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Research Article

ASSESSMENT OF DYE YIELDING POTENTIAL OF INDIAN LICHENS

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ABSTRACT
Lichens are symbiotic association of fungi and green/blue green algae that together form an independent physiological unit. Lichens produce unique secondary metabolites that have economic and pharmaceutical importance. The secondary metabolites are also well known source of colouring agent. However in India, the use of lichen dyes is not known.
In the present study dye yielding potential of some Indian lichen species was assessed using three methods viz. Boiling Water Method (BWM), Ammonia Fermentation Method (AFM) and DMSO Extraction Method (DEM). Colours obtained through different dyeing methods were recorded. The lichens used for extraction of dyes produced various shades of purple, pink, blue, green, yellow, orange and brown dyes. Out of the three methods used, the AFM produced most of the bright colours.

Keywords: *Lichens, Colours, Dyes, Secondary Metabolites*

INTRODUCTION
Lichens are self supporting organization of a fungus with one or more green or blue-green algae. The fungus is dependent upon algae for food and in turn provides shelter to the algae. The unique interdependence of two organisms enables lichens to produce variety of secondary metabolites, some of which are common in plants or in higher fungi but about 80% metabolites are specifically produced by lichens (Huneck and Yoshimura, 1996). The secondary metabolites, often called lichen acids, primarily produced by mycobiont and deposited externally on the hyphae. The lichen secondary metabolites are known to have biological and pharmaceutical activity such as antimicrobial, antiviral, cytotoxic and anticancer activities (Zambare *et. al*, 2012; Tiwari *et al*, 2011; Rankovic, 2011).
Earliest reports on use of lichens as source of dyeing came from Romans who used orchil, purple color pigment obtained from *Roccella spp.*, for dyeing. During eighteenth century dyeing stuffs made from lichens were economically important in Canary Islands (Muggia *et al.*, 2009). Lichens were also well known source of colouring agent among tribal people of South west United States (Brough, 1988) and chemist still make use of litmus paper made from extracts of lichen species to estimate pH values. However, in India the use of lichens as source of colouring agent is not known.
Natural dyes that are derived from plants including lichens, animals and minerals were the only source of colorant till the mid-nineteenth century. With the discovery of first synthetic dye in 1856 (Margareta, 1981), the use of natural dyes were replaced completely by synthetic compounds due to their easy extraction methods and cost effectiveness. As the synthetic dyes have tremendous environmental impact due to their toxic, carcinogenic and non-biodegradable nature, in the recent years several attempts are being made for the development of user-friendly pigments from the natural sources.
The aim of the present study is assessment of dye yielding potential of some Indian lichens from different dyeing methods so as to assess the applicability of lichen dyes as alternate source of colouring agent.

MATERIALS AND METHODS
Collection and identification of lichen Specimens
The lichen species were collected from different localities in Uttarakhand, Tamil Nadu, Maharashtra and Jammu and Kashmir states in India. The dried lichen specimens were identified based on morphological, anatomical and colour test details, using relevant keys and monographs (Divakar and Upreti 2005; Awasthi 2007) with the help of Leica S8APO stereo zoom microscope and Leica DM 500 micro-system.

Thin layer chromatography in solvent system C (180 ml toluene: 60 ml 1, 4 dioxane: 40 ml acetic acid) was performed to detect the secondary metabolites present in lichen specimens as described by Elix and Russel (1993) and Orange *et al.*, (2001). After authentic identification the voucher specimens were deposited in the CSIR- National Botanical Research Institute (NBRI), Lucknow herbarium (LWG) in Uttar- Pradesh, India, for future reference.

Extraction of dyes from Lichens

Lichens samples selected for extraction of dyes were thoroughly washed under tap water and dried at 40°C for 72 hrs. The dried samples were crushed with the help of mortar and pestle to powdered form and then weighed. For each of the three dye extraction methods, six gram each of the powdered lichen sample was used. The silk and tussar silk fibres, obtained from handloom shop at Lucknow, Uttar Pradesh, were dyed. The following three dye extraction methods were used for extraction of lichen dyes:

Boiling Water Method (BWM):

The powdered lichen samples were added to 50 ml distilled water and heated till boiling. The mixture was maintained at simmer for 1 hour. The content was filtered into a clean flask and the filtrate was again maintained at simmer for at least 2 hours until some colour was obtained. Pre-soaked fibres were then immersed in dye bath and were slowly heated at maximum 90˚C for 2 hours. The dye bath was cooled after dyeing; the threads were rinsed in cold water, dried and colours of the threads were recorded.

Ammonia fermentation Method (AFM):

The powdered lichen samples were 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 at room temperature. The extract was then filtered and fibres were added. After one month, fibres were removed from the flasks, rinsed and dried. The colours of the dried threads were recorded.

DMSO Extraction Method (DEM):

The powdered lichen samples were added to 50ml crude Di-methyl sulphoxide solution. The content was stirred vigorously and left for one month at room temperature. After one month, the content was filtered into another clean flask and pre-soaked threads were added for dyeing. The threads were removed from the flask after one month, washed with distilled water and were left for drying. Colours of the dried threads were recorded.

Mordents were not used in the study. Un-dyed colour fibres were used as control. After dyeing, the fibres were stored in envelopes at room temperature. The colours were named with those matching Ridgway colours (Ridgway, 1912).

RESULTS AND DISCUSSION

Among the three methods employed for extraction of lichen dyes, Ammonia Fermentation Method (AFM) and DMSO extraction method (DEM) (Figure 1) are better extraction methods as they produce better shades of colours than Boiling Water Method (BWM).

Ammonia fermentation method (AFM) yielded different shades of purple, blue, pink, brown and yellow colours, DEM yielded shades of green, brown, yellow while BWM yielded mostly shades of orange, brown and yellow. The details of secondary metabolites present in lichen species and the colours observed through different extraction methods are listed in Table 1.

Through AFM, the species of lichen genus *Xanthoria*, conataining parietin, produced pink shades of colour, the species of genus *Heterodermia* having zeorin in thallus, produced violet-blue while *Roccella montagnei* which contains erythrin, produced Purple colour. *Sticta nylandriana* produced pinkish-purple colour through AFM. *Dermatocarpon vellerum*, *Parmelaria subthomsoni* and *Parmelinella walluchiana* produced grey shades of colour and rest of the lichens produced various shades of yellow colour through AFM.

Most of the colours produced by BWM and DEM were brown, yellow, orange and green. The salazinic acid containing lichens such as *Bulbothrix setschwanensis*, *Everniastrum nepalense*, *Heterodermia leucomelos*, *Parmelinella wallichiana*, *Sticta nylandriana*, *Stereocaulon folilosum* and *Usnea undulata* produced brown colour through BWM.

Figure 1: Lichen thallus and silk fibres dyed from respective lichen dyes

a. Bulbothrix setschwanensis (Nyl.) Brodo and D. Hawksw., **b.** *Dermatocarpon vellerum* Zschacke., **c.** *Lobaria retigera* (Bory) Trev., **d.** *Parmelinella wallichiana* (Taylor) Elix and Hale, **e.** *Ramalina conduplicans* Vain., **f.** *Roccella montagnei* Bel. em. D. D. Awasthi, **g.** *Sticta nylandriana* Zahlbr. **h.** *Usnea ghattensis* G. Awasthi, **i.** *Xanthoria elegans* (Link) Th. Fries, **j.** *Xanthoria parietina* (Link) Th. Fries.

A-Lichen dye extracted through Ammonia Fermentation Method (AFM), **B**- Lichen dye extracted through DMSO Extraction Method (DEM).

Table 1: List of secondary metabolites present in lichens assessed for extraction of dyes and the colours observed through boiling water method (BWM), ammonia fermentation method (AFM) and DMSO extraction method (DEM)

S. No.	Lichens	Secondary Metabolites	Colour obtained through BWM	Colours obtained through AFM	Colour obtained through DEM
1	*Bryoria lactinea* (Nyl.) Brodo & D. Hawksw.	Fumaroprotocetraric acid	Pinkish buff	Chamois	Marguerite Yellow
2	*Bulbothrix setschwanensis* (Zahlbr.) Hale	Salazinic acid	Buffy brown	Mikado brown	Reed yellow
3	*Cetrelia braunsiana* (Mull. Arg.) W. Culb. & C. Culb.	Alectoronic and α-collatolic acid	Ivory yellow	Light yellowish olive	–
4	*Dermatocarpon vellerum* Zschacke.	No lichen substance present	Marguerite yellow	Buffy olive	Light turtle green
5	*Everniastrum nepalense* (Taylor) Hale	Salazinic and protolichesterinic acid	Chamois	Mikado brown	Reed yellow
6	*Flavopunctelia soredica* (Nyl.) Hale	Lecanoric acid	Orange-cinnamon	Buffy brown	Marguerite yellow
7	*Heterodermia diademata* (Taylor) D. D. Awasthi	Zeorin	White	Vinaceous russet	Cartridge buff
8	*Heterodermia leucomelos* (L.) Poelt	Zeorin, norstictic, salazinic acid and triterpenoids	Pinkish buff	Tawny olive	Primrose yellow
9	*Loberia retigera* (Bory) Trev.	Triterpenoids and thelephoric acid	Cartridge buff	Vinaceous buff	Clear fluorite green
10	*Parmelaria subthomsonii* D. D. Awasthi	Atranorin, alectoronic & α-collatolic acid	Ivory yellow	Isabella color	Marguerite yellow
11	*Parmelinella wallichiana* (Taylor) Elix & Hale	Salazinic and consalazinic acid	Clay color	Deep olive	Wood brown
12	*Peltigera rufescense* (Weiss) Humb.	No lichen substance present	White	Ivory yellow	Deep turtle green
13	*Ramalina conduplicans* Vain.	Usnic acid, sekikaic acid aggregate and salazinic acid	Cartridge buff	Isabella color	Turtle green
14	*Ramalina hossei* Vain.	Usnic acid and sekikaic acid aggregate	Pale pinkish buff	Olive yellow	White
15	*Ramalina sinensis* Jatta	No lichen substance present	White	Olive yellow	White
16	*Roccella montagnei* Bel. em. D. D. Awasthi	Erythrin	Ivory yellow	Naphthalene violet	Marguerite yellow
17	*Stereocaulon foliolosum* Nyl.	Atranorin and lobaric acid	Chamois	Isabella color	Colonial buff
18	*Sticta nylandriana* Zahlbr.	Atranorin, gyrophoric acid and unknown substances	Chamois	Dark vinaceous	Dark olive
19	*Sticta platyphylloides* Nyl.	No lichen substances	White	Isabella color	Olive buff
20	*Usnea ghattensis* G. Awasthi	Usnic acid	Pinkish buff	Dark dull violet blue	Reed yellow
21	*Usnea undulata* Stirt.	Salazinic acid, Usnic acid	Mikado brown	Light yellowish olive	Deep colonial buff
22	*Xanthoria elegans* (Link) Th. Fries	Parietin	Ivory Yellow	Corinthian red	Olive ocher
23	*Xanthoria parietina* (L.) Th. Fries	Parietin	Marguerite Yellow	Congo pink	Ivory yellow

The atranorin containing lichen *Parmelaria subthomsoni* and norstictic acid containing lichen *Flavopunctelia soredica* produced yellow colour through BWM. *Peltigera rufescence*, *Sticta platyphylloides* and *Ramalina sinensis* produced no colour through BWM and the dyed threads were remained uncoloured (white).

Dermatocarpon vellerum, Lobaria retigera, Peltigera rufescence and *Ramalina conduplicans* produced different shades of green colour through DEM. *Usnea undulata* and *Parmelinella wallichiana* produced brown colour through DEM.

The colours observed through different dyeing methods were mainly due to the presence of secondary metabolites in lichens (Upreti *et al.*, 2010). The side chain molecules of ringed structure of secondary metabolites undergo various chemical reactions (hydrolysis and decarboxylation) with the solvents in which dyes are extracted, resulting in the formation of colour producing compounds (Richardson, 1988). The secondary metabolites not only impart colour but also give unique aroma to the fibres. The lichen metabolites also have antimicrobial and insecticidal properties; hence lichen dyes have an inherent quality of insect resistance thus giving more life to the dyed fibres.

Since the lichens are slow growing organism unable to produce large amount of biomass, therefore there is a need to develop proper harvesting techniques of lichens for preparing lichen dyes and at the same time for conserving lichen biodiversity. Lichens that are already detached from the thallus or fallen on ground should be used instead of removing the whole thallus. Several experiments with algal free mycobiont cultures of lichens (Ahmadjian and Reynolds, 1961; Hamada *et al.*, 1996) have demonstrated that secondary metabolites are formed mainly by fungal partner. Thus, the culture of lichens in laboratory conditions particularly the mycobiont culture of potential dye yielding lichens as done by Upreti et al. (2012), would be a conservational approach for production of lichen dyes in India.

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