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Natural dyes from Himalayan lichens

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Eleven species of lichens collected from different sites of Gharwal region of Indian Himalayas were estimated for dye production using boiling water method (BWM), ammonia fermentation method (AFM) and Di-methyl sulphoxide extraction method (DEM). The dyes extracted were tested on silk, tussar silk, absorbent cotton and a co-relation of dye colour with the lichen substance present was also made. The lichens produced orange, yellow, blue-grey, purple and brown colour dyes. The effect of sunlight and the stability of colours after washing were also determined. Lichen dyes can be used in handlooms to serve local people in their livelihood.

Keywords: Himalayas, Lichens, Dyes, Colour

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Lichens are symbiotic organism composed of a photobiont usually algae or blue-green algae and a phycobiont which together form an independent physiological unit. The lichens have an ability to form wide range of secondary metabolites as a result of slow growth and harsh living conditions which are believed to serve as antimicrobial, anti-herbivore and antigrowth agents[1,2,3]. Besides the antibiotic properties, lichens have an inherent ability to produce natural dyes.

Lichens have been used as source of natural dyes since time immemorial. Orchil (purple dye) was the first documented dye produced from *Roccella spp.* through ammonia fermentation method. The purple dye from *Roccella* was historically very important as 'Royal-purple' throughout the Europe before nineteenth century until first synthetic dye came into existence in the year 1856[4].

There are about 20,000 lichen species described all over the world so far, and India represents 10% (2305) of the lichens known[5]. The Himalayan flora consists of large number of parmelioid lichen species that provide excellent source of dyes[6] and 157 Indian lichen species belonging to 65 genera have potential dyeing properties. Parmelioid lichens contain characteristic compounds known as depsides and depsidones that are formed by joining two or sometimes three phenolic units, derived through acetate-polymelonate pathway[7], are the main source in production of dyes which can colour natural fibres[8]. Except few records of lichen dyes from India so far[9], the dyeing properties of Indian lichens are not known. In India, the ethnic groups in Garhwal Himalayas are known to dye wool from dyes extracted from lichens species[10].

Thus in the present study an attempt has been made to screen out the most common and abundantly growing Himalayan parmelioid lichens for their potential of dyeing properties.

Materials and methods

Collection and identification of lichen sample

Lichens were collected from Ghursu Top, Auli, Joshimath and Chopta area of North-Western Himalayas of Uttarakhand state in Garhwal region during May, 2011.The specimens were identified after studying their morphology, anatomy using a Labomed ™ stereomicroscope and Leica ™ DM 500 optical microscope and chemical properties with the help of 'spot tests', UV light and standardized thin-layer chromatography (TLC) following procedure of John A. Elix *et al*[11] and Orange A. *et al*[12]. The specimens were authentically identified using relevant key and monographs by Divakar &

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Upreti[13] and Awasthi[14]. The voucher specimens were deposited at CSIR-National Botanical Research Institute (NBRI), Lucknow herbarium (LWG) in Uttar Pradesh.

Extraction of dyes from lichen sample

Lichen samples were segregated, cleaned of substratum, thoroughly washed under tap water and dried. Dried sample were crushed to powdered form with the help of mortar and pestle and weighed. The powdered lichen was then used for extraction of dyes and further dyeing experiments were carried out.

The dyes were extracted with ammonia fermentation (AFM), boiling water (BWM) and Di-methyl sulphoxide extraction method (DEM). Silk fibres and tussar silk fibres were obtained from silk handloom factory at Varanasi, Uttar Pradesh, and absorbent cotton was obtained from laboratory. Each fibre sample weighed and thoroughly washed in distill water before it was used for dyeing so that the dye penetrate and fix well into the fibre. Equal weights of dry lichen and fibres were used. Dye extractions and dyeing were done in 250ml Erlenmeyer flasks at room temperature (except in BWM). No mordents were used in the study because lichen dyes are unique in that they did not require any mordant or intermediary agent to be taken up by the fibres and mordanting substances are used to vary the colour in lichen dyeing[15]. The three dyeing methods used were:

Ammonia fermentation method (AFM)

Six gram lichen was added to diluted ammonium hydroxide solution (one part NH_4OH and 10 parts distilled water), the content was mixed thoroughly and was left for 1 month in a 250ml Erlenmeyer flask. The extract was then filtered using Whatman filter paper (No 1) in a Buchner funnel. Six grams each of silk, tussar silk and absorbent cotton (pre-soaked in distilled water) were added. After one month, fibres were removed from the flasks and were dried. The colours of the dried threads were recorded.

Boiling water method (BWM)

Six gram powdered lichen was added to distilled water and heated till boiling. The mixture was maintained at simmer for 1 hr. The content was filtered into a clean flask. The filtrate was again maintained at simmer for at least 2 hrs until some colour was obtained. Pre-soaked fibres were then immersed in dye bath and were slowly heated at maximum 90˚C for 2 hrs. The dye bath was cooled after dyeing; the threads were rinsed in cold water, dried and colours of the threads were recorded.

Di-methyl sulphoxide extraction method (DEM)

Six gram crushed lichen was added to 50 ml crude Di-methyl sulphoxide solution in a flask. The content was stirred vigorously and was covered with aluminium foil. The dye content was maintained at room temperature for one month to develop colour. After one month, the content was filtered into another clean flask and pre-soaked threads were added for dyeing. After one month, the threads were removed from the flask, washed with distilled water and were left for drying. Colours of the dried threads were recorded.

After dyeing the fibres were stored in envelopes at room temperature. The stability of dyes against light was tested by exposing the dyed fibres to sunlight for 8 hrs per day for a week (48 hrs) following the procedure employed by Sharma and Grover[16]. The fibres were also washed with detergent to observe the stability of the colour.

TLC of the selected eleven samples was done to determine the secondary metabolites present in them. The colours produced by lichens were then correlated with the combination of secondary metabolites observed in the lichen samples. The colours were named with those matching Ridgway colours[17]. Un-dyed colour fibres were used as control.

Results and discussion

The Indian Himalayan lichen flora has a rich diversity of foliose lichens that can serve as raw material for making dyes due to their unique chemistry and abundant biomass. Wide range of colour dyes like brown, orange, yellow, purple and grey were obtained from the lichens (Fig. 1). All the three methods employed have given different colours to the fibres. As tussar silk fibre itself had buff colour, so after dyeing the fibre appeared different from white silk and cotton fibres. Out of the three methods employed, dye colours extracted from ammonia fermentation method (AFM) were much brighter as compared to boiling water method (BWM) and di-methyl sulphoxide extraction method (DEM). Colour dyes obtained through AFM ranged from beautiful red-purple to grey-brown whereas BWM produced yellow to orange coloured dyes. The colours


a
A
B
b
A
B
c
A
B
d
A
B
e
A
B

Fig. 1—Lichens and silk threads dyed with lichen dyes extracted through Ammonia Fermentation Method (A) and Boiling Water Method (B) *a—Evernia mesomorpha* Nyl; *b—Everniastrum cirrhatum* (Fr.) Hale; *c—Flavoparmelia caperata* (L.) Hale; *d—Nepromopsis nephromoides* (Nyl.) Ahti & Randl; *e—Parmotrema nilgherrensis* (Nyl.) Hale; *f—Parmotrema reticulatum* (Taylor) Choisy; *g—Parmotrema tinctorum* (Despr. ex. Nyl.) Hale; *h—Punctelia reducta* (Ach.) Krog; *i—Usnea longissima* Ach.; *j—Xanthoparmelia somloensis* (Ach.) Ahti & Hawksw.

Table 1— Dye obtained from parmelioid lichens with three extraction methods

S.No.	Lichen species	Chemical present	Ammonia fermentation method	Di-methyl Sulphoxide method	Boiling water method	Reaction of dyed threads to sunlight
1.	*Evernia mesomorpha* Nyl	Divaricatic acid , Evrnic acid and Sekikaic acid	(a) Cameo brown (b) Dark naphthalene violet (c) Pale pinkish buff	(a) — (b) — (c) —	(a) Pale pinkish buff (a) Isabella Colour (a) Olive buff	AFM and BWM colours slightly faded
2.	*Everniastrum cirrhatum* (Fr.) Hale	Salazinic acid, Atranorin and Protolichesterinic acid	(a) Light yellowish olive (b) Brownish olive (c) Cream buff	(a) — (b) — (c) —	(a) Sayal Brown (a) Verona brown (a) Clay color	BWM colours slightly faded while AFM colours were stable
3.	*Flavoparmrelia caperata* (L.) Hale	Usnic acid, Protocetraric acid, Caperatic acid	(a) Light brownish olive (b) Brownish olive (c) Eoru-olive	(a) Cartridge buff (b) Isabella color (c) Marguerite yellow	(a) — (b) — (c) —	AFM colours slightly faded while DEM colours were stable
4.	*Nephromopsis nephromoides* (Nyl.) Ahti & Randl.	Lichesterinic acid and Protolichesterinic acid	(a) Eoru-olive (b) Dark greenish olive (c) Light yellowish olive	(a) Olive buff (b) Dark olive buff (c) Chamois	(a) Ivory yellow (b) Light brownish olive (c) Marguerite yellow	Colours from all the three methods were stable
5.	*Parmotrema nilgharrensis* (Nyl.) Hale	Atranorin, Alectoronic and α-collatolic acid	(a) Avellaneous (b) Dark olive (c) Pale olive buff	(a) — (b) — (c) —	(a) Ivory yellow (b) Buffy olive (c) Marguerite yellow	AFM and BWM colours slightly faded
6.	*Parmotrema reticulata* (Taylor) Choisy	Salazinic acid, Atranorin and Consalazinic acid	(a) Light brownish olive (b) Brownish olive (c) Buffy brown	(a) Deep olive buff (b) Army brown (c) Pale olive buff	(a) Sayal brown (b) Mikado brown (c) Cinnamon	BWM colours slightly fading while AFM and DEM colours were stable
7.	*Parmotrema tinctorum* (Despr. ex. Nyl.) Hale	Atranorin and Lecanoric acid	(a) Perilla purple (b) Seal brown (c) Pale pinkish buff	(a) Mikado brown (b) Warm sepia (c) Light brownish olive	(a) Chamois (b) Verona brown (c) Orange cinnamon	Colours from all the three methods were found to be stable
8.	*Punctelia reducta* (Ach.) Krog	Atranorin and Lecanoric acid	(a) Hay's brown (b) Warm blackish brown (c) Pale pinkish buff	(a) Cartridge buff (b) Isabella colour (c) Marguerite yellow	(a) Pale pinkish buff (b) Tawny olive (c) Pale olive buff	AFM and BWM colour slightly faded while DEM colours were stable
9.	*Usnea longissima* Ach.	Usnic acid, barbatic acid and fumaroprotocetrari c acid	(a) Dark mineral red (b) Dusky dull violet (2) (c) Dark livid brown	(a) Cartridge buff (b) Deep colonial buff (c) Marguerite yellow	(a) Pinkish buff (b) Verona brown (c) Pale pinkish buff	AFM and BWM colours faded while DEM colours were stable
10.	*Usnea stigmatoides* G.Awasthi	Stictic acid complex	(a) Light brownish olive (b) Deep olive (c) Wood brown	(a) — (b) — (c) —	(a) Pinkish buff (b) Verona brown (c) Light pinkish cinnamon	BWM colours slightly faded while AFM colours were stable
11.	*Xanthoparmelia somloensis* (Ach.) Ahti & Hawksw.	Salazinic acid, consalazinic acid and usnic acid	(a) Buffy olive (b) Brownish olive (c) Buffy olive	(a) Deep colonial buff (b) Isabella color (c) Reed yellow	(a) Mikado brownSnuff brownClay color	BWM colours slightly a faded while AFM and DEM colours were stable

(a) Recorded colour of silk thread; (b) Recorded colour of tussar silk thread;(c) Recorded colour of absorbent cotton; — when the colour appeared no darker than the control samples.

obtained through DEM ranged from light yellow to golden brown.

Out of eleven lichen selected for the screening of dye preparation (Table 1) four lichen species produced red-purple colour through AFM while three species produced grey shades of colour and another three species produced brown shades of colour from the same method. *Parmotrema nilgherrensis* produced grey-brown colour through AFM. The TLC data revealed that two purple colour producing lichens have lecanoric acid. Lecanoric acid is a *p*-depside that hydrolyses to orsellic acid and undergoes a series of

chemical reaction to form colour producing substance orcein. The Pink-purple dye produced by *Evernia mesomorpha* representing diluted purple may be due to the presence of evernic acid and divaricatic acid, where both are structural homologue of lecanoric acid. The blue-purple dye colour produced by *Usnea longissima* through AFM may be either due to *p*-depside barbatic acid or depsidone fumaroprotocetraric acid or it may be produced by the combined action of both. *Everniastrum cirrhatum, Parmotrema reticulatum* and *Xanthoparmelia solmonalis* contained salazinic acid in their thallus and produced brown dye through AFM and orange dye through BWM. Salazinic acid is responsible for the production of orange shades of colour[18] and due to increased fermentation time the orange shades became darker brown shades of dye colour. *Flavoparmelia caperata* and *Parmotrema tinctorum* produced orange dyes through DEM. *Usnea stigmatoides* produced brown colour through AFM which had stictic acid complex in the thallus. The grey dye was produced by *Flavoparmelia caperata* and *Nephromopsis nephromoids*, *F. caperata* contains ceparatic acid which may be responsible for the colour but no such ringed structure chemical was found in *N. nephromoids*. Lichens containing both atranorin and salazinic acid produced yellow colour while salazinic acid is responsible for orange and brown dye colours. Most of the secondary metabolites mentioned above have ortho-hydroxy aldehyde group which reacts with free amino group present in natural protein and forms stable schiff base which ultimately imparts colour to the fibres.

Change in dye colour was observed to some extent under the effect of sunlight in few lichen species. The change in colour is due to photo-oxidation of the colour producing structure, the chromophore[19]. It was observed that though the colours obtained through DEM were of light shade but they were stable against the effect of sunlight. Lichen dyes extracted from *P. tinctorum* and *N. nephromoids* through all the three methods did not fade in sunlight. Most of the colours remain stable after washing with detergent.

Traditional significance of study to the farmers/researchers and some constructive recommendations

The study provides significant information to the present state of knowledge of the use of lichens as natural dyes. So far few species of lichens in India are known for making dyes and the present study will broaden the scope of use of other parmeliod lichens as dyeing agents. Further, more colours could be obtained by mixing two or more species of lichens and varying the dyeing conditions like pH, solvents and use of mordents.

The Indian Himalayas and Western Ghats are huge reservoirs of parmelioid lichens, therefore local people can use them in dyeing handicrafts and rugs, after harvesting them sustainably. Since lichens are slow growing organisms unable to provide large scale biomass for commercial use, therefore mycobiont culture and whole thallus culture of lichens is the only method to get good biomass that will help in establishment of small cottage industries for employment to the poor villagers both in Himalayan and Western Ghats regions.

Conclusion

Parmelioid lichens are potential source of natural dyes and provide brilliant colours in different solvents. The ammonia fermentation method is best method to get wide range of colour dyes such as pink, violet, orange, grey, brown and yellow. The dye colours vary depending upon the soil properties, season of harvesting of lichen thallus and fermentation time. Lichen dyes not only provide colours but also imparts musky odour to the fibres. Furthermore, the dyed products are reputed to be insect-proof as the secondary metabolites render the fibres distasteful to the insects. Lichen dyes are environment friendly and give better quality of fibres than synthetic dyes but the tedious extraction and long dyeing time increases the cost of lichen dyes. Since no mordents have been used in the study, lichen dyes have limited colour fastness against sunlight. Probably more colour fast dyes could be obtained if mordents are used while extracting lichen dyes. It is emphasized that only naturally detached or found lichen should be used for dyeing and whole thallus must not be harvested in order to conserve them in their natural habitat. Being small in size as compared to higher plants and extremely slow growing organisms, lichens are not recommended to be used in textile industries on commercially large scale but certainly they can serve small scale handloom industries to offer employment to local people and villagers.

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