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Ethnobiology, Phytochemistry and Pharmacology of *Usnea Longissima*: A Review

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Abstract- Lichenized fungi or lichens are believed to be one of the most inconspicuous living forms on the earth. The traditional knowledge of numerous benefits which lichens possess has been transcended over the centuries within and among the humans. All the different growth forms of lichens have been used by man in one way or the other. These find their use as source of different medicines, food, fodder, dye and as ingredients in perfumery. They are pioneers in ecological succession and also serve as indicators of pollution. *Usnea longissima* is a fruticose form of lichen that has been used as a traditional medicine for centuries. Scientific studies have proved that the pharmacological properties of the species are due to presence of several important secondary metabolites. These secondary metabolites possess antioxidant, anticancer, antimicrobial activity and thus can be used for the control and treatment of several diseases. The present communication gives a review of the multifarious benefits associated with *Usnea longissima* along with the emphasis on its pharmacological properties.

Key Words: *Usnea longissima, Diffractaic acid, Usnic acid, phytochemistry, ethnobotany*

I. INTRODUCTION

Usnea longissima Arch. is also known as old man's beard and belongs to the family Parmeliaceae [1,2]. This fruticose lichen is widely distributed in Europe, Asia, North America, Africa [3,4] and in Russia as well [5]. The species, however, is rare and endangered in California [6]. In India, *Usnea longissima* is reported from the temperate regions namely Arunachal Pradesh, Assam, Himachal Pradesh, Sikkim, Uttaranchal and West Bengal at a varying altitude of 2100-3600 masl [4]. The thallus of *Usnea longissima* is pendulous, filamentous with terete and decorticated main branches upto several meters in length [7]. It appears as long, powdery thread or cobweb like structure growing luxuriantly over the twigs of several species of *Pinus* [8], *Quercus* [9] and on *Picea abies* [10]. Apothecia being uncommon, if present, is average 5mm in diameter with ascospores measuring 8×6µm [11,12]. This lichen species is very well known for its use in different home remedies and the Ayurveda and therefore has been the subject of research for the various pharmacological properties its possesses. Our work is an attempt to evaluate, review and organize the documented information related to this species in the form of a paper.

This paper is organized as follows, section I contains the introduction to the *Usnea longissima*, section II contains commercial and ethnic uses, section III lays emphasis on chemistry of *Usnea longissima*, section IV explains in detail the pharmacological properties of *U. longissima* which include anti-microbial, anti-fungal, insecticidal, anti-platelet, anti-thrombotic, anti-ulcerogenic, growth inhibitory, melanogenesis inhibitory, anti-oxidative, gastro-protective, hepato-protective, anti-cancerous and anti-mutagenic properties and the section V presents the conclusion of the paper.

Commercial and Ethnic uses

The species is endowed with multiple benefits and has been explored ethno-botanically in different parts of the world [13,14]. In Northern Anatolia (Turkey) it is used for treating cancer, tuberculosis, and ulcers [15,16]. In China, this lichen is used as a decongestant and for local treatment of ulcers and tuberculosis [17,18,19]. The Chhetri, Gurung, Lama, Limbu and Sherpa tribes of Nepal Himalayas use it for ritual, spiritual, bedding, aesthetic and decorative purposes [20]. Besides, the species finds its use for healing purposes in cases of bone fractures in Indo-Tibetan part of Himalayas [21]. However, in Unani system of medicine, it is either taken orally or inserted into the vagina to stimulate menstruation and even to induce abortion [22]. In Ayurvedic system of medicine in India, it is mixed in tobacco and also used as an occasional adulterant in chharila (*Parmelia perlata*) [23]. It is also known as 'Indian bandage' and therefore used for wound dressing [24]. The Baiga tribe of Madhya

Pradesh uses the lichen along with some other ingredients for treatment of bone fractures [8,25]. In Uttaranchal, Bhotia and Grahwal tribes use *Usnea longissima* as a spice, for stuffing nets and even as special ingredient of poultice for bone setting [26]. It is one of the important dietary components of the Musk deer found in Garhwal Himalayas region in India [27].

Chemistry

Numerous important chemical compounds have been extracted from *Usnea longissima*. These include atranorin, fumarprotocetraric acid, salazinic acid [28], barbatic acid [29], usnic acid [30], longissiminone A, longissiminone B, glutinol [31] (Figure 1), friedelin, beta-amyrin, beta-sitosterol, methyl-2, 4-dihydroxy-3, 6-dimethylbenzo, barbatinic acid, zeorin, ethyl orsellinate, 3-beta-hydroxy-glutin-5-ene, oleanolic acid, methylorsellinate, 4-methyl-2,6-dihydroxy-benzaldehyde, 3, 6-diacetyl-2, 7, 9-trihydroxy-8, 9b-dimethyl-1 [9bH]-dibenzofuranone and 1, 3, 8-trihydroxy4, 6-dimethyl-9, l0-anthracenedione [14], diffractaic acid [32] (Figure 1), protocetraric acid and evernic acid [7].
Different spectroscopic methods such as1D, 2D NMR experiments, UV, IR, EI-MS and HR-ESI-MS were used to elucidate

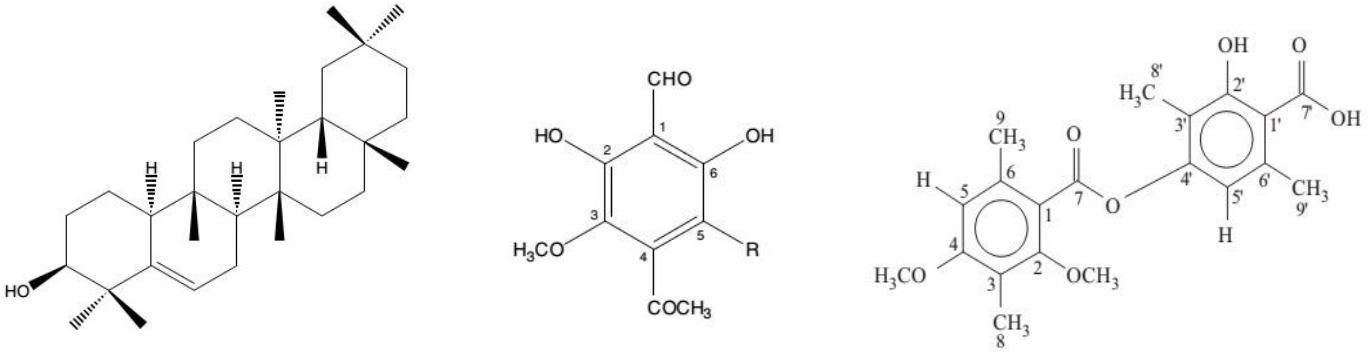

Figure 1. GultinolLongissiminone A (1) R=HDiffractaic acid

Longissiminone B (2) R=Cl

the structure of a new benzofuranone derivative, ethyl 2-(3,3-bis (7-acetyl-4,6-dihydroxy-3,5-dimethylbenzofuran-2-yl) acryloyl) from *Usnea longissima* [33]. Four new phytochemicals obtained and identified from *Usnea longissima* are (4aR,9bS)-2,6-diactyl-3,4a,7,9-tetrahydroxy-8,9b-dimethyl-1-oxo-1,4,4a,9b-tetrahydrodibenzo[b,d]furan, orcinol, 18R-hydroxydihydroalloprotolichensterinic acid and arabitol [34].

Pharmacological properties

Usnea longissima is considered to be a potent lichen with multifarious phytochemical and pharmacological properties [35].

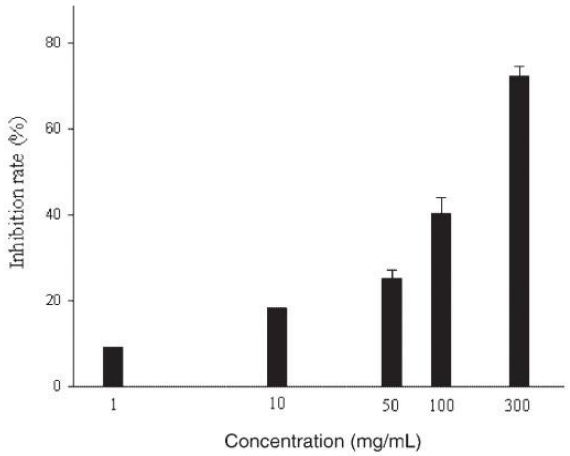

Figure 2. Effects of the methanol extract of *Usnea logissima* on ADP-induced platelet aggregation

Antimicrobial and Antifungal activity: The ethanolic extracts of *Usnea longissima* exhibited antibacterial activity against gram +ve bacteria namely *Staphylococcus aureus* and several gram –ve bacteria such as *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Shigella dysenteriae*, *Salmonella typhii* and *Escherichia coli* [36]. Likewise, the methanol extract of *Usnea longissima* showed antibacterial activity against *Salmonella typhii*, *Staphylococcus aureus* and *Escherichia coli* [37]. The methanolic extract possessed antifungal properties against *Trichoderma viridae* and *Candida albicans* [2]. The secondary metabolites usone and isousone exhibited inhibitory effect on the fungus *Trichophyton rubrum* [38]. Another study conducted by Oran and his co-workers revealed that *Escherichia coli* isolates were resistant to methanolic extracts of *Usnea longissima* and other two species as well [34]. Usnic acid can effectively inhibit the formation of bacterial biofilm on the surfaces of polymer [39]. Thus, usnic acid proves to be more effective against penicillin. The usnic acid extracted from *Usnea longissima* inhibited the growth of

some bacterial strains such as *Pneumococcus*, *Streptococcus* and *Staphylococcus* in dilutions of 1:20,000. Moreover, at dilutions of 1:200,000 – 1:2000,000 the growth of some strains of human TB was weakened and at 1:20,000 – 1:50,000 the growth of human TB was inhibited [36].

HPLC analysis of the usnic acid isolated from *Usnea longissima* collected from Anatolia and Turkey was performed. The acetone extract of usnic acid exhibited significant antimicrobial activity against *Escherichia coli* (ATCC 35218), *Enterococcus faecalis* (RSKK 508), *Proteus mirabilis* (Pasteur Ens. 235), *Staphylococcus aureus*, *Bacillus subtilis* and *Bacillus megaterium* [40]. The activity of novel multifunctional hydroxyphenylimino ligands (namely L1, L2 and L3) synthesized from usnic acid as well as their complexes (namely Cu(II), Co(II), Ni(II) and Mn(II) salts) was tested against some pathogen microbes by disc diffusion method. The ligand complexes of the metals showed significant zone of inhibition i.e. 11 – 32 mm against ten pathogenic microorganisms. The Mn(II) and Cu(II) complexes of the hydroxyl phenyl imino ligand along with the usnic acid showed the maximum antimicrobial activity [41].

In vitro microbial synthesis of bioactive nanoparticles from *Usnea longissima* exhibited potent activity against several humanoid pathogenic fungi. These nanoparticles effectively controlled the fungal infections [42].

Insecticidal effects: The secondary metabolites of the lichen when tested against *Sitophilus granarius* (Coleoptera: Curculionidae) showed insecticidal effects on them causing a high mortality rate [43].

Antiplatelet and Antithrombotic activities: The methanol extracts of *Usnea longissima* were investigated in vitro on platelet aggregation and in vivo on pulmonary thrombosis for determining antiplatelet and antithrombotic activities. The study showed concentration dependent inhibitory effects with an IC50 value of 3.6 mg /m of the extracts on the ADP-induced platelet aggregations shown in Figure 2 [56]. Longissiminone A isolated from *U. longissima* exhibited anti-inflammatory activity in a cell-based contemporary assay [31].

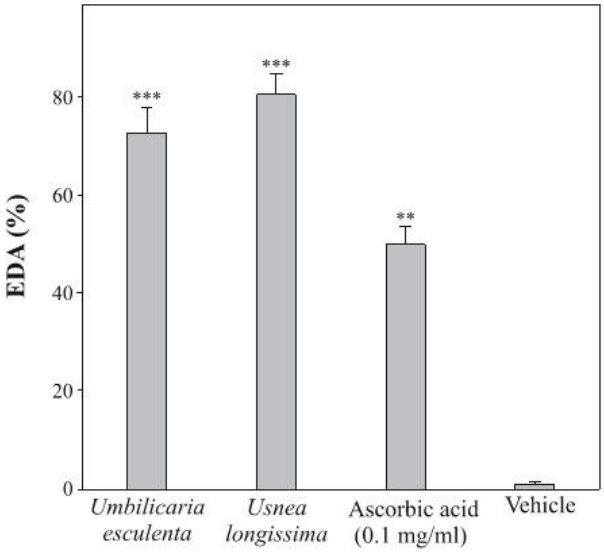

Figure 3. Electron donating ability (EDA) in methanol extracts of *U. esculenta* and *U. longissima.* Each value is expressed as Mean±SD in triplicate experiments.

Antiulcerogenic effect: The antiulcerogenic activity of water extract of *Usnea longissima* was determined by using Indomethacin - induced ulcer models in six rats. The negative and positive controls were treated with indomethican and rantidine respectively and the body weight doses administered were 50, 100 and 200 mg/kg. Although all the doses exhibited antiulcerogenic activity yet the highest activity was observed in case of 100 mg/kg body weight doses (i.e.79.8%). In the stomach tissues of rats the activities of antioxidant enzymes such as superoxide dismutase, catalase and glutathione S -transferase were also determined. This study was done in order to compare the effects of antioxidant enzymes on antiulcerogenic activity. The study also shows that the Indomethacin reduced the Superoxide dismutase and glutathione S - transferase enzymes activities. The activities of these enzymes were activated by the water extracts of *Usnea longissima*. However in case of CAT activity an opposite trend was observed which was increased by indomethacin and decreased by all doses of *Usnea longissima* and ranitidine [44].

Growth Inhibitory affect: The growth inhibitory-activity of some compounds isolated from *Usnea longissima* on lettuce seedling was observed. Depsides and orcinol derivatives which exhibited growth-inhibitory activity against lettuce seedlings were isolated. 4-O-demethylbarbatic acid exhibited the highest activity among the eight depsides compounds. However, β-Orcinol type depsides showed higher activity than orcinol type depsides. The nature of the hydroxy or methoxy substituents at the 2- and 4-positions affected the solubility in acetone and their biological activity. 3-α- Hydroxydiffractaic acid exhibited 90% radicle length and 60% hypocotyl length inhibitory activity to control at 4.0 x 10^{-4}M [45].

Melanogenesis Inhibitory Effect: The total phenolic compounds of methanolic extract of *Usnea longissima* were 1.46% and the electron donating abilities as well as the lipid peroxidation rates were also determined. The EDA values measured by the reduction of 1.1'-diphenyl-2-picrylhydrazyl (DPPH) was 80.7% for the extracts, with median scavenging concentration (SC50) of 1.03±0.06 mg/ml. Measurements of EDA using DPPH were conducted via the direct estimation method for antioxidation

activity. Extracts of *Usnea esculenta* and *Usnea longissima* evidenced 72.8% and 80.7% electron-donation effects, respectively, at a concentration of 6 mg/ml (Figure 3). The rates of inhibition of lipid peroxidation using linoleic acid was 97.3%, with IC 50 (median inhibitory concentration) value of 0.53 ± 0.06 mg/ml, as shown in (Figure 4). The methanolic extracts of *Usnea longissima* showed direct tyrosinase inhibition via inhibition of tyrosinase glycosylation thus exhibiting prominent melanogenesis inhibitory effects occurring through multiple routes. The inhibitory rate of extracts against tyrosinase was observed to be 84.8%. The extracts reduced melanin formation in human melanoma cells. Melanin contents in the samples treated with 0.01% and 0.1% *Usnea longissima* were 51.1% and 34.9%, respectively, whereas a value of 54.0% was observed when ascorbic acid was used as a positive control [46].

Activity against oxidative damage: *Usnea longissima* exhibited potent antioxidant activity [47]. The methanolic extracts of *Usnea longissima* suppressed the mutagenic effects of aflatoxin B_1 [48]. For evaluating antioxidative effect of methanolic extracts of *Usnea longissima* species, the activities of superoxide dismutase, glutathione peroxidase and malondialdehyde level were measured. This was followed by aflatoxin B_1 treatment which showed an increase of malondialdehyde level with a decrease of superoxide dismutase and glutathione peroxidase activities. The methanolic extract of lichen eliminated the genotoxicity and lipid peroxidation of aflatoxin B_1 by increasing the level of antioxidant enzymes activities. The adverse effects of aflatoxin B_1 in human blood cells is modified by methanol extract of lichen and shows strong antioxidative and antigenotoxic effects. This study reveals the antioxidant and free radical activity of ethanolic extract of *Usnea longissima* [49]. Polysaccharides of *Usnea longissima* were evaluated for their scavenging action against superoxide anion free radical (O^{2-}) using xanthine-xanthine oxidase system. Fenton reaction was used to study the Scavenging action on hydroxyl free radical (OH) and anti-lipid peroxidation effects. The scavenging action was characterized by the scavenging content of 50% for all the free radical (IC50). The IC50 for O^{2-} and OH were 0.45 mg/ml and 1.57 mg/ml respectively [50].

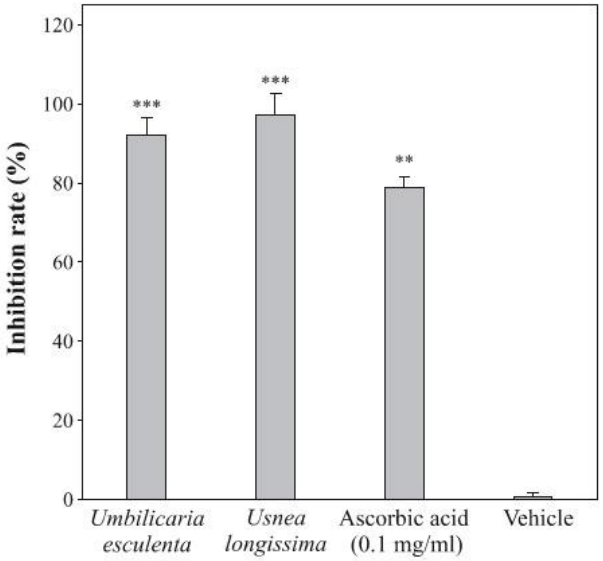

Figure 4. Inhibition of lipid peroxidation in methanol extracts of *U. esculenta* and *U. longissima.* Each value is expressed as Mean±SD in triplicate experiments.

Gastro-protective Effect: A study conducted by Bayir showed that the gastro-protective effect of diffractaic acid could be attributed to its enhancing effects on antioxidant defense systems and reducing effects of neutrophil infiltration [51]. The anti-ulcerogenic effect of diffractaic acid isolated from *Usnea longissima* on indomethacin induced gastric lesions was investigated in rats. Doses of 25, 50, 100 and 200 mg/kg of diffractaic acid and 50 mg/kg of ranitidine when administered reduced the gastric lesions in rats by 43.5%, 52.9%, 91.4%, 96.7% and 72.7% respectively. In all the treated groups of rats, in vivo activities of the antioxidant enzymes namely superoxide dismutase, catalase, glutathione peroxidase were evaluated. Also the levels of reduced glutathione and lipid peroxidation were evaluated. Indomethican caused oxidative stress in rats by decreasing the levels of glutathione peroxidase, Superoxide dismutase and glutathione as compared to healthy rats. Indomethican induced the gastric mucosal damage throughout the development in rats which also led to changes in activities of gastric mucosal nitric oxide synthases. However the gastric damage tissues induced by indomethican resulted in an increase in inducible nitric oxide synthases activity and decrease in constitutive nitric oxide synthases activity. The administered dose of 100 mg/kg of diffractaic acid and rantidine reversed the activities of inducible nitric oxide synthases and constitutive nitric oxide synthases. Usnic acid isolated from *Usnea longissima* showed gastro-protective and antioxidant effects on indomethican – induced gastric ulcer in rats [16].

Hepatoprotective activity: The methanolic extracts of *Usnea longissima* showed powerful hepato-protective and antioxidant activity against several experimental animals [52]. Hepatotoxicity study was carried out, in which ethanolic extract of *U. longissima* at doses 200 and 400 mg/kg body weight) were compared with Silymarin (25 mg/kg body weight). Hepatotoxicity was induced by CCl_4 (1ml/kg) and the study showed that the liver weights were significantly decreased. However ULE showed a dose dependent protection in liver weight. The result of the high dose (400 mg/kg) was compared with standard drug Silymarin (25 mg/kg) (Figure 5).

The hematological and antioxidant activity of chemical constituents isolated from *Usnea longissima* against CCl_4 iatrogenic acute liver damage in rats was investigated. This was disclosed by changes in level of LPO and GSH concentration in liver, additionally to the elevation of SOD, CAT and GPx activity. *Usnea longissima* extract ameliorates acute liver damage by the improvement in histopathological changes. The extract of *Usnea longissima* either causes stabilization of cellular membrane or showed anti-peroxidase activity. The study revealed that the extract of *Usnea longissima* exhibits powerful anti-oxidant and hepato-protective activity. Diffractic acid isolated from *Usnea longissima* at 3 doses 50, 100 and 200 mg/kg against CCl_4 induced hepatic fibrosis in Wistar rats whereas the daily dose of 50 mg/kg produced hepato-protective effect on Wistar albino rats and rest of the doses exhibited hepatotoxic effects. Diffractic acid is therefore potentially hepatotoxic at a low dose of 50mg/kg against acute liver toxicity induced by CCl_4 [53].

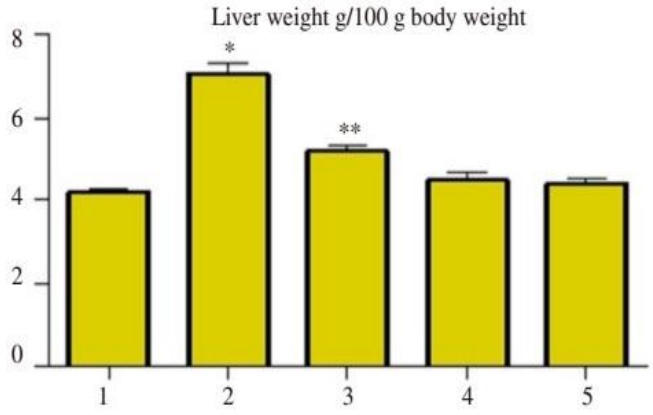

Figure 5. Effect of ethanolic *U. longissima* extract on liver weight in CCl_4 induced hepatotoxicity

1: Normal control; 2: CCl_4(1 mL/kg); 3: Slymarin (25 mg/kg) + CCl_4; 4: ULE (200 mg/kg) + CCl_4; 5: ULE (400 mg/kg) + CCl_4. All values are expressed as mean ± SEM (n = 6). One-way ANOVA was used.
*: Significant at $P < 0.05$,
**: Highly significant at $P < 0.01$ when compared with control.

Anti-cancerous properties: Usnic acid shows anti-proliferative activity against breast cancer cell lines namely the wild-type p53 (MCF7) and the non-functional p53 (MDA-MB-231) as well as against lung cancer cell line H1299. This non-genotoxic anti-cancer activity of usnic acid in a p53-independent manner needs to be further investigated. Thus, usnic acid has the potential for the treatment of tumors either as a systemic therapy or as a topical agent [54]. Cancer chemoprevention assay were designed using usnic acid to detect potential inhibitors of tumor promotion. It was shown that usnic acid unveiled strong inhibitory effects (ED_{50} 1.0µg/ml) against Epstein – Barr virus activation induced by teleocidin B – 4 potent tumor promoter [55].

Antimutagenic activity: Condensation of 2-aminophenol, 3-aminophenol and 4-aminophenol was done with usnic acid isolated from *Usnea longissima* to synthesize novel multifunctional hydroxyphenylimino ligands namely L1, L2 and L3. FT-IR, UV-Vis, (1)H-NMR, (13)C-NMR, 1D- and 2D NMR (, LC-MS and TGA,FT-MIR/FAR, UV-Vis, elemental analysis, ICP-OES and TG/DTA techniques were used to characterize the synthesized ligands and their Cu(II), Co(II), Ni(II) and Mn(II) complexes. The antimutagenic activities of all the ligands as well as their metal complexes were detected against Ames-Salmonella and E. coli WP2 microbial assay systems. The study revealed that the ligand complexes of Co and Mn possess potent antimutagenic activity [41].

II. CONCLUSIONS

The biochemical and physiological activities associated with the chemical entities present in *Usnea longissima* reveal their tremendous potential benefits which are yet to be explored fully. With the aid of modern equipments and latest biotechnological interventions, isolation and characterization of active compounds as well as elucidation of mechanism of their action has also become easier. Therefore, the secondary metabolites in lichens which are of natural origin can be isolated and utilized for the treatment of several diseases and ailments. Medicinal properties associated with *Usnea longissima* make it a very promising entity for drug development and in turn providing a great boon to the pharmaceutical industry

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Conflict of Interest

The authors declare no conflicts of interest.

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