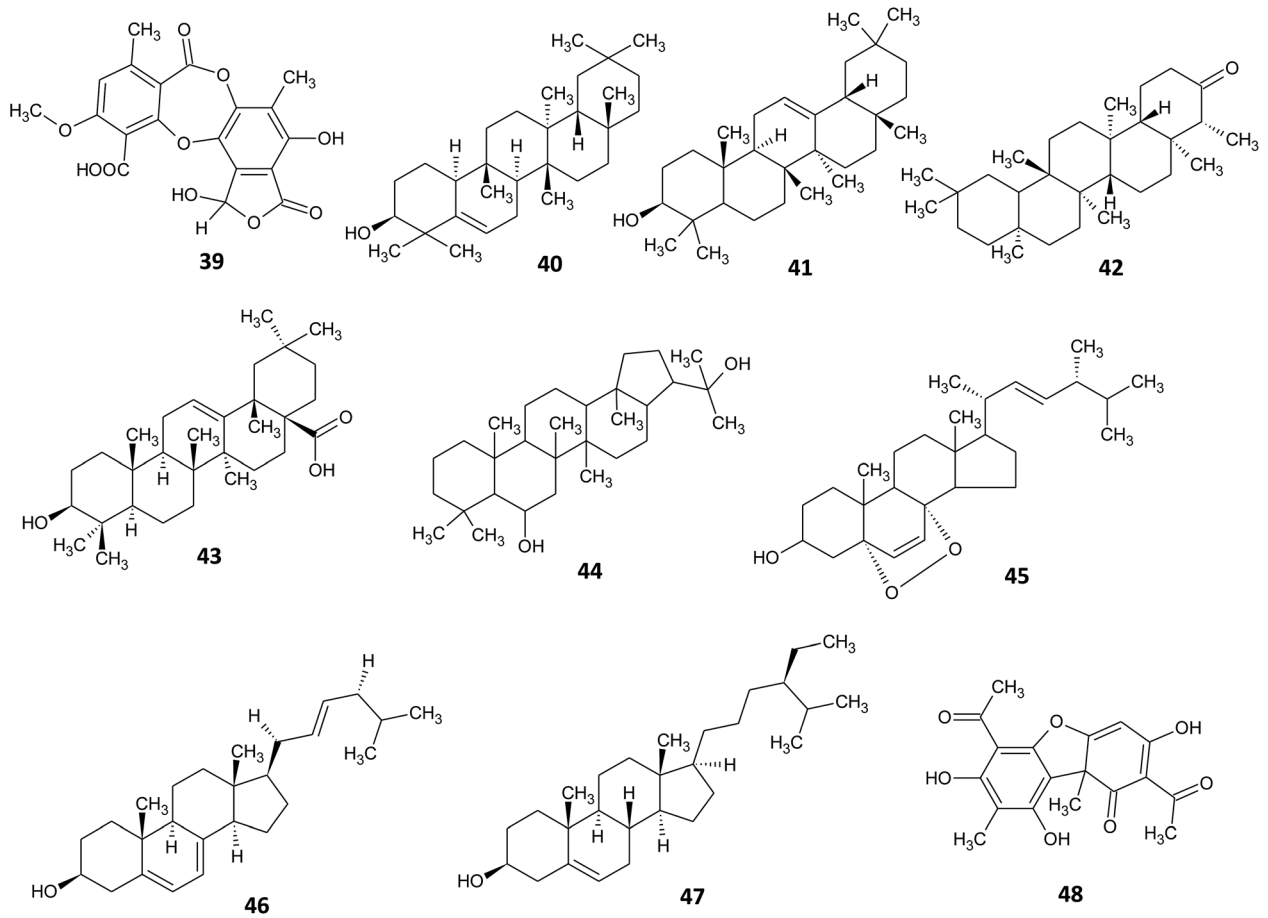

Fig. 5 Chemical structures of compounds 39–48 from *Usnea* spp.

Escherichia coli, *Bacillus megaterium*, *Pseudomonas aeruginosa*, *E. cloacae*, *Streptococcus aureus*, *C. albicans*, and *Saccharomyces cerevisiae*. The metal complexes of the ligands were found to be more effective against all of the microorganisms tested, exhibiting 11–32 mm inhibition zones around the ligands. On the other hand, a broad spectrum antimicrobial activity was observed for the Mn(II) and Cu(II) complexes of the hydroxyphenylimino ligand (L3) with usnic acid.[112]

Extracts of *U. ghattensis* were prepared using different organic solvents and their antibacterial activity was determined using a disc diffusion assay. The ethanolic extract was most effective against *B. cereus*, *P. aeruginosa*, *S. aureus*, and *Streptococcus faecalis* with MIC values of 3.125, 200, 6.25, and 25 μg mL^{-1}, respectively. Acetone and methanolic extracts presented almost similar effect against *S. aureus*.[113] L-(−)-Usnic acid was isolated from *U. subfloridana* and showed promising antibacterial against methicillin-resistant *S. aureus* (MRSA). The MIC of L-(−)-usnic acid against MRSA was recorded by 50 μg mL^{-1}. Similarly, a combined effect of L-(−)-usnic acid and 7.5% sodium chloride resulted in a reduced number of viable cells within 24 h compared to the control.[114] Furthermore, an *in vivo* study showed that L-(−)-usnic acid significantly ($p < 0.001$) reduced the microbial load of rat spleen in a dose-dependent manner (1 to 5 mg kg^{-1}).

The antibacterial activity of *U. steineri* was evaluated against *Mycobacterium tuberculosis*, *M. kansasii*, and *M. avium*. The (+)-usnic acid rich acetone extract displayed promising MIC values of 32 μg mL^{-1} for *M. tuberculosis* and 62 μg mL^{-1} for both *M. kansasii*, and *M. avium*.[21,115] Acetone and methanol extracts of *U. lapponica* were screened against four pathogenic bacteria, namely *S. aureus*, *E. coli*, *P. aeruginosa*, and MRSA. The extracts inhibited growth of all tested bacteria except *E. coli*. Usnic acid was identified as the major active antimicrobial compound in the extracts. The acetone extract was found to be particularly active against MRSA and *P. aeruginosa* with a MIC value of 15.6 μg mL^{-1}.[116] A new formulation of *U. barbata* extract was developed using alkyl polyglucoside surfactants as a vehicle to examine the antimicrobial potential for skin infections. This formulation has implausible potential against Gram positive bacteria.[117]

A study was performed to assess the *in vitro* effect of usnic acid isolated from *U. dasypoga* against clinical isolates and standard *Helicobacter pylori* strains. The dual susceptibility rate to usnic acid and clarithromycin was detected as very high (97.3%). Usnic acid had a strong and dose-dependent activity against *H. pylori* strains. The synergism between usnic acid and clarithromycin was also observed and it may be effective in the treatment of *H. pylori* infection.[118] Ethanolic and methanolic

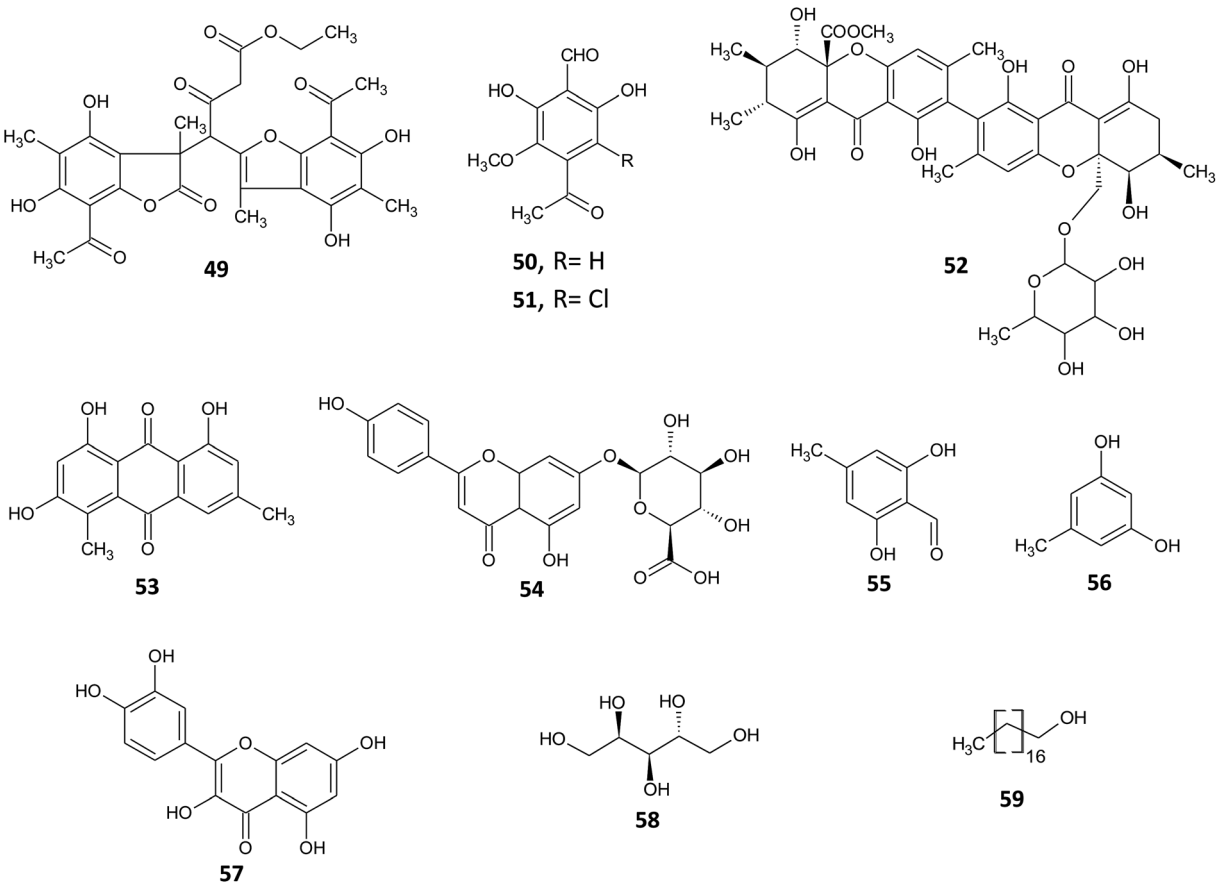

Fig. 6 Chemical structures of compounds 49–59 from *Usnea* spp.

extracts of *Usnea* species showed a zone of inhibition against some pathogenic bacterial strains, *S. aureus*, *P. aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhiand*, and *E. coli*.[112,119]

6.1.3. Anti-viral. The acetone extract of *U. complanta* exhibited significant antiviral activity against herpes simplex viruses (HSV) at a concentration non-toxic to the Vero cell line using cytopathic effect inhibition and virus yield reduction assays. The recorded IC_{50} value was 100 μg mL^{-1}.[120]

6.2. Antioxidant

The polyphenolic nature of the major secondary metabolites of the *Usnea* species is expected to afford antioxidant activity and a range of *in vitro* investigations have already been carried out on this issue with promising results. In general, antioxidant activity has been mainly evaluated based on some chemical *in vitro* assays, such as free radical quenching activity, reducing power and lipid peroxidation inhibition. Among organic solvent, ethanol and methanol have been used as the most efficient and suitable solvents for the extraction of metabolites with antioxidant properties from *Usnea*. Usnic and psoromic acids were extracted from the submerged cultivation of *U. complanata*. Different organic solvents including ethanol, methanol, ethyl acetate, and acetone were used for the preparation of extracts to determine their antioxidant activity. Except for the methanolic extract, other extracts exhibited antioxidative action in terms of free radical scavenging activity (FRSA), nitric oxide radical scavenging activity, and anti-lipid peroxidation potential with IC_{50} values ranging from 22.86 to

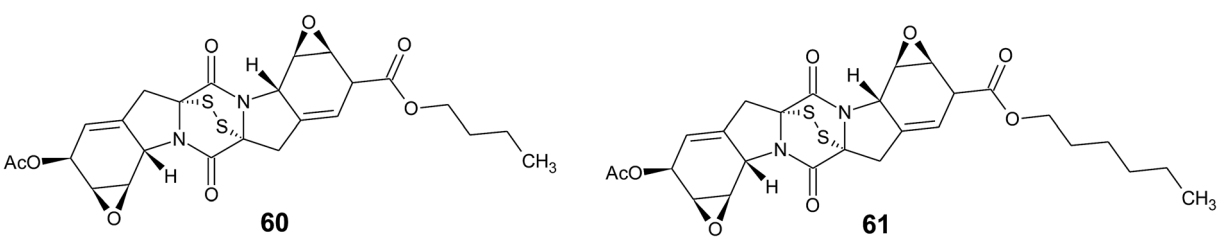

Fig. 7 Chemical structures of compounds 59–61 from *Usnea* spp.

Table 2 Bioactive constituents of *Usnea* spp.

S. No.	Chemical constituent	Source	References
	(A) Polysachharides		
1	Lichenan	*U. barbata*, *U. longissima*, *U. bayleyi*	86
2	Isolichenin	*U. fasciata*	87
	(B) Fatty acids		
3	18*R*-Hydroxydihydroallopr-otolichensterinic acid	*U. longissima*	175
4	Murotic acid	*U. longissima*	175
5	Iso-muronic acid	*U. longissima*	102
6	Lichesterinic acid	*U. longissima*	149
7	Neuropogolic acid	*U. longissima*	149
8	Bourgeanic acid	*U. esperatiana*, *U. florida*	4 and 89
9	Caperatic acid	*U. lapponica*, *U. angulata*, *U. nipparensis*, *U. orientalis*, *U. florida*, *U. sinensis*	89
10	Methyl 3,4-dicarboxy-3-hydroxy-19-oxoeicosanoate	*U. meridensis*	88
	(C) Depsides		
11	Aciculiferin A	*U. aciculifera*	95
12	Atranorin	*U. aciculifera*, *U. articulate*	95 and 96
13	Baeomycesic acid	*U. pacificana*	20
14	4-*O*-Demethylbarbatic acid	*U. longissima*	109
15	Barbatic acid	*U. diplotypes*, *U. fulvoreaquens*, *U. lapponica*, *U. pacificana*, *U. substerilis*, *U. wasmuthii*, *U. pangiana*, *U. dendritica*, *U. fragilis*, *U. norketti*, *U. nilgirica*, *U. certaina*	22
16	Diffractaic acid	*U. longissima*, *U. baileyi*, *U. aciculifera*, *U. certaina*, *U. fulvoreagens*, *U. diffracta*	22,97 and 109
17	3*b*-Hydroxy-glutin-5-ene	*U. longissima*	104
18	7-Hydroxy-5-methoxy-6-methylphthalide	*U. aciculifera*	95
19	Alectorialic acid	*U. dendritica*, *U. florida*, *U. subflorida*	22
20	Methyl hematommate	*U. aciculifera*	95
21	Ethyl hematommate	*U. longissima*	104
22	Ethyl orsellinate	*U. longissima*	104
23	Evernic acid	*U. madeirensis*, *U. longissima*	97
24	Barbatinic acid	*U. longissima*, *U. aciculifera*	95 and 104
25	Barbatolic acid	*U. barbata*	87
26	Methyl orsellinate	*U. longissima*, *U. undulate*, *U. aciculifera*	95 and 104
27	Methyl β-orsellinate	*U. aciculifera*, *U. undulate*	95 and 176
28	Methyl-2,4-dihydroxy-3,6-dimethylbenzoate	*U. longissima*	104
29	Thamnolic acid	*U. subfloridana*, *U. hirta*, *U. florida*	22 and 87
30	Squamatic acid	*U. pacificana*, *U. subfloridana*, *U. fragilescens*, *U. florida*, *U. longissima*	22
31	4-*O*-Demethylbarbatic	*U. dendritica*, *U. longissima*	36
32	Methyl β-orcinol carboxylate	*U. articulate*	93
33	Decarboxy stenosporic acid	*U. diffracta*	15
	(D) Depsidones		
34	2-*O*-Methylhypostictic acid	*U. undulate*	97 and 98
35	Menegazziaic acid	*U. undulate*, *U. aciculifera*	97
36	Norstictic acid	*U. baileyi*, *U. hakonensis*, *U. undulata*, *U. cornuta*, *U. flammea*, *U. frgilescens*, *U. fulvoreagens*, *U. hirta*, *U. wirthi*, *U. aciculifera*, *U. angulate*, *U. vulneraria*, *U. subfloridana*	95 and 97
37	Constictic acid	*U. aciculifera*	36
38	Protolichesterinic acid	*U. albopunctata*	22 and 87
39	Protocetraric acid	*U. albopunctata*, *U. articulta*, *U. glabrata*, *U. madeirensis*, *U. firmula*, *U. dasaea*, *U. maculate*, *U. trichodeoides*	87
40	Psoromic acid	*U. complanata*, *U. bornmuelleri*, *U. dasaea*, *U. inermis*, *U. pseudosinensis*, *U. subfloridana*	22,87 and 177
41	Hypocon stictic acid	*U. undulate*	97
42	Lobaric acid	*U. florida*, *U. barbata*	51
43	Salazinic acid	*U. rubrotincta*, *U. baileyi*, *U. trichodeoides*, *U. pangiana*, *U. longissima*, *U. complanata*, *U. compressa*, *U. corallina*, *U. dendritica*, *U. dasaea*, *U. himalayana*, *U. luridorufa*, *U. norketti*, *U. orientalis*, *U. pangiana*, *U. perplexans*, *U. picta*, *U. rigidula*, *U. robusta*, *U. sordida*, *U. rubicunda*, *U. splendens*, *U. subfloridana*, *U. trichodeoides*, *U. undulate*	87 and 97

Table 2 (*Contd.*)

S. No.	Chemical constituent	Source	References
44	Galbinic acid	*U. undulate*	97
45	Static acid	*U. aciculifera*, *U. cornuta*, *U. flammea*, *U. frgilescens*, *U. fulvoreagens*, *U. bismolliuscula*, *U. complanata*, *U. dasaea*, *U. eumitrioides*, *U. fischeri*, *U. himalayana*, *U. himantodes*, *U. indica*, *U. lucea*, *U. luridorufa*, *U. picta*, *U. pectinata*, *U. nipparensis*, *U. pseudojaponica*, *U. rigidula*, *U. rubicunda*, *U. spinosula*, *U. stigmatoides*, *U. stigmata*	22,87 and 176
46	Fumarprotocetraric acid	*U. articulate*, *U. glabrata*	22
(E) Terpenoids and triterpenes			
47	β-Amyrin	*U. longissima*	104
48	Zeorin	*U. longissima*	104
49	Oleanolic acid	*U. longissima*	104
50	Friedelin	*U. longissima*	104
51	Glutinol	*U. longissima*	99
(F) Benzofurans			
52	Ethyl 2-(3,3-bis(7-acetyl-4,6-dihydroxy-3,5-dimethylbenzofuran-2-yl)acryloyl)	*U. longissima*	102
53	7-Acetyl-*C*((7-acetyl-2,3-dihydro-4,6-dihydro-3,5-dimethyl-2-oxo)-3-benzofuranyl)-4,6-dihydroxy-3,5-dimethyl-*B*-oxo-, ethyl ester	*U. longissima*	175
54	Ethyl 4-(7-acetyl-4,6-dihydroxy-3,5-dimethyl-2-oxo-2,3-dihydrobenzofuran-3-yl)-4-(7-acetyl-4,6-dihydroxy-3,5-dimethylbenzofuran-2-yl)-3-oxobutanoate	*U. longissima*	175
55	(4*aR*,9*bS*)-2,6-Diactyl-3,4*a*,7,9-tetrahydroxy-8,9*b*-dimethyl-1-oxo-1,4,4*a*,9*b*-tetrahydrodibenzo[*b*,*d*] furan diethanone	*U. longissima*	175
56	(*Z*)-2-Acetyl-5,5-bis(7-acetyl-4,6-dihydroxy-3,5-dimethylbenzofuran-2-yl)-4-hydroxypenta-2,4-dienal	*U. longissima*	175
57	3,6-Diacetyl-2,7,9-trihydroxy-8,9*b*-dimethyl-1[9*bH*]-dibenzofuranone (longiusnine)	*U. longissima*	102 and 104
58	Usnic acid	*U. florida*, *U. barbata*, *U. longissima*, *U. rigida*, *U. hirta*, *U. subflorida*, *U. undulate*	97 and 109
59	2-Benzofuranbutanoic acid	*U. longissima*	7
(G) Sterols			
60	β-Sitosterol	*U. longissima*	104
61	Ergosterol	*U. longissima*	175
62	5,8-Epidioxy-5alpha,8alpha-ergosta-6,22*E*-dien-3beta-ol	*U. longissima*	175
(H) Others			
63	Longissiminone A & B	*U. longissima*	99
64	Atranol	*U. aciculifera*	178
65	Quercetin	*U. longissima*	28
66	Longissimausnone	*U. longissima*	104
67	Hirtusneanoside	*U. hirta*	103
68	Orcinol	*U. longissima*	175
69	Apigenin 7-*O*′-D-glucuronide	*U. longissima*	175
70	Eumitrin B, eumitrin A2, eumitrin A1	*U. baileyi*	36
71	Arabitol	*U. longissima*	175

25.0, 141.3 to 149.1, and 125 to 157.9 μg mL^{-1}, respectively. Isolated bioactive compound usnic acid showed FRSA with IC_{50} values ranging from 0.174 to 0.271 mg mL^{-1}.[20] Antioxidant and hepato-protective activities of a cultured lichen *U. ghattensis* have also been observed.[121] The obtained results revealed that at 20 μg mL^{-1} concentration the methanolic extract exhibits 67% inhibition of lipid peroxidation and 86% trolox equivalent antioxidant capacity. At the same concentration, it also showed superior superoxide ($O_2^{\bullet -}$), 1,1-diphenyl-2-picrylhydrazyl, nitric oxide, and hydroxyl ($^{\bullet}OH$) free radical scavenging activities of 89%, 89.6%, 94.8%, and 89.6%, respectively, compared to the synthetic antioxidants, butylated hydroxytoluene, butylated hydroxyanisol, and quercetin. $O_2^{\bullet -}$ scavenging activity and inhibition of lipid peroxidation potential of *U. longissima* was reported. The results were presented in terms of IC_{50} for $O_2^{\bullet -}$ (0.45 mg mL^{-1}) and lipid peroxidation (1.57 mg mL^{-1}).[122]

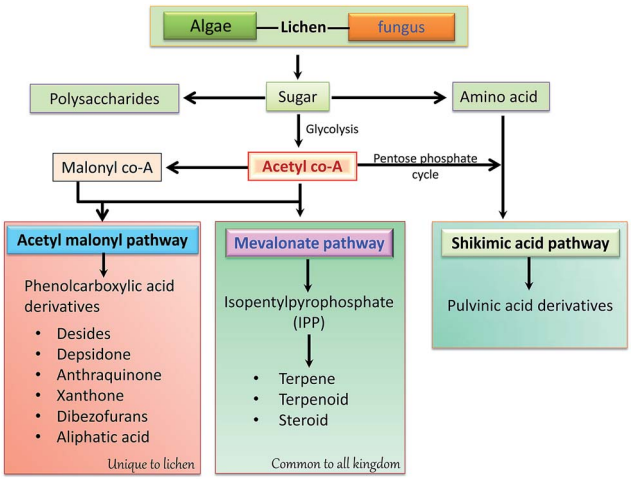

Fig. 8 Secondary metabolites of *Usnea* spp. synthesized by acetyl-polymalonate pathway, shikimic acid pathway and mevalonic acid pathway.

Usnic acid was isolated from the acetone extract of *U. barbata* and its *in vitro* antioxidant potential was examined. The IC_{50} values for the $O_2^{-\bullet}$ scavenging and reducing power were 102.65 and 130.73 μg mL^{-1}, respectively.[19] The methanol extract of *U. ghattensis* has shown good antioxidant potential by inhibiting lipid peroxidation and scavenging free radicals with pretty similar values to those of reference antioxidant compounds.[123] Moreover, *U. longissima* methanol extract was found to increase the level of antioxidant enzymes and inhibit lipid peroxidation[124,125] and its water extract was able to revert the effects of indomethacin *in vivo* through activation of SOD and GST activities and a decrease of CAT activity.[53] A depside, diffractaic acid was characterized as a major metabolite of *U. longissima* and also showed *in vivo* antioxidant properties.[126] Cakir and colleagues demonstrated that the methanol extracts of *U. articulata* and *U. lipendula* showed a protective role against AFB1 in human lymphocytes by enhancing SOD and GPx enzymatic activity and by decreasing lipid peroxidation.[64] Polysaccharides of *U. longissima* (PUS) scavenge the superoxide anion free radical ($O_2^{-\bullet}$) and hydroxyl free radical ($^{\bullet}OH$) with considerable IC_{50} values of 0.45 mg mL^{-1} and 1.57 mg mL^{-1}, respectively.[121] Authors concluded that PUS weakly inhibits the lipid peroxidation of the hepatocyte homogenate of mice. Various extracts of *U. complanata* showed DPPH free radical quenching properties (IC_{50}: 22.86–25 mg mL^{-1}), nitric oxide radical scavenging activity (72.52–149.1 mg mL^{-1}) and lipid peroxidation inhibition (74.58–157.9 mg mL^{-1}). Usnic and psoromic acids were identified as the active substances of the cultured symbiont. Usnic acid demonstrated better radical quenching potential while psoromic acid presented higher lipid peroxidation inhibition.[20]

6.3. Anti-cancer

Several crude extracts and isolated compounds from *Usnea* lichens have been screened against different cancer cell lines showing promising anti-cancer and cytotoxic activities.[127,128] The anti-proliferative effect of *U. filipendula* Stirt. on different human cancer cell lines, including lung cancer (A549 and PC3), liver cancer (Hep3B), and rat glioma (C6) was investigated. In a dose-dependent manner (1.56–100 μg mL^{-1}), the methanolic extract was observed to induce apoptotic cell death with a significant increase in genetic damage in the test cell lines.[129] The (+)-usnic acid and diffractaic acid isolated from the lichens *U. subcavata* and *Usnea* species were evaluated against melanoma UACC-62 and B16-F10 cells. The data from rgw sulforhodamine B assay revealed significant cytotoxic activity of diffractaic acid and usnic acid towards UACC-62 cells with IC_{50} values of 24.7 and 36.6 μg mL^{-1}, respectively. Moreover, IC_{50} values of diffractaic acid and usnic acid against B16-F10 cells were 24.0 and 25.4 μg mL^{-1}, respectively.[130] The bioactive metabolites in the acetone extract of *U. barbata* were investigated for their anticancer activity against FemX (human melanoma) and LS174 (human colon carcinoma) cell lines using the microculture tetrazolium test. Usnic acid was found to be potentially active against human melanoma FemX cells and human colon carcinoma S174 cells with IC_{50} values of 12.72 and 15.66 μg mL^{-1}, respectively.[19] *U. longissima* thallus strongly suppressed Epstein Barr Virus (EBV)-induced tumor promotion. Usnic acid, barbatic acid, 4-*O*-dimethyl-barbatic acid, diffractaic acid and evernic acid were responsible for this activity. Of these, usnic acid displayed the highest inhibitory activity (IC_{50} 1.0 mM).[131]

Two new heptaketides, including corynesporol (**1**) and 1-hydroxydehydroherbarin (**2**) along with herbarin (**3**) were isolated from an endolichenic fungal strain, *Corynespora* species BA-10763, associated with *U. cavernosa*. Aerial oxidation of corynesporol (**1**) yielded herbarin (**3**). The structures of **1–3** were elucidated from their spectroscopic data. Acetylation of **1** produced the naphthalene derivative **4**, whereas acetylation of **3** yielded the corresponding naphthoquinone **6** and dehydroherbarin (**5**). All compounds were evaluated for their cytotoxicity and observed inhibitory effect on the migration of human metastatic breast cancer MDA-MB-231 and prostate cancer PC-3 cell lines.[132] Diffractaic acid, a novel proapoptotic agent extracted from *U. longissima* and determined its *in vivo* anticancer activity. The orally and locally administered diffractaic acid showed the induction of apoptosis in tissues of titanium-implanted rabbits by activating initiator caspases (Cas-2, -8 and -9) and executioner caspase (Cas-3). It also showed strong effect on myeloperoxidase and inducible nitric oxide synthase activities, providing an alleviating effect.[133] The *in vitro* cytotoxicity assay of two new derivatives of phenylalanine diketopiperazine, ambewelamide A and B was examined against murine leukemia P388 cells. Only ambewelamde A exhibited significant cytotoxicity with IC_{50} value 8.6 ng mL^{-1}.[106]

Usnic acid obtained from *U. barbata* was examined for its anti-proliferative activity. L-Usnic acid caused moderate inhibition of murine P388 leukemia cells and also exhibited cytotoxic potential against cultured mouse leukemia Ll210 cells. It was inferred that the *p*-tri-ketone moiety was essential for optimum activity.[134] On the other hand, D-usnic acid (50 μg mL^{-1}) was found to reduce the cell counts of leukemic K-562 cells and endometrial carcinoma HEC-50 cells.[135,136] Different extracts of

Table 3 Pharmacological properties of extracts obtained from *Usnea* spp.

S. No.	Extract/compound	Source	Bioactivity	Target/system	Mode of action	Dose	References
(a) *In vitro* studies							
1	Methanol extract	*U. filipendula*	Anti-population	Human lung cancer (A549, PC3), liver cancer (Hep3B) and rat glioma (C6) cells	Induces apoptosis like cell death	1.56–100 μg mL^{-1}	129
2	Methanol, acetone extracts	*U. artarctica*, *U. auranticoatra*	Antioxidant	*In vitro* system	Scavenges free radicals	IC_{50}: 1 mg mL^{-1}	179
3	Acetone extract	*U. barbata*	Anti-cancer, anti-oxidant	FemX (human melanoma) and LS174 (human colon carcinoma)	Reduces cell viability	IC_{50}: 102.65 and 130.73 μg mL^{-1}	18
4	Acetone extract	*U. complanta*	Anti-viral	Herpes simplex viruses (HSV)	Exhibits cytopathic effect	IC_{50}: 100 μg mL^{-1}	120
5	Methanol extract	*U. longissima*	Melanogenesis inhibition	Human melanoma cells	Inhibits tyrosinase glycosylation	0.1%	147
6	Acetone, methanol extracts	*U. lapponica*	Anti-bacterial	*S. aureus*, *E. coli*, *P. aureginosa* and *Methicillin resistant S. aureus*	Kills bacteria	MIC: 15.6 μg mL^{-1}	116
7	Acetone extract	*U. barbata*	Anti-mycobacterial	*Mycobacterium tuberculosis*, *M. kansasii* and *M. avium*	Inhibits growth of pathogenic bacteria and fungi	MIC: 32 μg mL^{-1} and 62 μg mL^{-1}	18
8	Polysaccharide	*U. longissima*	Anti-lipid peroxidation	*In vitro* system	Scavenges free oxygen radicals and hydroxyl radical oxygen and reduces DNA damage	0.45–1.57 mg mL^{-1}	121
9	Methanol extract	*U. artarctica*	Anti-oxidant, anti-genotoxic	Human lymphocytes	Inhibits lipid peroxidation and enhances antioxidant enzyme activities	5–20 μg mL^{-1}	34
10	Supercritical CO_2-extract	*U. barbata*	Anti-inflammatory	HaCaT keratinocytes	Inhibits prostaglandin E2 synthesis and cyclooxygenase-2 (COX-2) expression	60 μg mL^{-1}	139
11	Acetone extract		Antioxidant	*In vitro* system	Scavenges free radicals	0.0008 to 0.5 mg mL^{-1}	18
12	Acetone, methanol, aqueous extracts		Antimicrobial	*In vitro* system	Inhibits the growth of bacteria and fungi	IC_{50}: 0.1 mg mL^{-1}	60
13	Supercritical CO_2-extract		Antimicrobial	*Malassezia furfur*, *S. aureus*	Inhibits the growth of bacteria and yeasts with dermatological relevance	—	180
14	Ethanol, methanol	*U. ghattensis*	Antioxidant	*In vitro* system	Inhibits lipid peroxidation	20 μg mL^{-1}	123
15	Diethyl ether, acetone, methanol, aqueous extract	*U. fasciata*	Cytotoxic	Sarcoma 180 and Ehrlich tumor cells	Decreases cell viability	—	87
16	Methanol extract	*U. filipendula*	Anti-oxidant, anti-genotoxic	Human lymphocytes	Inhibits lipid peroxidation and enhances antioxidant enzyme activities	5–20 μg mL^{-1}	34
17	Methanol, aqueous extracts	*U. longissima*	Antioxidant	*In vitro* system	Scavenges free radicals	—	124
18	Acetone, methanol, ethanol extracts	*U. ghattensis*	Antibacterial	Human pathogenic bacteria	Inhibits the growth of bacteria	MIC: 3–200 μg mL^{-1}	113
19	Acetone, dimethyl sulphoxide, methanol, light petroleum extracts		Antibacterial	Human pathogenic bacteria	Inhibits the growth of bacteria	MIC: 5–10 μg mL^{-1}	181
20	Different extracts of cultured mycobiont		Antioxidant	*In vitro* system	Inhibits lipid peroxidation and scavenges free radicals	0.2 mg mL^{-1}	123
21	Methanol extract		Antioxidant	*In vitro* system	Scavenges superoxide radicals	2–20 μg mL^{-1}	182
22	Methanol extract		Antioxidant	*In vitro* system	Quenches different types of free radicals	20 μg mL^{-1}	183
23	Methanol extract	*U. longissima*		Human blood cells		—	125

Table 3 (*Contd.*)

S. No.	Extract/compound	Source	Bioactivity	Target/system	Mode of action	Dose	References
			Antigenotoxic, antioxidant		Inhibits lipid peroxidation and induces antioxidant enzyme levels		
24	Acetone extract	*U. rubicunda*	Antitumor	*In vitro* system	Inhibits tumor promoter-induced Epstein–Barr virus activation	—	131
25	*n*-Hexane, diethyl ether, methanol extracts		Cytotoxic	Cancer cell lines	Reduces cell viability	IC_{50}: 20 μg mL^{-1}	184
26	Methanol extract	*U. siamensis*	Antifungal	*C. albicans*	Inhibits the growth of fungus	—	185
(b) *In vivo* studies							
1	Aqueous extract	*U. longissima*	Anti-ulcer	Rats	—	100 mg kg^{-1}	52
2	Methanol extract	*U. longissima*	Anti-platelet, anti-thrombotic	Mice	Anti-platelet activity	100–200 mg kg^{-1}	143
3	Methanol extract	*U. articulate*, *U. filipendula*	Anti-oxidative, anti-genotoxic	Rats	Increases the activities of superoxide dismutase, glutathione and glutathione peroxidase and decreases malondialdehyde formation	5–20 μg mL^{-1}	33
4	Methanol extract	*U. ghattensis*	Hepato-protective	Rats	Inhibits lipid peroxidation and induces antioxidant enzymes	20 μg mL^{-1}	122

U. fasciata containing usnic acid and isolichenin showed moderate anti-cancer activity against sarcoma 180 and Ehrlich tumor cells. However, high anti-tumoral activity, near 90% inhibition, was observed with the fraction containing raffinose.[87] Recently, Zuo and colleagues have elucidated the molecular mechanism through which usnic acid mediates anti-cancer activity. Usnic acid selectively killed the human breast cancer MCF-7 cells by inducing the generation of reactive oxygen species (ROS), which triggered the activation of c-Jun-N-terminal kinase (JNK), loss of mitochondrial membrane potential (MMP), release of cytochrome-c, and activation of the caspase-cascade.[137] Eventually, usnic acid was found to inhibit tumor growth in MCF-7 tumor-bearing mice without inducing significant toxicity. The authors suggested that usnic acid stimulated apoptosis through an ROS-dependent mitochondrial pathway in MCF-7 cells. Eumitrin A1, isolated from *U. blepharea* was evaluated for its cytotoxic activity against Murine Leukemia P388 cells. According to the observed IC_{50} value (4.5 μg mL^{-1}), it is reported as a very active toxic compound for cancer cell lines.[138]

6.4. Anti-inflammatory

Usnic acid extracted from *U. barbata* using supercritical fluid method has shown anti-inflammatory properties by inhibiting ultraviolet-B induced prostaglandin E2 synthesis and cyclooxygenase-2 (COX-2) expression in HaCaT keratinocytes. Moreover, a crude extract also inhibited prostaglandin E2 production at a half-maximal concentration of 60 μg mL^{-1} which contains 2.4 μg mL^{-1} of usnic acid. However, the extract did not affect the UVB-induced upregulation of COX-2, suggesting an effect on enzymatic activity rather than on protein expression.[139] Choudhary *et al.* succeeded in isolating new compounds, including longissiminone A, longissimone B and glutinol, from *U. longissima* and evaluated them for their anti-inflammatory activity. Longissimone A showed potential anti-inflammatory activity in comparison to standard drugs with IC_{50} 165.74 μg mL^{-1}.[99] Usnic acid has been demonstrated to be a potent anti-inflammatory agent.[140] Lichen metabolites such as atranorin, diffractaic, and protolichesterinic acids were found to attenuate LTB_4 biosynthesis in polymorphonuclear leukocytes, due to specific enzyme interaction rather than nonspecific redox mechanism.[141] The phenolic compound longissimone A, isolated from *U. longissima* displayed anti-inflammatory responses comparable to standard aspirin in a cell-based contemporary assay.[99]

6.5. Genotoxic, anti-genotoxic and anti-mutagenic

The genotoxic and anti-genotoxic potentials of two lichen methanolic extracts, *U. articulata* (UAE) and *U. filipendula* (UFE) against aflatoxin B1 (AFB1)-induced genotoxic and oxidative damage were studied. It was observed that the methanolic extracts of UAE and UFE decrease the frequencies of sister chromatid exchange and malondialdehyde level and increase the level of antioxidant enzymes such as superoxide dismutase, glutathione, and glutathione peroxidase in a concentration-dependent manner (5 to 20 μg mL^{-1}).[34] A concentration-dependent anti-mutagenic potential of usnic acid ligands (L1, L2 and L3) and their complexes were examined for the first time against known mutagens, NaN3, 9-AA and MNNG in *S. typhimurium* TA1535, TA1537, and *E. coli* WP2uvrA, respectively. The results were evaluated using the standard plate incorporation method. The results showed that the ligands and their

Table 4 Pharmacological properties of chemical constituents isolated from *Usnea* spp.

S. No.	Extract/compound	Source	Bioactivity	Target/system	Mode of action	Dose	References
(a) *In vitro* studies							
1	Quercetin	*U. longissima*	Anti-fungal	*Candida albicans*	Suppressor of biofilm formation and hyphal formation	0.2–1.0 mg mL^{-1}	28
2	Isodivaricatic acid	*U. florida*	Anti-fungal	*Microsporum gypseum*, *Trichophyton mentagrophytes* and *T. rubrum*, *C. albicans*, *C. tropicalis*, *Saccharomyces cerevisiae*, *Aspergillus niger*, *A. flavus* and *A. fumigates*	Inhibits growth of human pathogenic fungal pathogens	50–100 μg mL^{-1}	111
3	Usnic acid	*U. longissima*	Anti-bacterial	*E. aerogenes*, *B. brevis*, *M. luteus*, *E. coli*, *B. megaterium*, *P. aeruginosa*, *E. cloacae*, *S. aureus*, *C. albicans* and *S. cerevisiae*	Increases the synthesis of some novel multifunctional hydroxyphenylimino ligands (L1, L2 and L3)	0.25–2 mg mL^{-1}	112
4	Usnic and diffractaic acids	*U. subcavata*	Anti-proliferative	UACC-62 and B16-F10 melanoma cells	Increases genetic damage in the cell lines	IC_{50}: 24.7–36.6 μg mL^{-1} (UACC-62) and 25.4 μg mL^{-1} (B16-F10)	130
5	Usnic, psoromic acids	*U. complanata*	Anti-oxidant	*In vitro* system	Scavenges free radicals	IC_{50}: 22.86 to 25.0 μg mL^{-1}	19
6	Heptaketides, corynesporol, 1-hydroxydehydroherbarin	*U. cavernosa*	Anti-cancer	Human metastatic breast and prostate cancer cell lines including MDA-MB-231 and PC-3M MDA-MB-231 and PC-3M	Inhibits the migration of cancer cells	5.0 μM	132
7	Heptaketides, corynesporol, 1-hydroxydehydroherbarin	*U. cavernosa*	Anti-cancer	Human metastatic breast and prostate cancer cell lines including MDA-MB-231 and PC-3M MDA-MB-231 and PC-3M	Inhibits the migration of cancer cells	5.0 μM	132
8	L-Usnic, D-usnic acids	*U. barbata*	Anti-proliferative	Leukemic cells (K-562) and endometrial carcinoma cells (HEC-50)	Reduces cell viability	50 μg mL^{-1}	135,136
9	Usnic acid	*U. dasypoga*	Anti-helicobacter pylori	*Helicobacter pylori*		MIC: 0.128–2 μg mL^{-1}	118
10	Usnic acid	*U. longissima*	Anti-mutagenic	*S. typhimurium* TA1535, TA1537 and *E. coli* WP2uvrA	Prevents mutation	20–100 μg per plate	112
11	Eumitrin A1	*U. blepharea*	Cytotoxic	P388 cells	Inhibits cell viability	4.5 μg mL^{-1}	138
12	Ambewelamide A, B	*Usnea* sp.	Cytotoxic	Cancer cell lines	Reduces cell viability	—	106
13	Barbatic acid, 4-*O*-demethylbarbatic acid, diffractaic acid	*U. longissima*	Antitumor	Tissue culture	Inhibits tumor promoter-induced Epstein–Barr virus activation	>1 μM	131
14	Diffractaic, usnic, norstictic, psoromic acids	*U. subcavata*	Cytotoxic	UACC-62 and B16-F10 melanoma cells and 3T3 normal cells	Decreases cell viability	24.7 to 36.6 μg mL^{-1}	130
15	Diffractaic, norstictic, usnic, hypostictic, protocetraric acids	*U. subcavata*	Antimycobacterial	*Mycobacterium tuberculosis*	Inhibits the growth of bacterium	15.5–125 μg mL^{-1}	186
16	Diffractaic, norstictic, usnic, hypostictic, protocetraric acids	*U. longissima*	Antioxidant, antimicrobial	DPPH radical system Gram-positive and Gram-negative bacteria and fungi	Scavenges free radicals and inhibits the growth of pathogenic microbes	—	187
17	Usnic acid	*U. ghattensis*	Antibacterial pro-apoptotic	*Bacillus licheniformis*	Inhibits the growth of bacteria	0.005–0.01%	187
19	Evernic acid	*U. longissima*	Antitumor	Tissue culture	Inhibits tumor promoter-induced Epstein–Barr virus activation	>1 μM	131
20	Galbinic acid	*U. undulata*	Antimicrobial	*B. cereus*, *B. subtilis*, *S. epidermidis*	Shows inhibitory effect	31–62.5 μg mL^{-1}	176

Table 4 (*Contd.*)

S. No.	Extract/compound	Source	Bioactivity	Target/system	Mode of action	Dose	References
21	Gautinol	*U. longissima*	Anti-inflammatory, cytotoxic	Spectroscopic model system	Reduces cell viability	200 μg mL^{-1}	99
22	Hirtusneanoside	*U. hirta*	Antibacterial	Gram-positive bacteriaS	Shows growth inhibitory activity	—	103
23	2′-*O*-Methyl hypostictic acid	*U. undulata*	Antimicrobial	*B. cereus*, *B. subtilis*, *S. epidermidis*	Shows inhibitory effect	31–62.5 μg mL^{-1}	176
24	Psoromic acid	*U. camplanata*	Antioxidant, cardiovascular protective	*In vitro* system	Scavenges free radicals and inhibits lipid peroxidation	0.174–0.271 mg mL^{-1}	20
25	Norstictic acid	*U. undulata*	Antimicrobial	*B. cereus*, *B. subtilis*, *S. epidermidis*	Shows inhibitory effect	31–62.5 μg mL^{-1}	97
26	Usnic acid	*U. longissima*	Anti-inflammatory	LPS-stimulated RAW264.7 macrophages	Decreases the TNF-alpha level	IC_{50}: 12.8 μM	140
27	Methyl β-orsellinate	*U. undulata*	Antibacterial	*B. cereus*, *B. subtilis*, *S. epidermidis*	Shows inhibitory effect	31–62.5 μg mL^{-1}	97
(b) *In vivo* studies							
1	Diffractaic acid	*U. longissima*	Pro-apoptotic	Rabbits	Activates the expressions of initiator caspases (Cas-2, -8 and -9) and executioner caspase (Cas-3)	30 mg kg^{-1}	133
2	Usnic acid	*Usnea* species	Anti-genotoxicity	Mice	Modulation of enzyme activity (ALT and AST)	60–120 μg mL^{-1}	142
3	Ambewelamide A	*Usnea* species	Cytotoxicity	Swiss mice and V79 cells	Reduces cell growth	8.6 ng mL^{-1}	106
4	Diffractaic acid	*U. longissima*	Hepatoprotective	Mice	Induces levels of antioxidant enzymes	50 mg kg^{-1}	148
5	Diffractaic, usnic acids	*U. diffracta*	Analgesic, anti-pyretic	Mice	—	500–1 g kg^{-1}	109

coordination compounds exhibited various anti-mutagenic effects ranging from 25.2–82.5%.[112] Usnic acid, a major bioactive compound of the *Usnea* genus was evaluated for genotoxicity and MMS-induced genotoxicity in a conc. dependent manner in *in vivo* (Swiss mice) and *in vitro* systems (V79 cells). It was demonstrated that usnic acid exhibits a protective effect against MMS-induced genotoxicity by reducing the frequencies of micronuclei and DNA damage.[142]

6.6. Anti-platelet and anti-thrombotic

The antiplatelet and antithrombotic properties of a methanolic extract of *U. longissima* were determined. The test was performed on platelet aggregation *in vitro* and on pulmonary thrombosis *in vivo*.[143] A concentration dependent inhibitory effect was seen on ADP-induced platelet aggregation, with an IC_{50} value of 3.6 mg mL^{-1}. For the *in vivo* studies, a thrombotic model was used in which mice were injected intravenously with a mixture of collagen and epinephrine. The oral administration of the extract prior to the injection produced a significant inhibition of thrombotic death or paralysis at 100–200 mg kg^{-1} body weight. The results revealed that the antithrombotic activity of *U. longissima* extract might be due to antiplatelet activity rather than anti-coagulant activity.[143]

6.7. Others

Many other biological activities for the *Usnea* species have also been reported. Diffractaic and usnic acids of *U. diffracta* were identified as analgesic and antipyretic components in mice.[109] Halici *et al.* confirmed the potential gastroprotective effect of the aqueous extract of *U. longissima* against indomethacin-induced gastric lesions in rats through an antioxidant mechanism.[144] The acetic acid-induced writhing and tail-pressure methods were performed to examine the effects. Both compounds showed an analgesic effect for acetic acid-induced writhing and tail-pressure methods in mice. Diffractaic acid showed significant effects at 500 mg kg^{-1} and 1 g kg^{-1}, while usnic acid was found to be effective at 100 mg kg^{-1}.[144] One year later, the same research group isolated the diffractaic and the usnic acids from an organic extract of *U. longissima* as promising anti-ulcerogenic agents.[145,146] This effect was investigated using indomethacin-induced ulcer models in rats by comparing the negative (treated only with indomethacin) and positive (ranitidine) control groups. The extract showed significant anti-ulcerogenic activity compared to the negative control groups in a dose-dependent manner. The highest activity (79.8%) was observed with 100 mg kg^{-1} body. It was associated with the inhibition of oxidative damage and neutrophil infiltration.

The methanolic extract of *U. longissima* was also applied to determine *in vitro* melanogenesis inhibitory effects.[147] The extract was found to reduce melanin formation in human melanoma cells in concentration-dependent manner. Inhibition of melanin content by 51.1% and 34.9% were recorded at 0.01% and 0.1% solutions of the extract, respectively. The obtained results were compared to ascorbic acid. It has also determined that the extract affected the activity of tyrosinase *via* inhibition of glycosylation process.[147]

7. Toxicity

Apart from the analysis of phyto-constituents as a traditional medicine, researchers have also carried out toxic studies on *Usnea* species. Until now, usnic acid, a major constituent of the genus *Usnea* was reported only for severe hepatotoxicity and allergic cross reaction, but because it is poorly and slowly absorbed when in either a tea or alcoholic solution, there is little cause for concern. The LD_{50} is 25 mg kg^{-1} in mice. It is best not to swallow the alcoholic tincture without diluting it, as it can be irritating. Large quantities of a strong tea of some lichens could cause gastro-intestinal upset, because of the irritating nature of the lichen compounds. Recently diffractic acid, isolated from *U. longissima* was investigated for carbon tetrachloride-induced hepatic damage *in vivo* and all biochemical and histopathologically assays were performed. Diffractic acid was found to be hepato-protective agent at low dose 50 mg kg^{-1} daily but at high dose (100 and 200 mg kg^{-1}) it showed hepatotoxicity.[148] Dobrescu and colleagues studied about the acute toxicity of *U. barbata* and *U. hirta*. The hydro-alcoholic extracts of *U. barbata* and *U. hirta* exhibited toxicity with LD_{50} values of 22.53 g kg^{-1} and 21.02 g kg^{-1}, respectively after intraperitoneal administration in rats. LD_{50} values of 7.43 g kg^{-1} for *U. barbata* and 4.52 g kg^{-1} for *U. hirta* were recorded after intravenous administration.[149] Recently, in Spring 2003, several reports appeared indicating that ingestion of usnic acid, as the suspect compound in LipoKinetix (a product of Syntrax Innovations, USA), caused liver failure that was complicated by cerebral edema in one individual who took the product, and liver damage in six other cases. The duration of ingestion for this adverse effect is just a few weeks. The first FDA warning appeared in 2001 and was updated in spring of 2002,[150] and formally reported in Annals of Internal Medicine at the same time.[151] There may have been other cases of liver damage from this same product,[152] based on retrospective studies.

Chemical constituents of *Usnea* species exhibit acute toxicity against larvae of the polyphagous insect herbivore *Spodoptera littoralis* revealed the LD_{50} at 8.6 μM for (−)-usnic acid, 90.8 μM for (+)-usnic acid and 111.0 μM for vulpinic acid.[153] Pramyothin *et al.* demonstrates that usnic acid showed no serum transminase activity when inducing swell in liver mitochondria and endoplasmic reticulum at a dose level of 50 or 200 mg kg^{-1} intraperitoneally for 5 days. Meanwhile a dose level of 1 mM usnic acid in rat primary hepatocytes triggered the release of hepatic transaminases, decreased the content of reduced glutathione, and caused a loss of cell membrane integrity.[154] It was observed that the administration of 5 μM usnic acid for 16 h in mouse primary hepatocytes exhibited 98% necrosis rather than apoptosis by generating oxidative stress and acting directly on the uncoupling of oxidative phosphorylation of the electron transport chain in mitochondria. Usnic acid administration in sheep triggered serum creatine kinase, aspartate aminotransferase, and lactate dehydrogenase activities. It was also estimated that 485 and 647 mg kg^{-1} d^{-1} median toxic doses (ED_{50}) in domestic sheep.[155] Sheu and colleagues studied allergic content dermatitis by applying lichen acid mixture and usnic acid to four patients and observed that all patients showed positive results for patch test.[156]

8. Conclusions and future prospects

Within the fungus kingdom, lichens produce a wide array of both primary (intracellular) and secondary (extracellular) compounds with different biological properties. However, the knowledge of the biological potential of many lichens and their metabolites is very narrow compared to other fungi. Moreover, this knowledge is very recent and limited investigations have been conducted for a deeper understanding of the mechanism and cellular sites of action of lichen substances responsible for the different pharmacological properties described so far. Thus, the aim of this review is to provide up-to-date information about traditional uses, phylogeny, phytochemistry, pharmacology, and toxicology of the most numerous and widespread genus of lichens, *Usnea* which comprises of approximately 350 species based on scientific literatures.

Usnea is a lichen; a combination of an algae and a fungus growing together. *Usnea* species are endemic to many parts of Asia, Africa, Europe, and America and are widely used in traditional medicine for various applications. It is used to treat stomachache, bronchitis, sterility, pneumonia, pulmonary diseases, strep throat, colds, flues, urinary tract, kidney, and bladder infections. *Usnea* is also beneficial for women with yeast infections, trichonomosas, bacterial vaginosis, and chlamydia. It could be useful for people with chronic fatigue, HIV, herpes, and other chronic conditions related to depressed immune systems. The phytochemical results have indicated a significantly diversity of structural types of chemical constituents. Pharmacological studies indicated that *Usnea* lichens and their bioactive constituents possess various biological properties, especially in the areas of anti-microbial, anti-cancer, anti-proliferative, anti-oxidant, anti-inflammatory, anti-ulcer, hepatoprotective, and anti-genotoxicity *etc.* To a certain extent, pharmacological results have shown that (a) traditional uses for the treatment of flues, gastroenteritis and bacterial/fungal infections, and strep throat were related to antimicrobial activities; (b) use for wounds, ulcers, and fevers were associated with anti-inflammatory activity; (c) the anti-cancer activity was due to the regulation of molecular targets including caspases; (d) the anti-oxidant, anti-ulcer, and anti-genotoxic properties have been investigated by *in vitro* and *in vivo* experiments. Regarding the constituents contributing to therapeutic values, the findings indicated that depsides, depsidones, and benzofurans are key phytochemicals for the treatment of microbial infections, oxidative stress, cancer, ulcer, and inflammation. It

is imperative to discuss the stereo-chemistry and structure–activity relationships of depsides, depsidones, and benzofurans for evaluating medicinal properties of *Usnea* species. However, relating mode of action with chemical structure is difficult since, up to date, the number of investigated chemical constituents for each of these substance classes and pharmacological activities is not very large and the techniques applied and parameters estimated are very variable. As an example, for assessing cytotoxic activity, most of the current research evaluated the capacity of crude extracts and active compounds to inhibit cancer cell proliferation, without focusing on the mechanism of action. For anti-microbial activity, some researchers have focused on Gram positive bacteria, others on Gram negative bacteria, on fungi, on mycobacteria and some other on mixture of them. For estimating antioxidant activity, some researchers have examined the capacity of certain active compounds to scavenge free radicals, others assess their anti-lipidperoxidative property and others determine the level of endogenous antioxidants including CAT, SOD, and GST. Moreover, existing information on the relationships between chemical structure and pharmacological mechanism of action is very limited within natural products, taking as an example the plant kingdom, which is much more known than fungus.

It is noteworthy that current studies on the chemical constituents and pharmacological mechanisms of *Usnea* species lack depth and more investigations on phytochemistry and the mechanisms of the main active ingredients in demonstrating certain biological activities should be encouraged to fully understand the compounds responsible for the pharmacological effects and the mechanisms of action. The great progress on the phytochemistry and pharmacology of the genus *Usnea* that has been made confirm its traditional uses. However, there is a pressing need to investigate more conclusive molecular and clinical studies on the safety, efficacy, and toxicity of extracts as well as pure phytochemicals to gain a better understanding of this genus. Furthermore, a significant proportion of the collected pharmacological research has been performed on lichen extracts with promising results, being of interest for the determination of active principles.

Several hurdles were initially faced in the *in vitro* culture of lichens in order to obtain substantial quantities of lichen substances for various applications. However, advanced techniques such as mycobionts under adjusted culture conditions and heterologous expression of polyketide synthase gene in filamentous fungi, yeasts, and bacteria have recently contributed to great progress in lichen research. These could contribute to future pharmaceutical applications of selected substances of *Usnea* species, obtained in suitable amounts.

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