CASE STUDY

Indigenous Knowledge of Lichens can Improve Human Life in Tribal Areas of Arunachal Pradesh: A Case Study

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Abstract

The higher eastern Himalayan regions of India exhibit the luxuriance of different lichen taxa together with other group of plants. The "Punpun" is a common name of lichens used by the Sherdukpen tribe of West Kameng District of Arunachal Pradesh in Eastern Indian Himalayan regions. The punpun or lichens are gorgeous in nutrients and contains biologically active compounds belonging to various chemical clades. The tribal people have gigantic traditional knowledge about lichens thus allowing them to use different types of lichens to fulfil their dietary need along with medicinal prospects. The present information was collected during the field visit to the area between the years 2017-2021. The tribal people in the area prepared some delicious diets from the punpun and served as nutritive ethnic food to tourists visiting there. Though tribal people have great knowledge of lichen uses, but they lack the acquaintance of appropriate species proficiency. The tribal people provided the detailed methodologies and application of lichens which may be useful in near future for bioprospection and herbal drug development. The present case study is presented with an aim to encourage the tribal people to investigate further applications of lichens to improve the life of surronding inhabitants and to make them aware of the importance and conservation of lichen species.

Keywords: Herbal drugs, Indigenous food, Entrepreneurship, Disease remedies, Conservation.

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Introduction

The indigenous and ethnic people in the world have been cultured to live in the most unsympathetic environmental circumstance in the universe. The most fascinating feature associated with the indigenous and ethnic groups is that, they live in areas that are vastly rich in biodiversity. It is predictable that about 300 million indigenous people are living in the world, out of which nearly half i.e. 150 million are living in Asia, whereas about 30 million are living in Central and South America and a significant number of them live in Australia, Europe, New Zealand, Africa (Elkhateeb *et al.,* 2022).

It is well known that the ethnic people play a pivotal role in the conservation of environment as well as management and development process as they posse's traditional knowledge which is quite useful in eco-restoration (Elkhateeb, *et al*. 2022). In India, 68 million people belonging to 227 ethnic groups, comprising of 573 tribal communities resulting from six racial stocks namely - Negroid, Proto-Australoid, Mongoloid, Mediterranean, West Breachy and Nordic exists in different part of the country (Pushpgandhan, 1994).

The Indigenous information inherited by native and local communities is unique for a culture or a society. The scientific investigation and proper documentation of indigenous knowledge on wild resources are imperative tools to recognize the traditional living and food systems of local residents (Bharucha and Pretty, 2010). It is estimated that around one billion people worldwide consume wild foods in their diet, among them wild plants play a crucial role in the subsistence strategy of rural communities in developing countries (Burlingame, 2000; Zemede and Mesfin, 2001). Among wild plants, the lichens are being used in traditional foods and medicines since millennia and play vibrant roles in ecosystem function and human welfare (Crawford, 2019). However, in mid-eighteenth century, common crops were badly affected in Europe by frosts and droughts causing famine, and as a consequence, where lichens were used as food because of their easy availability, cheapness and nutritive value (Llano, 1948), excluding a few which are poisonous, e.g. *Bryoria fremontii, B. tortuosa, Cetraria pinastri and Letharia vulpina*, and used to kill enemies and animals (Spribille *et al.,* 2016).

It is well known that lichen is a symbiotic association between fungi (mycobionts) and photoautotrophic, algal partners (photobionts). According to Hawksworth and Grube (2020), lichen is a self-sustaining ecosystem shaped by the interaction of an exhabitant fungus and an extracellular arrangement of one or more photosynthetic partners and an indeterminate number of other microscopic organisms and upper surface microorganism called lichenosphereic. The

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mycobiont is unique in the symbiotic association and usually dominates the association, lichens are traditionally classified as a life form of fungi. The fungal partnership is mostly (98%) accorded by ascomycota and remaining by the Basidiomycota and anamorphic fungi (Gilbert, 2000). Approximately 21% of all fungi are able to act as a mycobiont; thus, lichens form the largest mutualistic group among fungi (Honegger, 1991). Forty algal genera are involved as photosynthetic partners in lichen formation, 25 are green algae and 15 are cyanobacteria (Kirk, *et al*. 2008). The number of accepted species of lichens reported from all over world is 19,387 under 995 genera, 115 families, 39 orders and 8 classes (Lücking, *et al*. 2017). However, in India a total of 2,961 species are reported, which is approximately 14% of the world known lichen species as well as 5.41% of the plant diversity of India (Mao, *et al*. 2021).

The lichens are important constituents of primary producers and have a wide range of substrates and habitats, comprising some of the most extreme conditions on earth like Himalaya is singular, poles (North and South), desert (cold and hot), even on man-made artifacts, glass surfaces and others. Several lichens such as *Usnea, Cladonia, Hypogymnia*, *Evernistrum* and *Parmelia* are sensitive against environmental instabilities and can be used to assess air pollution in the areas (Garty, 2001; Shukla, *et al*. 2013). Contrasting, with simple dehydration in higher plants and animals, lichens may experience a complete loss of body water in dry periods called poikilohydric nature. Recent research reported that lichens contribute nitrogen availability to soils either by forming litter, or predation by herbivores, e.g., snails, which then defecate, providing nitrogen to the soils. In hot deserts and arid/semi-arid areas, lichens are part of wide, biological soil crusts, which is essential for sustaining the soil structure of an area (Zambare and Christopher, 2012). On the basis of thallus morphology lichens are grouped as: *Crustose* (phytobiont in a distinct layer below an upper mycobiont cortical layer with no lower cortex); *Leprose* (groups of phycobiont surrounded by mycobiont); *Foliose* (leafy; phycobiont in a layer below an upper cortex with a discrete cortex below, separate from the substratum on which it grows; *Filamentous* (phycobiont surrounded by a sheath of mycobiont); and *Fruticose* (shrubby; erect, vertical or trailing; radial in structure, often attached at the base, with the phycobiont in a layer inside the outer cortex) (Yang,*et al.* 2021). All the growth forms of lichens are successfully used in the food industry and for preventing or treating diverse human diseases. Furthermore, lichens produce secondary metabolites as a defense mechanism to protect themselves against external predators, harmful UV radiations, pollutants and abiotic/biotic stresses (Devkota, *et al*. 2017).

It is well-recognized that, the lichens are important diet for humans and animals since ancient time. In China, overall 38.4% diet of *Rhinopithecus roxellana*, depends only on lichens and it is also reported that the regional lichen diversity will affect living habitat of *R. Roxellana* (Liu, *et al*. 2013). Additionally, in most of the temperate and arctic regions of the world, people frequently use lichens as food, spices, and pharmaceutical products, as well as in various ethnic practices (Crawford, 2019). Earlier, *Cetraria islandica* (Iceland moss) was an important source of food for humans in northern Europe and the lichens were cooked as bread, porridge, pudding, soup, or salad. The tribal people in North America and Siberia traditionally consume the partially digested reindeer lichen (*Cladonia* sps.) after they remove it from the rumen of caribou or reindeer that have been killed. The rock tripe lichens *Umbilicaria* and *Lasallia* sps., are frequently used as an emergency food in North America whereas *Umbilicaria esculenta* was used as traditional Korean and Japanese foods (Llano, 1994). In eastern Indian regions *Leptogium denticulatum* Nyl., due to presence of wide lobes of the thallus, the local ethnic group named the species as "Eki Nyaru" meaning "Dog ear". Dog ear lichen is widely used in soup by Adi tribes in Arunanchal Pradesh (Rout, *et al*. 2010).

In India, both scientists and ethnic people have used lichens in various sense, such as, food (Upreti *et al*. 2005; 2015), fodder (Upreti *et al*. 2005), environmental monitoring studies (Shukla *et al*. 2013), climate change studies (Bajpai *et al*. 2019), bioprospection studies (Prateeksha, *et al*. 2016; 2020), dye preparation (Shukla *et al*. 2014), and so on. Scientists have exploited the lichens for brewing, distilling, tanning, ingredients in cosmetics and perfumes, pharmaceuticals, aromatic fixatives, incense and environmental monitoring studies worldwide (Maraseni and Shivakoti, 2003). However, ethnic groups besides lacking scientific knowledge about the chemical constituents of lichens have cleverly used lichens for their medicinal value, food value, ritual and spiritual value, aesthetic and decorative value, bedding value, and ethno-veterinary values (Devkota, *et al*. 2017). The traditional uses of lichens involve boiling the lichen thallus to produce a mucilage (carbohydrates such as isolichenins and galactomannans) which is drunk for lung or digestive ailments, or applied topically for other issues (Nayaka, *et al*. 2010; Upreti, *et al*. 2015; Crawford, 2019).

The majority of the Asian ethnic groups which use lichens currently reside in the massive Himalayan areas of Bhutan, China, India and Nepal, and have principally adopted the classical systems of medicine following Ayurveda, Yoga, Naturopathy, Unani, Siddha (AYUSH), along with Indian Traditional Medicine (ITM) and continued their traditional uses of lichens for food, beverages and medicines, because of which the trade of lichens for folk uses, especially for manifestly health-promoting tea, dry snacks, local secret spices, has increased remarkably since one decade.

Among the Indian Himalayan regions (IHR), the tribes of eastern Himalaya have used lichens of that region in different ways to improve their economy gradually. Since the year 2017, authors have studied folk uses of lichens together with investigation on the lichen flora of Eastern Himalaya, with aims to reveal the traditional uses. The diverse uses of lichens in the area were studied based on published records and interviewing the local people through the questionnaire (Annexure I). The study initiated with an aim to improve the knowledge of the local peoples, regarding pros and cons of lichen species which lead to conserve the lichen diversity in the area. The present study will not only help to understand the use of lichens by the tribes of eastern parts of the Indian Himalaya but also serve as a key reference for superfluous researches of lichen in near future.

Materials And Methods

The ethnic practices of lichens were chronicled during eight field trips, each of roughly 12-15 days in five repeated years,

concluded through direct questionnaires to more than 100 persons. Questions were asked to local people about lichen uses and ethnic market places were visited in order to purchase lichen samples offered for sale by ethnic people's edict to ensure the acceptability, relevance and credibility of the given substantiation. The lichen collected by tribal as well as market samples were identified using standard microscopic techniques and hand-sectioned under a Leica EZ4 dissecting microscope. Anatomical descriptions are based on observations of these preparations under a Nikon microscope. However, the secondary metabolites of all the specimens were identified using spot tests and thin-layer chromatography (TLC) (Orange, *et al.* 2001). The detailed methodology is presented in Fig. 1.

Results And Discussion

A total of 82 species of lichens were collected by tribal people from forest as non-timber forest products (NTFP) and sold in the local market (Table 1). Among market samples, species of lichen genus *Usnea* exhibit their dominance followed by *Bryoria, Cetraria, Parmotrema, Rematotrachyna* and *Ramalina*. The tribal people provided the detailed methodology from lichen collection to its application. The local people visit forest for collection of lichens during the period when oak trees shed leaves and branches (mostly women prefer to go in group and collect it). Further lichens which are blown off by the running wind and are also being collected from the forest floor (Fig 2 A-F). The tribal people are more aware about the lichen resource conservation, and this can be justified by: 1) they never pick thallus directly from tree bark, and always collect lichens from fallen twigs as well as soil, 2) they create some natural space in the forest in order to conserve lichens, and 3) they don't repeat the same path for collection of lichens in the same year.

After collection, the lichens are segregated for various purposes and rinse with natural water to avoid dust and debris (Fig 3 A-D). The segregated lichen thallus is transferred to a large container with 1/3 of water and allowed to boil (10-15 minutes). Then 250 gm wood coal ash per kg of lichen is added during this boiling procedure (Fig 4 A-H). The coal affords good taste as well as neutralized the pH and provide golden brownish colour to the lichen thallus. After some time the lichen changes its colour and texture and it is then transferred to another container, where it is washed with clean water so as to remove ashes. The cleaned lichens are then kept for drying on bamboo woven mat (Mandro). The drying process after boiling help to keep lichens fresh which can be stored for months and used whenever required. The preserved lichen is boiled as well as fried and mixed with rice, soup, daal and stuffed in wheat flour and used as food and health supplement (Fig. 5 A-E).

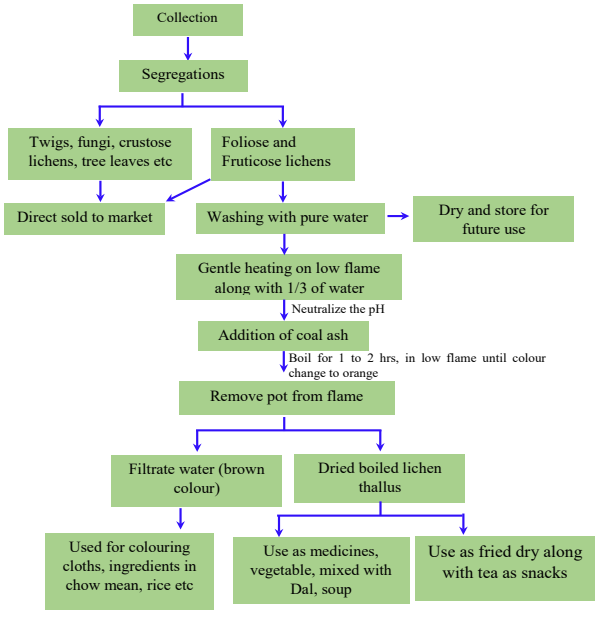


Fig. 1: Method applied by tribal people for lichen harvesting.

Past Works Resembles with Present Study

In past, it was well known that the edible lichens were mostly assembled for private consumption (Wang *et al.,* 2001) as traditional/local food to tourists as a commodity in the Himalaya (Upreti, *et al.* 2005). The *Lethariella* and *Thamnolia* species, are widely used as health-promoting teas in tribal areas of the Himalaya. The species of *Lobaria, Umbilicaria*, *Nephromopsis* and *Ramalina* having greater thallus which are used as food and are relatively common in the local supermarkets and some restaurants of the country. Generally, summer and autumn are the best seasons to harvest edible lichens, which are used as fresh or dried for later use. Usually, stewing with burns, steaming, cooking soup and other methods are used to make dishes, such as "Liang Ban" from *Ramalina* species (Upreti, *et al.* 2005). Whereas, *Lobaria pulmonaria* is frequently used in the area as traditional medicines effective for treating pneumonia, due to their lung-like appearance (applied because of the doctrine of signatures, suggesting that herbs can treat body parts that they physically resemble) (Wang and Qian, 2013). *Lobaria* species have also been reported to serve as a valuable source of proteins, having a protein content higher than that of kelp or edible fungi, such as *Tremella*. In addition, the content of dietary fibre in *Lobaria* species is significantly higher than in other fungi and edible algae, it is also rich in calcium (Cui and Duan, 2000). Similarly, *Peltigera leucophlebia* (Nyl.) Gyeln., is used to cure for thrush (Aphtha, Candidiasis), due to the resemblance of its vegetative organs cephalodia to the appearance of the disease (Dobson 2011). The earliest report about traditional medicine of lichen could be the *Usnea longissima* Ach., in the Chinese Qing

Fig 2(A-F): People collecting lichen from forest

Table 1: Lichen species used by tribal people in Arunachal Pradesh and their applications described by earlier workers

Lichen taxa collected by tribal peoples	*Modes of consumption*	*Ethnobiological aspects*	*References*
Bryoria asiatica (Du Rietz) Brodo & D. Hawksw., *Bryoria bicolor* (Ehrh.) Brodo & D. Hawksw., *Bryoria confusa* (D.D. Awasthi) Brodo & D. Hawksw., *Bryoria himalayensis*(Mot.) Brodo & D. Hawksw., *Bryoria lactinea* (Nyl.) Brodo & D. Hawksw., *Bryoria levis* D.D. Awasthi, *Bryoria nepalensis* D.D. Awasthi, *Bryoria nitidula* (Th. Fr.) Brodo & D. Hawksw., *Bryoria variabilis* (Bystr.) Brodo & D. Hawksw, *Bulbothrix isidiza* (Nyl.) Hale, *Bulbothrix meizospora* (Nyl.) Hale, *Bulbothrix setschwanensis* (Zahlbr.) Hale	Either boiled or used dry. Boiled and used as poultice for arthritis.	Applied as poultice to reduce swellings, hair and rub ashes on hair and scalp to stop hair from going gray. Used for healing skin abrasions, diarrhoea, and vaginal discharge. Used for kidney deficiency and general weakness, dizziness, heart palpitation, involuntary ejaculation, night sweats, difficulty urinating, edema, impetigo, draining pus, and improving eyesight. Drink decoction; or apply decoction or powdered lichen to affected area	Wennekens 1985, Wang and Qian 2013
Cetraria islandica (L.) Ach., *Cetraria laevigata* Rass., *Cetrelia braunsiana* (Müll. Arg.) Culb. & Culb. , *Cetrelia cetrarioides* (Delise ex Duby) W.L. Culb. & C.F. Culb., *Cetrelia chicitae* (W.L. Culb.) W.L. Culb. & C.F. Culb., *Cetrelia collata* (Nyl.) W.L. Culb. & C.F. Culb., *Cetrelia olivetorum* (Nyl.) W.L. Culb. & C.F. Culb., *Cetrelia pseudolivetorum* (Asahina) Culb. & Culb., *Cetreliopsis rhytidocarpa* (Mont. & Bosch.) M.J. Lai subsp.	Salads, mush, flavouring agents, ingredients of bread	Prevent the diseases in lung, kidney, and gastrointestinal tract, expectorants, treat mouth and throat irritation, colds, indigestion, fevers, and infections; moisturizing; sedatives	Airaksinen, *et al.,* 1986; Kalle & Souk, 2012; Meli *et al.,* 2018
Cladonia furcata (Huds.) Baumg., *Cladonia rangiferina* (L.) Weber	Salads	dry throats, jaundice, dystopian blurring of vision	Simkova & Polesny, 2015, Wang, *et al.,* 2001
Dermatocarpon miniatum (L.) W. Mann	Dishes and soups	Improve digestion; dispel wind-damp; relieve pain; treat abdominal distention	Huang *et al.* 2018
Flavoparmelia caperata (L.) Hale	Spices and flavoring agents	Treat blurred vision, bleeding, sores, swelling, and dermatitis Vermifuges	Salin Raj *et al.,* 2014; Siddiqi *et al.,* 2018
Hypotrachyna cirrhata (Fr.) Divakar, A. Crespo,Sipman, Elix & Lumbsch, *Hypotrachyna nepalensis* (Taylor) Divakar, A. Crespo, Sipman, Elix & Lumbsch, *Hypotrachyna vexans* (Zahlbr. ex W.L. Culb. & C.F. Culb.) Divakar, A. Crespo, Sipman, Elix & Lumbsch	Vegetable, spices, and flavoring agents	Astringents, resolvent, laxatives, carminative, aphrodisiacs, and antiseptics; treat bleeding piles, leprosy, excessive salivation, bronchitis, and disorders of blood, heart, and stomach; heal wounds	Kekuda *et al.,* 2011
Lobaria pulmonaria (L.) Hoffm., *Peltigera leucophlebia* (Nyl.) Gyeln., *Leptogium denticulatum* Nyl.	Mush and ingredients of bread Dishes and soups	Treat indigestion, wounds, malnutrition, bloating, swelling, lung inflammation, and infections; relieve pain and itching, Antiseptics; healing agents; treat pulmonary diseases, diarrhea, and bleeding; stimulate appetite. Treat stomach diseases, eye problems, sores, wounds, arthritis, and inflammation	Süleyman *et al.,* 2003, Malhotra *et al.,* 2008, Rout *et al.* 2010
Parmotrema cooperi (J. Steiner & Zahlbr.) Serus, *Parmotrema cristiferum* (Taylor) Hale, *Parmotrema eunetum* (Stirt.) Hale, *Parmotrema hababianum* (Gyeln.) Hale, *Parmotrema latissimum* (Fée) Hale, *Parmotrema mesotropum* (Müll. Arg.) Hale, *Parmotrema nilgherrense* (Nyl.) Hale, *Parmotrema ravum* (Krog & Swinscow) Sérus., *Parmotrema reticulatum* (Taylor) M. Choisy, *Parmotrema robustum* (Degel.) Hale, *Parmotrema sancti-angelii* (Lynge) Hale, *Parmotrema subtinctorium* (Zahlbr.) Hale, *Parmotrema thomsonii* (Stirt.) A. Crespo, Divakar & Elix, *Parmotrema tinctorum* (Despr. ex. Nyl.) Hale, *Parmotrema zollingeri* (Hepp) Hale	Spices and flavoring agents, Mush and ingredients of bread –Tea	Treat bleeding, ulcers, burns, and scalds, Treat respiratory diseases, asthma, hypertension, and congestion	Simkova & Polesny, 2015, Emsen, *et al.,* 2018
Ramalina conduplicans Vain., *Ramalina hossei* Vain., *Ramalina nervulosa* (Müll. Arg.) Abbayes, *Ramalina pacifica* Asahina, *Ramalina pollinaria* (Westr.) Ach, *Ramalina roesleri* (Hochst) Hue, *Ramalina sinensis* Jatta	Dishes, Mush and ingredients of bread	Reduce inflammation, Astringents; treat fevers, wounds, burns, eczema, and hemorrhoids	Luo *et al.*, 2010, Simkova & Polesny, 2015

Remototrachyna adducta (Nyl.) Divakar & A. Crespo, *Remototrachyna awasthii* (Hale & Patw.) Divakar & A. Crespo, *Remototrachyna flexilis* (Kurok.) Divakar & A. Crespo, *Remototrachyna infirma* (Kurok.) Divakar & A. Crespo, *Remototrachyna koyaensis* (Asahina) Divakar & A. Crespo, *Remototrachyna rigidula* (Kurok.) Divakar & A. Crespo, *Remototrachyna scytophylla* (Kurok.) Divakar & A. Crespo, *Remototrachyna thryptica* (Hale) Divakar & A. Crespo	Dishes, Spices and flavoring agents, Mush and ingredients of bread –Tea	Treat bleeding, ulcers, burns, and scalds, Treat respiratory diseases, asthma, hypertension, and congestion	Emsen, *et al.,* 2018
Umbilicaria spp.	Dishes, soups, and salads	Treat inflammation and poisoning; hemostasis; antifebrile; moisten lung; dysentery, Treat tuberculosis and prolonged bleeding	Kim & Cho, 2007, Du *et al.,* 2015; Kim & Lee, 2006; Xu, *et al.,* 2011
Usnea aciculifera Vain., *Usnea bismolliuscula* Zahlbr., *Usnea bornmuelleri* J. Steiner, *Usnea cineraria* Motyka, *Usnea dendritica* Stirt., *Usnea eumitrioides* Motyka, *Usnea glabrata* (Ach.) Vain., *Usnea himalayana* Bab., *Usnea himantodes* Stirt., *Usnea lucea* Mont., *Usnea luridorufa* Stirt., *Usnea maculata* Stirt., *Usnea nepalensis* D.D. Awasthi, *Usnea nilgirica* G. Awasthi, *Usnea nipparensis* Ashina, *Usnea orientalis* Motyka, *Usnea pangiana* Stirt., *Usnea perplexans* Stirt., *Usnea pictoides* G. Awasthi, *Usnea rigidula* (Stirt.) G. Awasthi, *Usnea robusta* Stirt., *Usnea rubicunda* Stirt., *Usnea spinosula* Stirt., *Usnea splendens* Stirt., *Usnea stigmatoides* G. Awasthi, *Usnea subfloridana* Stirt., *Usnea thomsonii* Stirt., *Dolichousnea longissima* (Ach.) Articus, *Eumitria baileyi* Stirt.	Mush and ingredients of bread, Dishes	Antiseptics, Treat insomnia, bleeding, jaundice, nausea, and whooping cough, Dishes Antiinflammation; detoxification; treat dyspepsia, amenorrhea, ulcer, vomiting, ascariasis, female problems, and injuries of legs, loins, and bones, Treat cancers, ulcers, and tuberculosis, Pectorals; diuretics; febrifuge; treat burns, colds, indigestion, and mouth ulcers	Dobrescu *et al.,* 1993, Bai, *et al.,* 2014; Kim & Cho, 2007; Siddiqi *et al.,* 2018, Guzman, 2008, Emsen *et al.*, 2019

dynasty (ca. 1500); it was reported that the *Usnea* species were used to treat cold, swelling and pain (Zhao, 1765).

The lichens have their abode in current pharmaceutical research as they produce metabolites which has potential therapeutic or diagnostic value (Prateeksha, *et al.* 2020). Certain metabolites like usnic acid, fumarprotocetraric acid, artanorin produced by lichens are structurally and functionally similar to broad-spectrum antibiotics, while a few of them are associated with antiseptic similarities (Müller, 2001).

The species of lichen genera *Evertiastrum, Flavoparmelia, Lobaria, Usnea, Thamnolia, Lethariella, Cladonia* distributed between altitudes of 2000-4500 m in the IHRs, is mainly sold in local market and exported to nearby area (Upreti and Negi, 1996). The Tawang and Bomdela localities in Arunachal Pradesh are the holy places of Buddhist and have great demand for incense. The species of lichen genera *Evertiastrum, Usnea, Lethariella, Sulcaria* and *Thamnolia will* used as raw material as essential component of Buddhism incense because of its unique fragrance. Further the species of lichen genera *Cladonia* are widely used as garden decoration.

However, Devkota *et al*. (2017) reported ritual and spiritual value (RSV), aesthetic and decorative value (ADV), bedding value (BV) and ethno-veterinary values (EVV) of lichens, together with medicinal value (MV) and food value (FV), among different collectors and indigenous people and local communities (IPLCs) in Indian nearby Nepal. Conversely, *Cetrelia collata* used as a sacrificial fiber, together with *Melanelia infumata, Everniastrum cirrhatum* and *Parmotrema nilgherrense, Usnea ghattensis* for coloring hair, *Thamnolia vermicularis* with its spiritual value in India and Nepal (Devkota,*et al.* 2017; Upreti *et al.*2015). The *Buellia subsororioides, have been used* to color palms and lips as a substitute for Heena, mostly by the Garhwali Herdsman in Uttarakhand, India (Upreti and Negi, 1996). Furthermore, Shukla *et al.* (2014) have also highlighted the use of eleven lichen species as dying agents in Gharwal region of India. The tribal people throughout the world are widely using lichens in different ways but it is not well documented for Arunachal Pradesh. Apart from the common and traditional uses of lichens, account of different aspects which can play a vital role in improving the socio-economic status of local inhabitants in the east Indian Himalayan regions in near future are as follows.

Food and fodder

There is an undoubted indication that, over many decades, people from numerous cultures in extensive parts of the world have eaten or drunk lichens as well as consumed other products developed from lichens. However, there is no proper evidence that lichens have regularly formed a major part of the diet within any society. Some lichens have been seen as delicacies but more often they appear to have been a food of last resort or have been used to eke out other food supplies. There are long lists of species that have been consumed by the tribal people (Upreti, *et al.* 2015). In India the species of *Parmelia* lichens has been used as a curry and *Lecanora esculenta* has been eaten by inhabitants of the Middle Eastern deserts. Lichens as food have also been used by man during famines because they are rich in polysaccharides, certain enzymes and some vitamins (Table 2). *Cetraria islandica* is taken as food in Sweden, Norway, Scandinavian countries and Iceland. However, *Lecanora esculenta*

Fig 3(A-D): Segregation and washing of lichens

Fig 4(A-H): Process of boiling and addition of coal ash.

Fig 5(A-E): Lichen ready to serve for food

is used as food in Israel and *Umbilicaria esculenta* in Japan. Species of *Parmelia* are used as curry powder in India. In France the lichens are used in confectionary for making chocolates and pastries. *Cladonia rangiferina* is the main food for reindeers (a kind of deer) in polar countries. The lichens are serving as an important source of food for invertebrates. The *Cetraria islandica* is used as fodder for horses whereas *Stereocaulon, Evernia,*

Fig. 6 A-C: lichens used in Homeopathic medicines (photo by RB); D-G: lichen medicines available in market (photo by internet)

Parmelia and *Lecanora are used* as fodder for several animals as well as mites, caterpillars, termites, snails and slugs, as they feed directly or indirectly (Manoharachary and Deshaboina, 2021).

The Limbu community of eastern Nepal, prepared traditional food items called Yangben curry and Sargyangma from the boiled and dried lichens (Bhattarai, *et al.* 2000; Devkota, *et al.* 2017). Limbu call the edible lichens as Yangben whereas lichens commonly are called as Jhyau in Nepal. The species are namely *Hypotrachyna nepalensis, Hypotrachyna cirrhata, Parmotrema cetratum, Ramalina conduplicans, R. farinacea, R. subfarinacea, R. sinensis, Usnea thomsonii*. They prepared such food items on festivals like Udhauli (Chashok tangnam), Ubhauli (Yakwa sewa) Maghe Sankranti (Kakphekwa tangnam), Saune Sankranti (Sisekpa tangnam), Dashain, Tihar, and other special gatherings, ceremony, fairs, or even when a pig is slaughtered at home. Limbu people often offered such traditional food items to the guests with Tongba (millet-based alcoholic beverage), Raksi (distilled alcoholic drink) or other drinks for hospitality.

Yang, *et al.* (2021) have documented 42 species of edible lichens in the Himalaya and southwestern part of China that are consumed as vegetables and tea. It is also reported that Limbu community cook dried lichens with mixed pork, pork's blood, pieces of intestines, heart, liver, etc. They often cook it after mixing with internal parts and blood of buffalo, and sometimes with egg. The boiled and dried lichens are soaked and added to well-cooked meat. The powdered lichens are consumed after mixing it with pulses such as blackgram, rice bean. The vegetarians can have it as it is or can mix it with other vegetables too. The *Cetraria islandica*, contain carbohydrates in the thallus and in Sweden and Russia the species is used to produce alcohol as well as used in confectionary (Carette, 2000; Elkhateeb, *et al.* 2021).

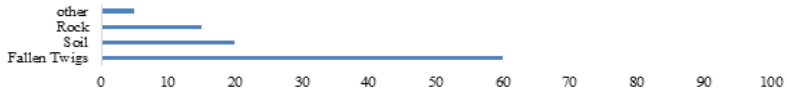
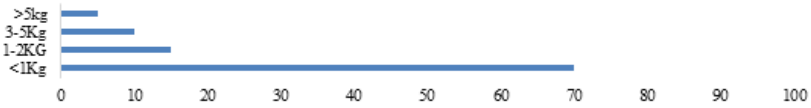
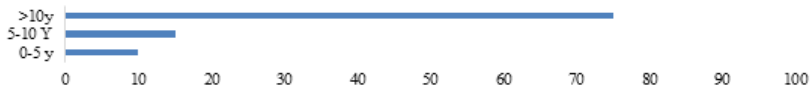
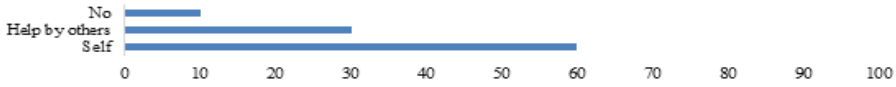
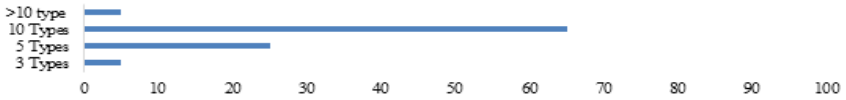
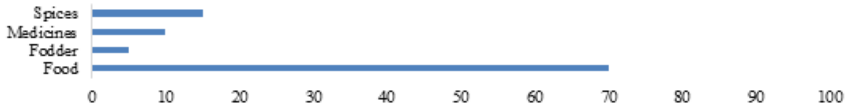
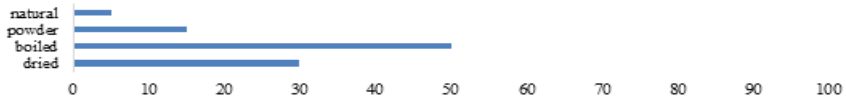
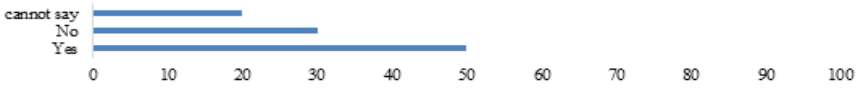
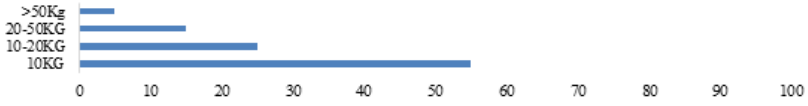
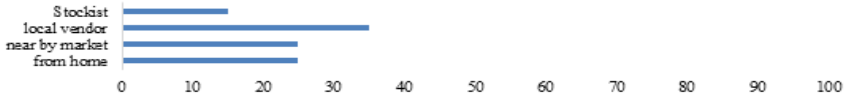
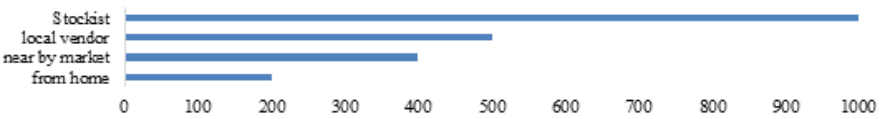
Colouring Agents

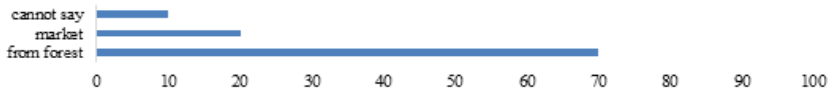
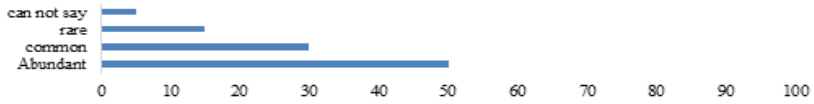
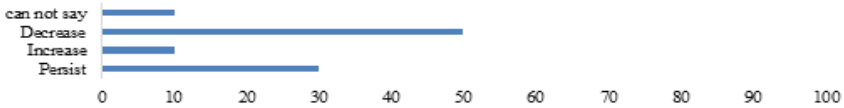
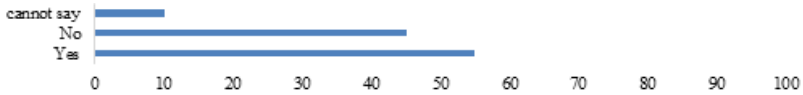
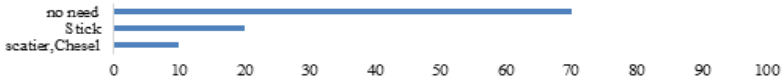
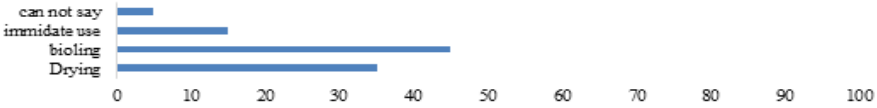
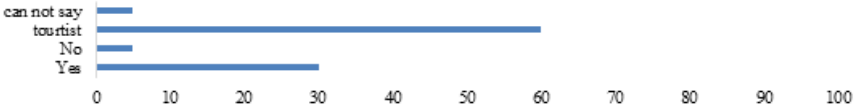
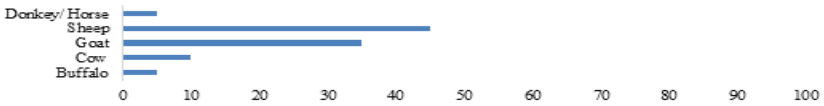
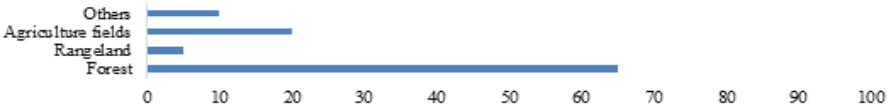
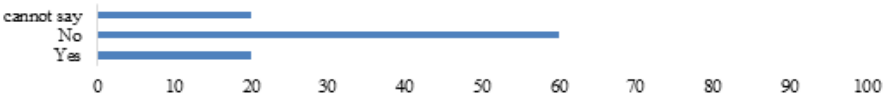
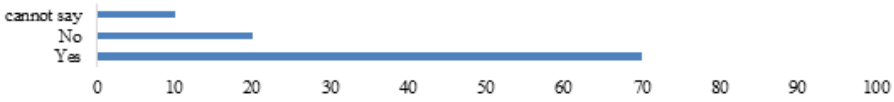
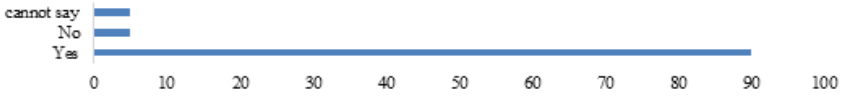
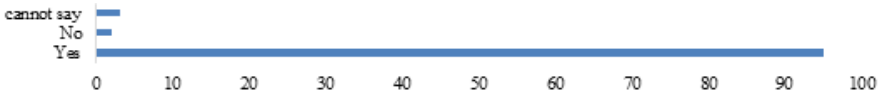
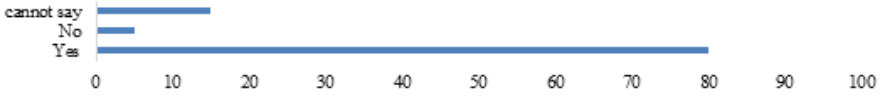
The dyes obtained from lichen, will play a significant role in the industrial revolution since of the use of natural dye for food,

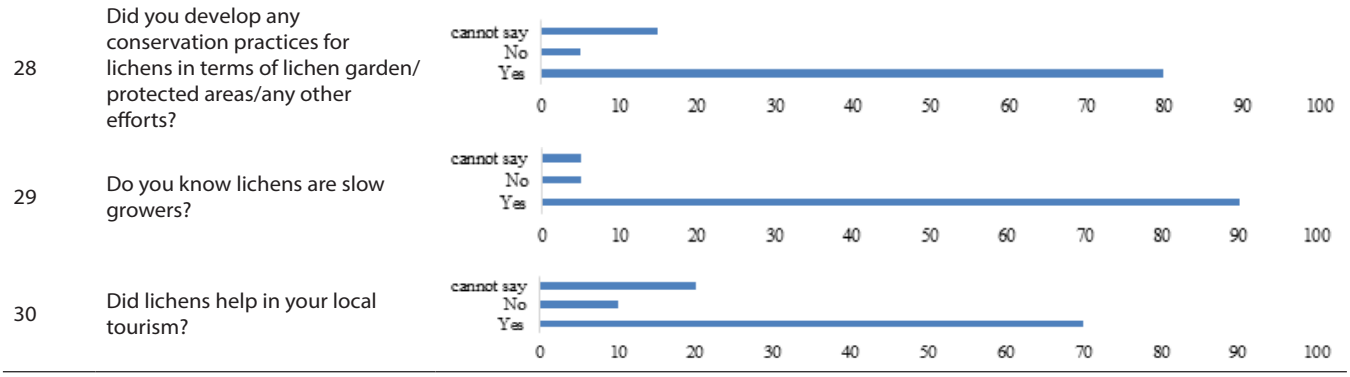
Table 2: Composition of some edible lichens

Parameters	*Unit*	*Lichen taxa*												
		Ramalina conduplicans	*Parmotrema tinctorum*	*Parmotrema pseudotinctorum*	*Ramalina hossei*	*Cetraria islandica*	*Hypotrachyna cirrhata*	*Usnea pictoides*	*Lobaria orientalis*	*Thamnolia vermiculars*	*Alectoria sarmentosa*	*Bryoria fermontii*	*Bryoria fuscescens*	*Bryoria pesudofuscescens*
Moisture	%	8.86	9.12	15.2	16.0	14.20	-	-	-	-	-	-	-	-
Total carbohydrates	%	79.08	72.13	53.2	59.9	82.5	-	-	-	-	-	-	-	-
Water soluble carbohydrates	%	1.3	1.8	1.1	0.8	1.9	-	-	-	-	-	-	-	-
Cellulose	%	4.2	3.5	2.1	3.8	39	-	-	-	-	-	-	-	-
Protein	%	5.95	9.1	11.3	16.2	8.8	-	-	-	-	3.8	-	1.4	-
Crude fibres	%	5.86	16.36	12.0	10.8	-	-	-	-	-	-	-	-	-
Acid detergent fibres (ADF)	%	22.5	21.6	19.8	35.6	24.8	-	-	-	-	-	-	-	-
Neutral detergent fibres (NDF)	%	18.9	28.7	32.9	15.6	24.8	-	-	-	-	19.0	26.0	30.0	28.0
Ash	%	4.01	6.15	8.9	12.1	-	-	-	-	-	1.5	1.7	2.1	2.2
Fat	%	2.1	1.3	6.5	3.2	-	-	-	-	-	-	-	-	-
Calcium	ppm	7040.20	2334.13	-	-	17190.0	5191.0	17087.60	21.98	15147.0	1215.0	1472.0	2594.0	1742.0
Phosphorous	ppm	737.22	852.08	970.0	650.0	642.0	24.67	-	1340.0	682.0	637.0	927.0	309.0	861.0
Sulphur	ppm	-	-	-	-	-	-	-	1521.0	2818.0	-	-	-	-
Potassium	ppm	2548.92	2931.82	800.0	800.0	2091.0	1542.0	1474.14	5497.0	2818.0	1657.0	2274.0	1360.0	2874.0
Sodium	ppm	154.31	370.58	-	-	-	-	259.68	11.0	279.0	59.0	31.0	102.0	719.0
Magnesium	ppm	561.98	884.34	-	-	-	1506.0	240.86	834.0	265.0	288.0	258.0	264.0	402.0
Iron	ppm	1009.64	8250.52	19573.0	10358.0	667.0	893.7	1937.11	751.0	311.0	-	-	-	-
Manganese	ppm	32.23	233.75	123.5	34.7	23.5	53.13	36.9	84.0	33.0	-	-	-	-
Copper	ppm	30.33	30.15	114.0	180.0	4.3	5.83	23.78	5.2	4.3	-	-	-	-
Zinc	ppm	46.11	55.81	74.1	32.8	31.2	66.3	83.42	31.0	40.0	-	-	-	-
Iodine	ppm	-	-	-	-	4.05	-	-	-	-	-	-	-	-
Nickel	ppm	1.92	9.85	-	-	-	-	4.50	-	-	-	-	-	-
Chromium	ppm	6.14	21.60	-	-	-	-	12.70	-	-	-	-	-	-
Lithium	ppm	-	-	-	-	-	-	4.73			-	-	-	-
Cobalt	ppm	0.03	0.01	0.08	0.02	0.02	1.05	1.01	0.58	0.02	-	-	-	-
		Kambar et al. 2014		Vinayaka et al 2009	Kumar, et al. 2010	Meli et al. 2018	Kekuda et al. 2011	Vinayaka et al 2009	Luo et al. 2010		Dubay et al. 2008			

Table 3: Result of questionnaire asked to tribal 150 people and data presented as mean percentage (100%)

S. no.	*Questions*	*Answers (mean percentage)*
1	Habitat of the lichen species collected?	
2	Quantity harvested per month/ family?	
3	For how long you know lichens?	
4	Did you segregate lichens self/or with any aid?	
5	Did you know macro and micro lichens?	
6	How many types of lichen are found in your area?	
7	Did you know the uses of lichens?	
8	If yes, then how you use them (dired/fresh)?	
9	How do you prepare them for use?	
10	Whether you sold them in the local market?	
11	If yes, then how much / year?	
12	To whom you sell it?	
13	Accessibility of lichen selling?	

14	Whether your requirement are fulfilled from forest or from market?	
15	Is the lichens perceived by the informant is abundant/common/ rare?	
16	Availability status of the lichens over the past 10 years.	
17	Did you used any method to regenerate? or involve in conservation of lichens?	
18	What kinds of tools are being used for collection?	
19	What kinds of traditional methods are being used for the processing of lichens after harvesting?	
20	Whether lichens are used by the local people?	
21	Preference for kind of livestock?	
22	Where do you graze your livestock?	
23	Whether livestock eat lichens?	
24	Whether lichens uplift your income?	
25	Do you want to develop entrepreneurship with lichens in your area?	
26	Do you want a small-scale industry for lichen in your area?	
27	Any conservation efforts by Collector/ Community peoples?	



cosmetics and textile industries are the major challenges in the present time (Rather, *et al*. 2016). The dyeing of fabric materials is commercially the most valued application of lichen dyes had high economic value for many decades. Lichens were used for the dyeing wool and silk in ancient times and probably the most famous are the lichens known collectively as the orchil lichens which are known to develop purple and violet colours (Cardon, 2007). However, yellowish, brownish, and reddish-brown colours were obtained using simple dyeing method in which lichens were boiled in water together with mordanted and un-mordanted wool (Shukla, *et al.* 2014; Devkota, *et al.* 2017). Moreover, atranorin found in *Parmelia* sp., and *Xanthoria parietina* whereas gyrophoric acid in *Ochrolechia tartarea* and *Lasallia pustulata*. Salizinic acid, the derivative of atranorin, is found in *Parmelia saxatilis* and several other *Parmelia* spp., are widely used to extract the dye by local peoples. Moreover, orchil dye was used to dye wool, silk, wood, feathers, marble, and even leather whereas litmus is still used as a reagent for orchil to react to alkalis and acids obtained from *Roccella* and *Lecanora* sp., (Rather, *et al*. 2016). The orchil is a complex mixture of several other orceins e.g., β- and γ-aminoorcein, β- and γ-hydroxyorcein and β- and γ-aminoorceimine and *Rocella* sp., contain lecanoric acid up to 3-4 % on dry weight basis (Schweppe, 1993; Cardon, 2007).

Cosmetics and Perfume

Baby creams to deodorants to face and body lotions utilise, extracts from lichens and other botanicals are becoming common additives in personal care products at present. The lichen plagiaristic products are extensive in the United States and Europe. It is well known that lichens have both primary and secondary metabolites and lead to provide path for advance research in near future. One estimate counted more than 90 deodorants and perfumes or colognes that explicitly list lichen-derived ingredients (Schalock, 2009). The usnic acid is the most commonly extracted product of lichens, chemically, usnic acid is a dibenzofuran, which is related to the furocoumarins but is rarely photosensitizing (Sheu, *et al.* 2006). However, extract from *Evernia prunastri* is used as a fragrance component. For custom extended series patch testing, a lichen acid mix is available from Chemo technique (Malmö, Sweden); it contains usnic acid, atranorin, and everinic acid, all at < 0.1% in petrolatum. The usnic acid at 0.1% in petrolatum is available from Hermal (Reinbeck, Germany). Oakmoss absolute is one of the eight components of fragrance mix and is also available separately from Chemotechnique and Hermal. Conversely, *Evernia*, *Ramalina, Pseudorina* are reported to have perfumed volatile oils. Due to the aromatic substances present in the thallus, the lichens are used in the preparation of various cosmetic articles, perfumery goods, dhoop, hawan samagri (Singh, *et al.* 2015; Sahu, *et al.* 2015).

Minerals

The involvement of lichens in rock weathering (pedogenesis) has been deliberated since the end of the 19th century (Julien, 1982).The physical mechanism is mainly characterized by hyphae penetration and thallus expansion and contraction, while the chemical action, oxalic acid and several other lichens substances are important. It is well known that the lichen secondary metabolic products are powerful metal complexing agents leading to a fragmentation of the rock surface. Lichens living on carbonate or silicate rocks, in which calcium oxalates were found, can be both epilithic and endolithic. It is well known that the release of oxalic acid by the mycobiont of lichens and crystalline salts of oxalic acid, in particular calcium oxalate monohydrate (whewellite, $Ca(C_2O_4)\ H_2O$) and calcium oxalate dihydrate (weddellite, $Ca(C_2O_4)\ (2.5–x)\ H_2O$), are the most common mineral phases forming in the presence of lichens. The presence of Ca, Mg, Fe and other essential elements in the substratum will come into the thallus by the physical and chemical methods can be used as food to fulfill the nutrient demand of the body. *Lecanora esculenta* is found growing on limestone deserts and yields large amount of calcium oxalate crystals , of the order of about 60% of its dry weight (Manoharachary and Deshaboina, 2021).

Sources of New Medicines

Lichen-derived bioactive compounds hold great promise for biopharmaceutical applications as antimicrobial, antioxidant and cytotoxic agents and in the development of new formulations as green molecules for the advantage of human life. Lichens are an untapped source of biological activities of industrial importance and their potential is yet to be fully explored and utilized. Lichentherapy or Ethnolichenology is a branch of ethnobotany that studies the uses that man makes of lichens traditionally (Elkhateeb and Daba, 2019; Elkhateeb, *et al.* 2021). Several other applications of lichens are related to treating gynaecological diseases, treatment of eye afflictions and use in smoking mixtures to treat teeth pain (Ahmed, *et al.* 2017; Elkhateeb and Daba, 2019; El-Garawani *et al.* 2019).

In India, lichens have been used in Traditional systems of medicine including Traditional Indian Medicine (TIM), Homeopathic and Western Medical Herbals (Fig. 6). Further,

Annexure I: A questionnaire for ethnolichenological studies

(A): Informants' details:

Name:	*Gender:*
Occupation:	Age:
Location/residence:	Education:
Ethnic group:	Mobile No.
Collection site:	Date & Time:

(B): Questions:

S. No	*Questions*
1	Habitat of the lichen species collected?
2	Quantity harvested per month/family?
3	For how long you know lichens?
4	Did you segregate lichens self/or with any aid?
5	Did you know macro and micro lichens?
6	How many types of lichen found in your area
7	Did you know the uses of lichens?
8	If yes, then how did you used them (dried or fresh)?
9	How do you prepare it for use? (tea, infusion, topical application, food, fodder etc.)
10	Whether you sold them in the local market?
11	If, yes then how much/year?
12	To whom you sell it?
13	Accessibility of lichen selling:
14	Your requirement are fulfilled from forest or from market:
15	Is the lichens perceived by the informant is abundant/common/rare?
16	Availability Status over the past 10 years:
17	Did you used any method to regenerate? or involve in conservation of lichens?
18	What kinds of tools are being used for collection?
19	What kinds of traditional methods are being used for the processing of lichens after harvesting?
20	Whether lichens are used by the local people?
21	Preference for kind of livestock?
22	Where do you graze your livestock?
23	Whether livestock eat lichens?
24	Whether lichens uplift your income?
25	Do you want to develop entrepreneurship with lichens in your area?
26	Do you want a small-scale industry for lichen in your area?
27	Any conservation efforts by Collector/Community peoples?
28	Did you develop any conservation practices for lichens in terms of lichen garden/protected areas/any other efforts?
29	Do you know lichens are slow growers?
30	Did lichens help in your local tourism?
Other optional questions:	
1	Use of lichen as food in the area (names):
2	Use of lichen as medicines in the area (names):
3	Use of lichen as vegetable in the area (names):
4	Use of lichen as spices & Condiments in the area (names):
5	Use of lichen as dye yielding in the area (names):
6	Use of lichens in ornamental purposes in the area (name):
7	Name of the most threatened lichen species in the area (earlier present but absent now):

8	Any hands-on training on lichens conducted in the area?
9	Which season is appropriate for harvesting in the area?
10	Other observations if any:

(Signature of counsellor)

lichens been used in the treatment of different diseases like arthritis, alopecia, constipation, kidney diseases, leprosy, pharyngitis rabies, infection, worm, wound healing and microbial infestation (Malhotra, *et al*. 2008; Upreti, *et al.* 2015; Prateeksha, *et al*. 2016, 2021, 2019).

The lichen *Xanthoparmelia scabrosa*, is an ingredient in various aphrodisiac formulations sold in the international market. Traditionally, *Cetraria islandica* was used to treat mild inflammation of the oral and pharyngeal mucosa, dyspepsia, and loss of appetite. In European folk medicine, *Cetraria islandica* was used in cancer treatment (Dandapat and Paul, 2019). In India, *Parmelia chinense* finds applications as a diuretic, as a liniment for headaches and is used in a powdered form, to heal wounds, whereas the *Tinea* (Ringworm) like disease is treated with *Parmelia sancti-angeli. Parmelia nepalense* is used in Nepal for the treatment of toothache and sore throat. *Usnea longissima* is used as a natural antioxidant, antitumor and healing agent (Prateeksha, *et al.* 2016, 2019, 2020, 2021; Crawford, 2019; Elkhateeb, *et al.* 2021, 2020; Elkhateeb and Daba, 2020; El-Garawani, *et al.* 2020).

Future Prospect for Native People

The present case study investigates the commercial collection of lichens, quantifies the traded volume and relates it to a market scenario, tourism aspects and discusses conservation measures in Arunachal Pradesh. The lichen harvest and trade are closely associated with the livelihood of most of the rural people in the area. It was seen that the commercial grading of lichens was not common in North East, but this practice is established in other parts of the India. According to Pant (2014) three types of grading systems are reported from Kumaun Himalaya; Grade A is the best and contains no moss or bark; Grade B contains Grade A lichens mixed with mosses, twigs and bark; and Grade C mainly consists of saxicolous lichens commonly called ''Patthar Chura''. Similarly, four grades were reported by Shah (2014) from Uttarakhand, *viz*. Grade I, Grade II, Grade III and Grade IV, on the bases of different lichen species compositions.

The lichens are found on every continent and have ancient history of its applications as food, medicine, dyes, livestock feed and for other applications. The pharmaceutical potential of lichens is high nowadays, and several companies are now attempting to commercialise these unique attributes. Industrial production of lichen metabolites is yet to progress. Approaches towards axenic cultivation of the lichen mycobionts have been successfully performed to analyse the secondary metabolites produced by the lichen forming fungi as well as endolichenic fungi (Stocker-Wörgötter, 2008; Prateeksha, *et al.* 2019, 2020). This has given hope in identifying novel molecules that can be of pharmaceutical importance and in industrial applications. Lichens grow very slowly and hence the optimization of its culturing conditions in the laboratory towards obtaining abundant biomass production is a challenging task (Behera, *et al*. 2005). Once this can be solved, access to lichen-derived substances for possible applications in different fields to help in human life can surely be a breakthrough phenomenon. The large-scale production of important compounds such as sekikaic acid, salazinic acid, fumarprotocetratic and usnic acid needs further improvements to increase yield of such medically important compounds. Optimal conditions required for the growth of the two partners sharing to produce the lichen need to be further investigations.

The techniques such as metabolic engineering, genetic manipulation, and other biotechnological approaches should be used to overcome the limited availability of medically and industrially important lichen producing compounds and reduce the dependency on natural thallus (Elkhateeb and Daba, 2019). Further research is required in order to isolate and identify bioactive compounds responsible for such biological activities and other industrial applications. Moreover, clinical trials and more *in-vivo* experiments have to be carried out to confirm lichens capabilities as sources of compounds having medical applications.

Consciousness of Tribal People about Lichens in the Area

The case study is based on the questionnaire presented in Annexure (I). A total of 30 questions were asked to the local people and they provided valuable information for the report. The graphical results of about major thirty questions are presented in Table (3). As local people of this region are aware about the illegal trading hence a single person collects about 1 to 5 kg lichen per month from fallen twigs as well as from forest floor just to fulfil their need. The excess amount of lichens is sold to the local market. The biomass of lichens have declined in the present localities during the last 10–15 years but no action has so far been taken for their conservation. The people are eager to know more about the current application of lichens and showed interest to develop some entrepreneurship with lichens in the area along with conservation aspects. Mostly the local people collect lichens and prepare local food dishes to attract tourist to show the novelty of the area as well as traditional food.

Notwithstanding the ban on lichen collection in the country, lichens are collected and traded illegally, with no documentation of population sizes, carrying capacity of forests or species identities, and no application of scientific tools or management. The competition among poor collectors to collect more and more lichen also forces them to cut branches and spend days and nights in the forest to collect lichens (Maraseni and Shivakoti 2003; Maraseni, *et al.* 2006). This trend definitely leads to the decline of lichens in the wild due to overexploitation. After a hard day of collecting, collectors cannot deal directly to the main traders and mainly have to depend on middlemen. Ineffective price-regulating practices cause the exploitation of large volumes of lichens for small amounts of money.

Though legally regulated for the last few years, biological resources have been heavily extracted and exploited for trade (Olsen and Larsen, 2003). Property rights of collectors and equity benefit sharing must be ensured for the sustainable management of lichens as well as non-timber forest produce

(Maraseni, *et al.* 2006). For example, the collection and trade of lichens in Uttarakhand, India has been more regulated since October 2004: the Forest Department and Uttarakhand Van Vikas Nigam (UKVVN) under Uttarkahnd Development Corporation provide training to registered collectors on sustainable harvesting practices and also play a crucial role in marketing and in creating a healthy relationship between collectors and buyers (Shah, 2014).

Presently in north east particularly in Arunahcal Pradesh there is no such guidelines available for lichen trading, thus in coming years the vast exploration of lichens will lead to great loss of lichen diversity in the area. In North Eastern Himalayan regions, similar practices could be adopted rather than a complete ban on commercial lichen collection. Banning the use of resources without scientific investigation does not guarantee their protection over the long term (Olsen and Larsen, 2003). It was also proved that primeval forests are more species rich in lichens than secondary forests (Bergamini, *et al.* 2005). Overexploitation of lichens in the Himalaya for household and commercial uses is a major threat. It will cause a decrease in the local lichen population density. Therefore, steps for lichen conservation should start with the maintenance of forest habitat, forest area and functional connectivity, together with sustainable management (Scheidegger and Goward, 2002; Upreti, *et al.* 2005; Scheidegger and Werth, 2009; Søchting, 2015; Zedda and Rambold, 2015). Lichen diversity should be protected from the destruction caused by anthropogenic activities, as habitat loss and fragmentation of natural forest landscapes cause tremendous declines in forest lodging lichen populations.

Conclusion

The present case study reveals that lichens are of great interest as foods, traditional medicines/therapeutic values, and aesthetic and spiritual values to the local inhabitants of Arunachal Pradesh. There is a long tradition of using lichens among the tribal communities residing in the area and there is a need to uplift their knowledge through scientific inputs. Collection, consumption, and marketing of lichens in sustainable ways would lead to the long-term availability of these resources in the area. Furthermore, there is a need to provide hands-on training, workshop and field visits with local people for sustainable harvesting of lichen resources. Meanwhile, some scientific standards to precede, on how to harvest lichens in a sustainable way, could be a better option to generate a reasonable income for people who traditionally use this uncommon biological resource. Thus, it is recommended to carry out further advanced research on their application, abundance, stock estimation, and collection impact on economically and socially important species from the area.

Declaration

Ethics Approval and Consent to Participate

This article does not contain any studies with human or animals' participants. All authors have given their consent to participate in the manuscript.

Human and Animal Ethics

Not applicable

Consent for Publication

All authors have given their consent for publication and have no conflicts of interest.

Availability of Supporting Data

The lichen voucher specimen was deposited in the herbarium CSIR National Botanical Research Institute Lucknow (LWG).

Conflicts of interest/Competing interests

The authors declare that they have no conflict of interest.

Authors' Contributions

RB – original concept, collection, analysis of data and drafting of manuscript; LTT organise field trips and fill the questionnaire, DKU and CPS critically reviewed the manuscript.

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