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# BIOPROSPECTION OF UV-SCREENING COMPOUNDS FROM LICHENS INHABITING THE INDIAN STATE OF SIKKIM

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**ABSTRACT**

Lichens show mutualistic relationship between algae/cyanobacteria and fungi and are found in diverse environmental conditions ranging from sea levels to high alpine elevations. They can tolerate and survive in harsh environmental conditions such as habitats having low temperature, desiccation, high temperature and ultraviolet radiation (UVR). Lichens have developed several photoprotective mechanisms such as light scattering, radiation screening, activation of antioxidants and macromolecules, thermal dissipation, membrane repair and synthesis of UV protective compounds such as despides, diphenyl ether, xanthones, anthraquinones, mycosporines, mycosporine-like amino acids (MAAs) and scytonemin to cope up with damaging UVR. MAAs are water-soluble molecules that absorb short wavelength solar UVR and disperse the energy as heat. Scytonemin is a small hydrophobic alkaloid pigment present in the extracellular sheath of several cyanobacteria as a protective mechanism against UVR. In the present study, bioprospection of lichen was done for screening of UV-absorbing compounds MAAs and scytonemin from different regions of Sikkim, India. Tentative identification and partial purification of these compounds was done with the help of high-performance liquid chromatography. Scytonemin showed absorption maxima at 252, 278, 385 nm and peaks at 347 and 410 nm showed the presence of scytonemin-3a-imine and gloeocapsin respectively. MAAs showed absorption maxima at 325.1 and 308 nm corresponding to mycosporine-NMA:Ser and mycosporine-taurine respectively. Scytonemin and MAAs display multiple roles, functioning as a potent UV-sunscreen and antioxidant molecules, and can be exploited in cosmetic and other industries for the development of novel drugs and pharmaceuticals, hence their bioprospection from different sources becomes crucial.

***Keywords:*** Cyanobacteria, High performance liquid chromatography (HPLC), Lichens, Mycosporine-like amino acids (MAAs), Scytonemin, Ultraviolet radiation (UVR)

## INTRODUCTION

Lichens inhabit a wide range of habitats globally, and are good indicators of environmental pollution (Nguyen *et al.,* 2013). They show three major growth patterns crustose, fructose and fruticose (Singh *et al.,* 2019). In crustose lichens thallus is tightly adhered to the substratum. In fructose lichens thallus is loosely attached to substratum by hair-like rhizines. Fruticose lichens are bushy and erect or pendulous (Awasthi, 1988, 1991, 2000, 2007; Nayaka, 2004; Singh *et al.,* 2019; Upreti *et al.,* 2005, 2008). Lichens are considered as pioneer organisms at rock surfaces, dead woods, living bark of trees and surfaces of old buildings exposed to high ultraviolet radiation (UVR) (Chen *et al.,* 2000; Stocker-Wörgötter, 2015). Lichen represents symbiotic association of fungus (mycobiont) and alga/cyanobacterium (phycobiont). About 18,500 different lichen species have been described all over the world which can be found in very cold and dry environments, either at polar latitudes or at extreme altitudes (up to 7400 m) (Boustie and Grube, 2005). Despite this extreme range of ecological adaptations, most lichens are sensitive to changes of their preferred ecological conditions and can hardly grow in non-native habitats (Boustie and Grube, 2005). In lichens photobiont contains chlorophyll and necessary photosynthetic apparatus involve in primary metabolites production that is required for the synthesis of secondary metabolite products (lichen substances) by mycobiont having many biological roles, and protect thalli from deleterious effects of radiations, low temperature and desiccation (Asahina and Shibata, 1971; Culberson, 1969). Some of these secondary metabolites, synthesized by photobiont or mycobiont in lichens act as photoprotective shield against lethal UVR (Büdel *et al.,* 1997; Huiskes *et al.,* 1999; Kennedy, 1995; Wynn-Williams, 1994).

Since the birth of life on the Earth, UVR threatened the fragile equilibrium between a physiological need of UVR and their harmful effects on DNA acting by a direct energetic effect or by generation of reactive oxygen species (ROS) (Legouin *et al.,* 2017; Lucas *et al.,* 2006; Pathak *et al.,* 2019a). Incidence rates of melanoma, one of the most deleterious skin cancers on humans, are projected to rise caused by acute and chronic UVR exposure (Reed *et al.,* 2012). Among the preventive strategies, UV blockers remain commonly used. These compounds are expected to act like filters covering the broad UV range and also possess antioxidant properties (Legouin *et al.,* 2017). They must also be photostable without any dermal toxicity. Currently, a sunscreen is only guaranteed by a

combination of different organic and mineral filters, these latter being essential to reach a high index of protection. Many concerns about UV filters safety exist and their role in damaging aquatic/terrestrial life (Couteau *et al.,* 2012; Danovaro *et al.,* 2008; Downs *et al.,* 2014; Gilbert *et al.,* 2013). Thus, it is mandatory to find new compounds responding to a growing consumer demand focused on natural sources.

Some natural ingredients, mainly from plants or algae, can already be found in commercialized sunscreens (Downs *et al.,* 2014; Korać and Khambholja, 2011; Schmid *et al.,* 2006; Serafini *et al.,* 2015; Torres *et al.,* 2004). Indeed, among strategies implemented by animals and plants to counteract the deleterious effects of UVR, the production of mineral physical UV blockers such as calcium oxalate crystals or organic UV-absorbing metabolites can be observed (Edwards *et al.,* 1997). Lichen produces a large number of photoprotective compounds, among them polyphenols and anththraquinone pigments are well known (Hawksworth and Hill, 1984; Solhaug and Gauslaa, 1996). Some of the naturally occurring secondary metabolites metabolites could additionally neutralize the ROS, thus, flavonoids can be cited for plants, mycosporines or eumelanins for animals and fungi (Nguyen *et al.,* 2013) and MAAs and scytonemin from cyanobacteria (Ahmed *et al.,* 2021; Rastogi *et al.,* 2013).

As pioneer species in extreme places, with high light-irradiance and thus elevated UV exposure, lichens are of interest to this field. Lichen compounds can be a good source of inspiration for potent UV blockers for organic chemists and biotechnological options can be envisaged in some cases (Balskus and Walsh, 2010). However, a possible shortage of this low growing resource has to be considered. The extreme resiliency of lichens is partly based on the biosynthesis of various unique compounds, comprising a wide array of photoprotective compounds. With rise in the field of lichenology, a compilation of data for about 430 substances, including their occurrences in lichen species have been reported (Culberson, 1969, 1970, 1977) and the number of known substances from lichens is increasing continuously in recent years, while many more still need to be characterized (Huneckand Yoshimura, 1996; Huneck, 2001). According to their chemical structures, most lichen substances are phenoliccompounds (orcinol and β-orcinol derivatives), depsides (barbatic acid), dibenzofuranes and usnic acids (usnic acid), depsidones (salazinic acid), depsones (picrolichenic acid), lactones (protolichesterinic acid, nephrosterinic acid), quinones (parietin), and pulvinic acid derivatives (vulpinic acid) (Boustie and Grube, 2005). Lichens apparently evolved diverse biosynthetic pathways to produce this diversity of compounds, mainly polymalonate, mevalonic acid and shikimic acid pathways (Boustie and Grube, 2005).

The occurrence of UV-absorbing substances like scytonemin and mycosporine-glycine was reported for the first time from cyanobacterial lichens of the genera *Collema*, *Gonohymenia*and *Peltula*, all coming from high-light-intensity habitats (Büdel *et al.,* 1997). Except for *Collema* with the filamentous *Nostoc*, all other cyanobionts belong to the unicellular genera *Chroococcidiopsis*, *Cyanosarcina*, *Gloeocapsa*or *Myxosarcina*. From transmission electron microscope studies, it was found that the pigmentation (scytonemin) is located extracellularly in the sheath of the outer thallus parts (Büdel *et al.,* 1997). Fluorescence microscopy and microprobe measurements clearly showed UVR into the lichen thallus and hence the relevance of UV-sunscreens for the protection of these organism (Büdel *et al.,* 1997). An unknown compound was reported in cyanobacterial lichen of genus *Collema*which absorbed UVR strongly (Adams *et al.,* 1993).

Scytonemin is indole alkaloid present in extracellular sheath in several cyanobacteria (Garcia-Pichel and Castenholz, 1991; Pandey *et al.,* 2020; Pathak *et al.,* 2020; Proteau *et al.,* 1993; Rastogi *et al.,* 2013) and mycosporine-like amino acids (MAAs) are concentrated intracellularly (Ahmed *et al.,* 2021; Garcia-Pichel and Castenholz, 1993; Karsten and Garcia-Pichel, 1996; Richa and Sinha, 2015). MAA linking to oligosaccharide covalently had been isolated and chemically characterized in *Nostoc commune* (Böhm *et al.,* 1995). Scytonemin absorbs UVR strongly at 386 nm while MAAs mainly absorb at lower ranges between 310 and 360 nm (Garcia-Pichel and Castenholz, 1993; Pathak *et al.,* 2017a, b, 2019b). UV-protective compounds MAAs and scytonemin are found in organisms inhabiting environments having high levels of UVR and increased level of UVR increases the biosynthetic rates. They are comparatively photostable having good filtering power of their photoproducts and evade DNA damage by transferring energy to thymine singlets (Hidalgo *et al.,* 2002; Rancan *et al.,* 2002). MAAs are polar, water soluble compounds having low molecular weight (Bernillon *et al.,* 1990; Pattanaik *et al.,* 2008; Sampedro, 2011; Singh *et al.,* 2008) which have been present in many taxonomically marine lichens and their symbiotic partners (Řezanka *et al.,* 2004) as shown in table 1. These compounds consist of common chromophore unit corresponding to a cyclohexanone ring and substituents are present at different positions like alcohol, carboxylic acid, glycone that involve in their absorption. Balkus and Walsh (2010) have revealed their biosynthesis from sedoheptulose-7 phosphate *via* pentose phosphate pathway.

Natural UV-B absorbing mycosporine, Collemin A has been isolated from *Collemacristatum* and is capable of preventing UV-B damage such as cell destruction, pyrimidine dimer formation and erythema (Torres *et al.,* 2004). Mycosporine-glycine and mycosporine-taurine can help the organisms to tolerate both UV-A and UV-B and another MAA shinorine is prominently induced under UV-B irradiation (Singh *et al.,* 2008; Sinha *et al.,* 2001, 2003). Hence, scytonemin and MAAs play crucial role in photoprotection of the organisms and may also act as potential pharmacophores. Therefore, bioprospection of these novel class of compounds (Scytonemin and MAAs) from different possible sources is important for their

commercial use as antioxidants and natural sunscreens. Sikkim, the northeastern state of India, is one of the suitable habitats for the growth of lichens and reports of UV-screening compounds (MAAs and scytonemin) from lichens dwelling different habitats from this region of India are scarce. Considering above facts and reviewing the available concerned literature, we framed a simple field study to find the new possible sources of these UV-screening compounds from lichens inhabiting the hilly regions of Sikkim, India.

## MATERIALS AND METHODS

### Sample collection

Lichen samples were collected from different regions of Sikkim, India, during the winter season in 2019 (Fig.1), which is located between latitudes of 27º 5'N to 20º9'N and longitudes of 87º 59'E to 88º 56'E. It is situated in northeastern part of India and is the second smallest state of India. Sikkim is sandwiched between Nepal in West and Bhutan in the East, China in North and West Bengal in South and covers an area of 7,096 $km^{2.}$ Average annual temperature of Sikkim is around 18º C (64º F). Sikkim has diverse climate ranging from tropical to tundra and has rich collection of flora and fauna. Lichen samples were placed in plastic bags and brought to the laboratory for further analysis.

### Identification of organisms

The collected lichen samples were soaked in double distilled water (DDW) for few hours and washed repeatedly with sterile DDW. Then small amount of sample was taken on slide and observed under the compound microscope (OLYMPUS: Model no. CX21i-TR). Lichens were identified based on the morphology and anatomy of thallus structure with the help of available literature (Awasthi, 1988, 1991, 2000, 2007; Nayaka, 2004; Upreti *et al.,* 2005, 2008). Some online keys like website of Botanischer Garten under Botanisches Museum, Germany, were also used for identification of lichens. Members of cyanophyceae were identified by standard monographs and taxonomic keys (Desikachary, 1959; Komárek *et al.,* 2014).

### Extraction of UV-absorbing compounds MAAs and scytonemin

Lichen samples (0.4 g) were suspended in 100% HPLC-grade methanol for extracting MAAs by incubating the samples overnight at 4°C. The aliquots were then centrifuged at 8000 g for 5 min. Supernatants, which were taken in fresh Eppendorf tubes were evaporated at 38°C by vacuum evaporator. Redissolved the residues in DDW (600 µL) and added chloroform (100 µL) along with gentle vortexing. Centrifuged the mixture at 8000 g for 5 min and transferred the uppermost water phase (Photosynthetic pigment-free) carefully into fresh Eppendorf tubes and filtered the solution through sterilized syringe filters (0.2 µm pore-size) (AxivaSichem Biotech., New Delhi) (Richa and Sinha, 2015). Extraction of scytonemin was done in methanol/ethyl acetate (1: 1, v/v) by incubating the samples overnight at 4°C followed by sonication (2011-Sonic, cycle 30%, Power 40%) for 4 min. Samples were centrifuged at 10,000g for 5 min and supernatants were evaporated at 38°C in a vacuum evaporator and redissolved in 1:1 (v/v) methanol: ethyl acetate (500 µL) (Rastogi *et al.,* 2013). Finally, the samples were filtered through sterilized syringe filters (0.2 µm pore-size) (AxivaSichem Biotech., New Delhi) prior to the HPLC analysis. These filtered samples were further subjected to HPLC analysis (Waters 2998, Photodiode Array, pump L-7100, USA). For HPLC, a reverse phase semi-preparative column was used which was equipped with a Licrospher RP 18 column and guard (5-µm packing; 250 mm×4 mm inside diameter).

### HPLC analysis of UV-protective compounds

Further analysis of partially purified UV-screening compounds (Scytonemin and MAAs) was done using the HPLC system. MAAs sample (50 µL) was injected into the column of the HPLC system by an autoinjector (Waters 717 plus). Acetic acid (HPLC grade) (0.02%) constituted the mobile phase which ran isocratically with a flow rate of 1.0 mL $min^{-1}$. The PDA scan wavelength ranged from 200-400 nm and detection wavelength for MAA was at 330 nm (Richa and Sinha, 2015). For scytonemin, the flow rate of elution was maintained at a 1.5 mL $min^{-1}$ using the mobile phase consisting of solvent A (ultra-purewater) and solvent B (acetonitrile-methanol-tetrahydrofuran, 75:15:10, v/v). Gradient elution programme of 30 min was set with a linear increase (0-15 min) from solvent A (15%) to solvent B (100%) and 15-30 min at 100% solvent B. In the HPLC column, samples (50 µL) were injected and the detection wavelength for scytonemin was at 380 nm with PDA scan wavelength ranging from 250-750 nm. Identification of MAAs and scytonemin was done in the solvent through their characteristic absorption maxima and appropriate retention times (Garcia-Pichel and Castenholz, 1991; Pathak *et al.,* 2017b; Rastogi *et al.,* 2013; Richa and Sinha, 2015).

## RESULTS AND DISCUSSION

### Identification of organisms

Lichens samples collected from different habitats of Sikkim, India, were identified as *Heterodermia*sp., *Caloplaca*sp. and *Cetrelia*sp. and associated cyanobacteria were found to be *Gloeocapsa* sp., *Nostoc* sp. and *Solentia*sp. as depicted in Fig. 2.

### HPLC analysis and partial purification of MAAs and scytonemin

UV-absorbing compounds MAAs and scytonemin were extracted and partially purified from the collected lichen samples. Absorption spectra of 90% (v/v) methanolic extract was observed at 665 nm for chlorophyll *a*, 470 nm for carotenoid, 309-362 for MAAs, 386, 278 and 254 for scytonemin (Fig. 3 and 4).

Figure 3 shows the presence of MAAs as confirmed by HPLC chromatogram of the aqueous solutions and their corresponding absorption maxima. Prominent peaks at 1.28 and 1.55 min were recorded in HPLC analysis of partially purified MAAs (Fig. 3A and 3C) from *Cetrelia*sp.


**Figure. 1** Sampling sites of lichens collected from Sikkim state, India. Photographs showing lichens inhabiting rocks (A, H, I, J) and tree barks (B, D, F).

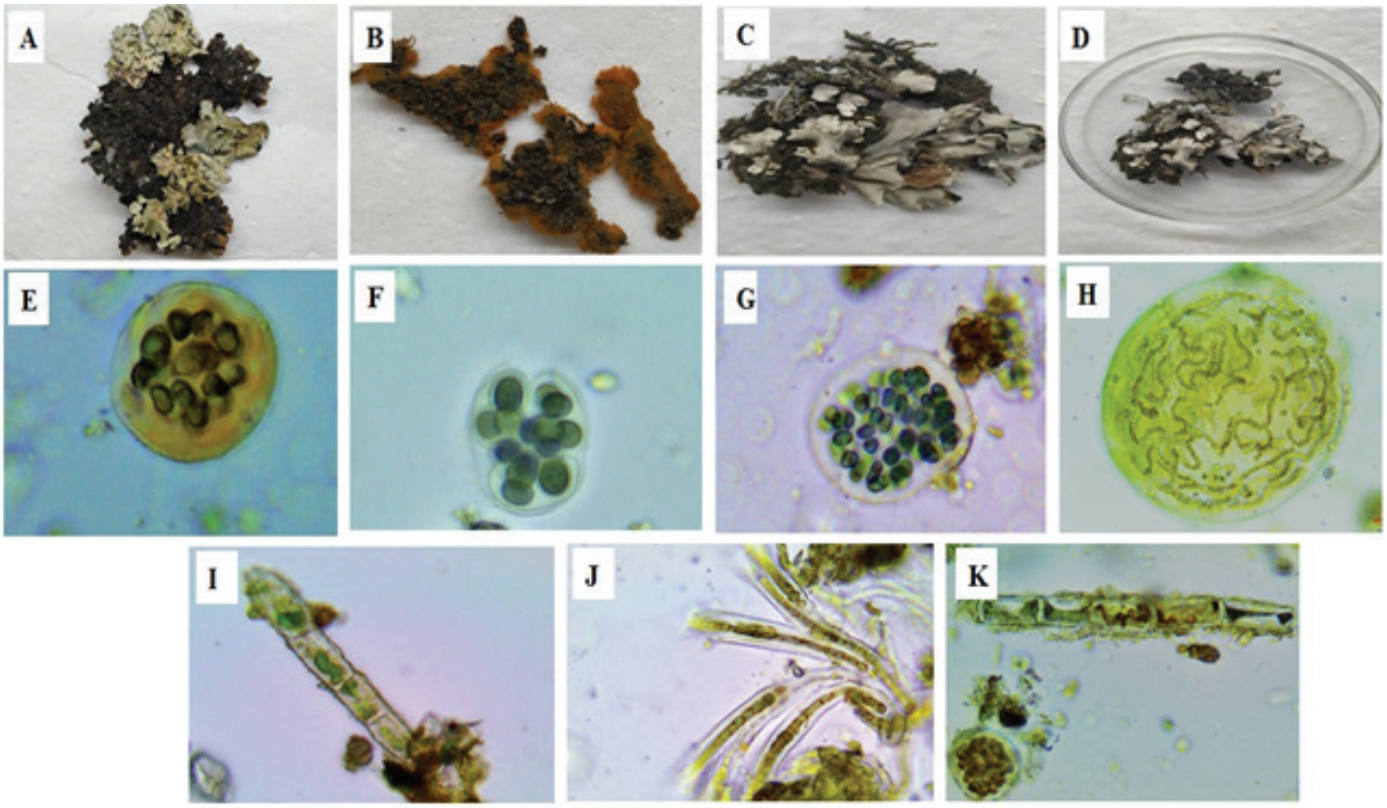


**Figure. 2** Microphotographs of lichens collected from natural habitats of Sikkim state, India (A) *Heterodermiasp*. (B) *Caloplaca* sp. (C-D) *Cetrelia* sp. and associated cyanobacteria (E-G) *Gloeocapsa* sp. (H) *Nostoc* ball (I-K) *Solentiasp*.

and *Heterodermia*sp. respectively with UV absorption maxima (UV$\lambda_{max}$) at 308.3 and 325.1 nm, which were identified as mycosporine-taurine ($\lambda_{max}$=308.3 nm; Fig. 3B) and mycosporine-NMA:Ser ($\lambda_{max}$=325.1 nm; Fig. 3D) from *Cetrelia*sp. and *Heterodermia*sp. respectively. Scytonemin was found in high concentration in the sampled lichens which were identified as *Caloplaca*sp. HPLC chromatograms of extracted scytonemin from lichens showed aprominent peak at the retention times of 5.25, 9.20 and 8.19min for scytonemin, scytonemin-3a-imine and gloeocapsin respectively (Fig. 4A) with absorption maxima at 384.7, 437.0 and 410.1nm respectively (Fig. 4B). Scytonemin showed absorption maxima at 252, 278, 385 nm which was found to be consistent with the earlier findings. Table 2 summarizesthe overall result of this bioprospection study which aimed at screening of

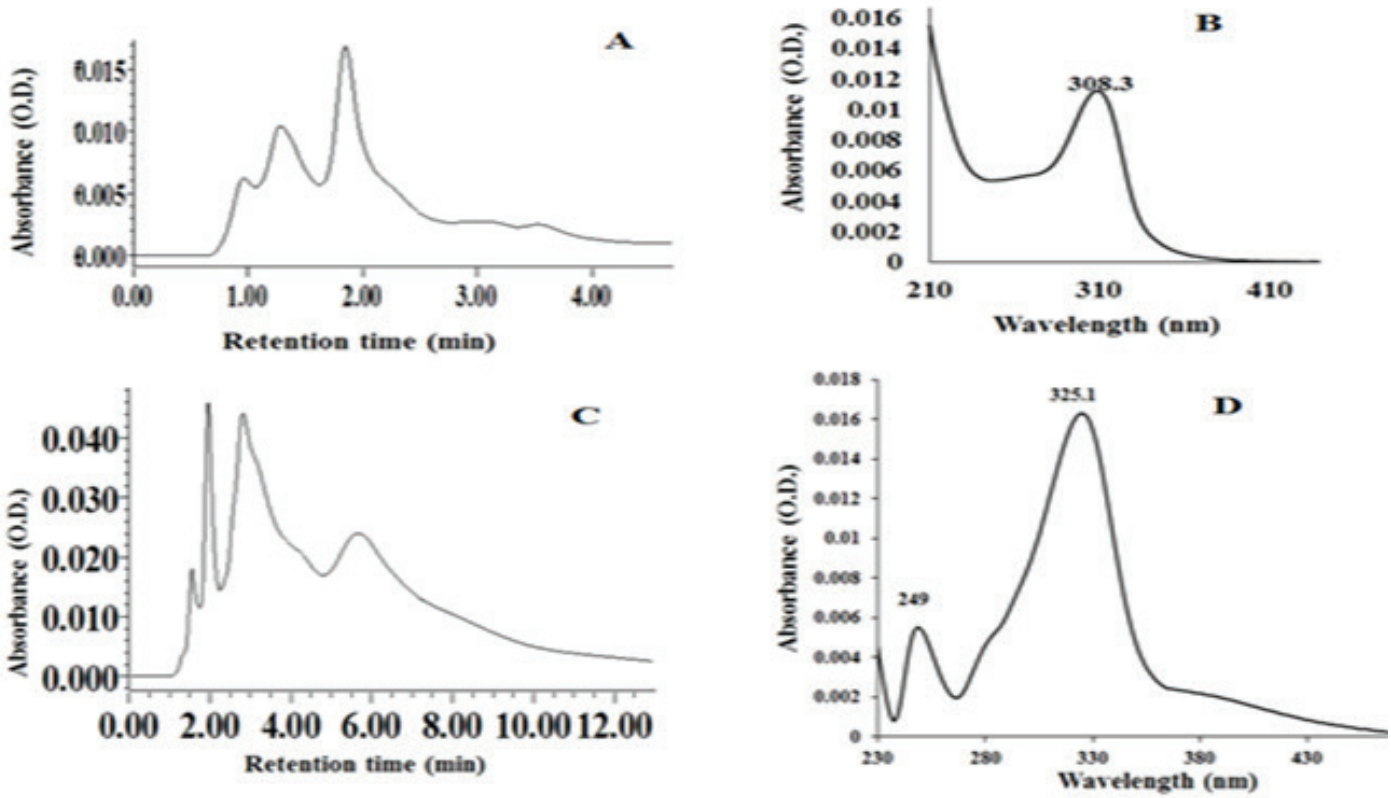


**Figure. 3** HPLC chromatograms of partially purified *mycosporine*-like amino acids and their corresponding absorption spectrum from *Cetrelia* sp. (A, B) and *Heterodermia* sp. (C, D)

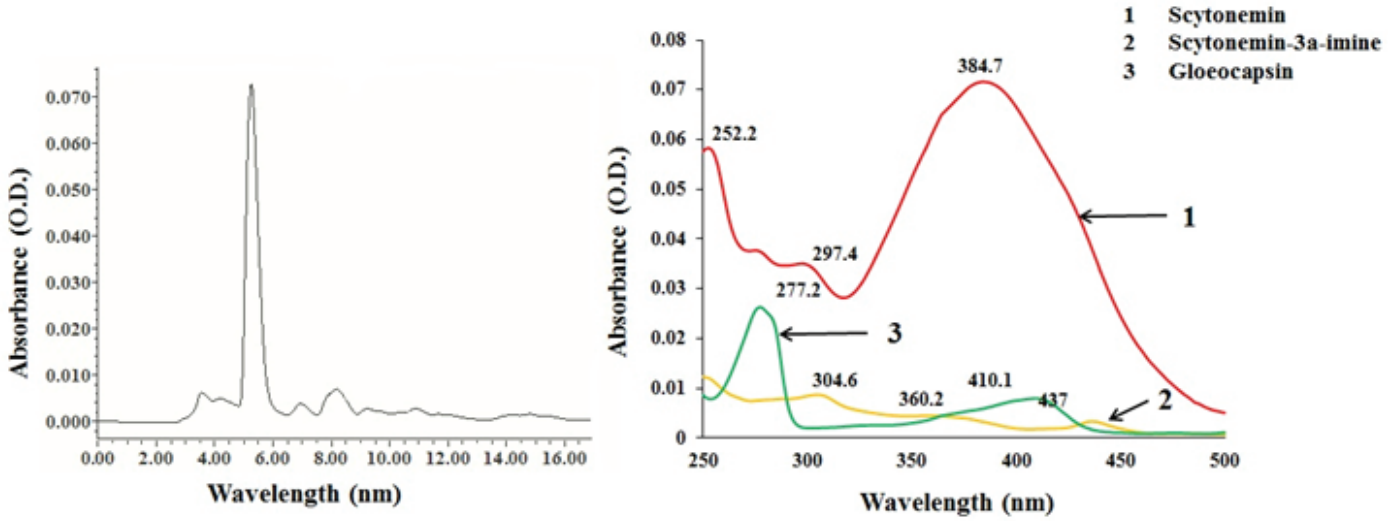


**Figure. 4** HPLC chromatogram of partially purified scytonemin, scytonemine-3a-imine and gloeocapsin (A) and their corresponding absorption spectra (B) from the lichen *Caloplaca* sp.

photoprotective compounds present in different lichens collected from diverse habitats of Sikkim, India and tentative identification of these compounds with the help of HPLC chromatogram considering their absorption maxima and retention times.

## CONCLUSION

Lichens are characterized as a stable and self-supporting association between fungi and photoautotrophic algal/cyanobacterial partners. Lichenization is a successful strategy for fungi, almost 20% of all species being lichenized with a wide variety of photobionts and photobiont arrangements (Honegger, 2009). Cyanolichens are usually stated to be bipartite (mycobiont plus cyanobacterial photobiont). Analyses revealed green algal carbohydrates in supposedly cyanobacterial lichens (*Peltigera*, *Pseudocyphellaria* and *Sticta*) (Büdel and Scheidegger, 2008; Nash III, 2008). The exact form of the symbiosis in lichens remains still controversial, but is commonly suggested to be mutualistic. Lichens survive in high UVR by several adaptive mechanisms like membrane and macromolecule repair systems, thermal dissipation, antioxidant defense, light scattering and radiation screening through biosynthesis of photoprotective compounds (Nguyen *et al.,* 2013). Lichens are the unique organisms which produce a vast array of compounds, with more than

**Table 1.** Molecular structure, absorption maxima, extinction coefficient of photoprotective compounds reported in cyanolichens/ lichens.

| Organisms | Photoprotective compounds | $\lambda_{max}$(nm)and ε ($M^{-1}$ $cm^{-1}$ ) | References |
|---|---|---|---|
| Cyanolichens Collagen, Gonohymenapeltula | Mycosporine -glycine | 310 (28800) | Büdel and Scheidegger 2008, Franceschi and Horner 1980 |
| Lichina pygmaea, Lichina confinis, Peltigera praetextata, Peltigera horizontalis, Polydectillum, | Mycosporine serinol | 310 (27270) | Roullier *et al.,* 2009, Roullier et al 2011 |
| Nephroma laevigatum Arch | Mycosporine hydroxyglutamicol | 310 | Roullier *et al.,*2011 |
| Collema cristatum | Collemin A | 311 (34400) | Torres *et al.,* 2004 |
| Collemacf. coccophorum, Petulaclavata, Petulaeuploca, Petulapatellata, Petulatortuoso, Peltulaumbilicata, Gonohymenianigritella | Scytonemin | 386 | Büdel *et al.,* 1997 |

**Table 2.** Photoprotective compounds present in different lichens and their corresponding absorption maxima and retention times.

| Lichen | Scytonemin | | Mycosporine-like amino acids | | |
|---|---|---|---|---|---|
| | $\lambda_{max}$(nm) | Retension time (min) | $\lambda_{max}$(nm) | Retension time (min) | Photoprotective compounds present |
| Caloplacasp. | 384.7<br>410.1<br>437.0 | 5.25<br>8.19<br>9.20 | - | - | Scytonemin<br>Gloeocapsin<br>Scytonemin-3a-imine |
| Cetreliasp. | - | - | 308.3 | 1.28 | Mycosporine-taurine |
| Heterodermasp. | - | - | 325.1 | 1.550 | Mycosporine-NMA:Ser |

1000 secondary metabolites known.

An important protective mechanism of lichens is the production of unique and efficient UV absorbing compounds such as depsidones, depsides, diphenyl ethers, bisxanthones, mycosporines, MAA sand scytonemin along with classical pigments such as melanin and carotenoids (Büdel *et al.,* 1997; Nguyen *et al.,* 2013). While the most abundant lichen polyfunctionalized aromatic compounds, belonging to orsellinic derivatives, are UV-B screens, these organisms produce strong UV-A filters such as calycin (pulvinic acid derivatives), bisxanthones (secalonic acids), scytonemin or mycosporines and MAAs with the latter ones exhibiting attractive properties as photoprotectants (Nguyen *et al.,* 2013). Due to the harmful effects of the UVR wavelengths of sunlight, the search for new sunscreens remains important.

We herein have endeavoured for the presence of UV-protective compounds specifically MAAs and scytonemin from the lichen's samples collected from Sikkim, India. The MAA profile of collected samples showed presence of MAAs mycosporine-taurine ($\lambda_{max}$=308.3 nm) and mycosporine-NMA:Ser ($\lambda_{max}$=325.1 nm). A significantly higher amount of scytonemin was observed in some of the lichen samples. HPLC chromatograms of extracted scytonemin revealed the presence of a prominent peak at the retention times of 5.25- and 9.20-min having absorption maxima at 384.7 and 437.0 nm for scytonemin and scytonemin-3a-imine respectively. HPLC chromatogram also showed a prominent peak at the retentiontime of 8.19min with absorption maxima at 410.1nm indicating presence of another important UV-protective pigment gloeocapsin in the lichen samples.

Environmental factors also affect the production of these UV-protectant compounds in which lichens grow. These compounds allow the visible lights which are responsible for photosynthesis and help in absorbance of UV light followed by energy dissipation and thus give consideration to the ecological feedback of organisms to the environment. These compounds should be associated with other UV filters and antioxidants which protect DNA damage that occurs by putative singlet oxygen species formed after photodegradation, so these could not be used alone (Büdel *et al.,* 1997). Cyanobacteria have various defense strategies which helps them to tolerate high UV irradiations and one such important strategy is synthesis of UV-protective compounds (Scytonemin and mycosporine-like amino acids) (Ahmed *et al.,* 2021; Pandey *et al.,* 2020; Pathak *et al.,* 2017a, b, 2019a, b, 2020; Rastogi *et al.,* 2013; Richa and Sinha, 2015). Scytonemin minimizes cellular damage from UV-induced ROS and thymine dimer formation thus helps in photoprotection and genome maintenance (Pathak *et al.,* 2020; Rastogi *et al.,* 2014). Scytonemin is induced not only by UVR as its synthesis is also enhanced by several abiotic factors such as high temperature, periodic desiccation stress, osmotic stress, photo-oxidative stress, light: dark cycle (12:12), high illumination intensity, nitrogen deficiency and salinity (Chen *et al.,* 2013; Dillon *et al.,* 2002; Fleming and Castenholz, 2007, 2008; Rath *et*

*al.,* 2012). MAAs have good absorption under UV-A and UV-B regions and also have high photostability (Ahmed *et al.,* 2021; Rastogi and Madamwar, 2016; Richa and Sinha, 2015).

Environmental factors such as temperature, salinity, ammonium concentration also affect the MAAs and their related compounds production (Ahmed *et al.,* 2021). In the past few years, both MAAs and scytonemin isolated from different organisms have become one of the most promising naturalsubstances in the field of biotechnology and biomedical research due totheir non-toxic nature, strong UV absorption, ROS scavenging, potential antioxidant, and antiproliferative properties (Ahmed *et al.,* 2021; Misonou *et al.,* 2003; Pathak *et al.,* 2020; Rastogi *et al.,* 2013, 2016; Rastogi and Madamwar, 2016; Stevenson *et al.,* 2002). Hence, continuous bioprospection of these multipurpose UV photoprotectants from different organisms including lichens is much required area of research.

## AUTHOR'S CONTRIBUTIONS

NK: Performed the experiments and wrote the manuscript; JP: Helped in performing experiments, analyzed the experimental results, helped in writing and critically reviewing the manuscript; AKD: Performed the sampling of lichens from Sikkim, India; RPS: Designed the experiments, provided laboratory facilities and reviewed the manuscript. The authors accepted the final version of the manuscript.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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