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FIRST REPORT OF DYE YIELDING POTENTIAL AND COMPOUNDS OF LICHENS; A CULTURAL HERITAGE OF HIMALAYAN COMMUNITIES, PAKISTAN

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Abstract

Lichens are well-known dye yielding organisms since ancient times. The present study investigates the dye yielding potential of nineteen lichen species belonging to eleven genera (*Flavopunctelia, Flavoparmelia, Cladonia, Parmelia, Umbilicaria, Xanthoria, Ochrolechia, Hyperphyscia, Hypogymnia, Dermatocarpon* and *Parmotrema*) of Himalayan region (Abbottabad) Pakistan. Wool and silk were dyed using the 3 different methods i.e. dimethyl sulphoxide (DEM), ammonia fermentation (AFM) and boiling water (BWM). Over 57 different dye tests were made on silk. Predominant color was cerise but yellow, brown, purple, green, pink and olive were produced. COSMIN software was used to detect HEX Colour codes with RBG and HSL values. These dye colors were further altered by modifying: exposure to light, temperature and subsequent additional extractions using the different method or the same one. After dying samples were tested for stability in sunlight and the action of soap, some samples were faded to some degree and some of them changed color. Most dyes obtained through the AFM and DEM method were stable while dyes from boiling water method were light stable. A correlation of dye color with lichen secondary metabolites was also attempted. Spot test results showed the presence of different lichen substances (gyrophoric, lecanoric acid, umbilicaric acids, usnic acid, atranorin, chloroatranorin, salazinic acid and parietin).

Key words: Lichens, Extraction, Secondary metabolites, Dye, Cultural heritage, Himalayas.

Introduction

Lichens are beautiful organisms; once one starts looking for them he may be surprised at the diversity and quantity. These are symbiotic organisms composed up of a phycobiont and a photobiont (usually an alga) together form an independent and unique physiological unit. They are growing in terrestrial habitat on wood, leaves, rocks, soil and other fixture (Shah, 2011). They show slow growth in harsh living conditions that's why they are able to produce a variety of secondary metabolites (Abdullahi, 2009; Santiago *et al*., 2010; Marijana & Rankovic, 2010; Krystle *et al*., 2010), which are believed to serve as anti-herbivore, antimicrobial and antigrowth agents (Gupta *et al.,* 2007; Manojlovic *et al.,* 2005). Besides these properties, these unique entities have an inherent ability to produce beautiful dye colors. A dye can be defined as a coloring substance that has an affinity to the applied substrate. The dye generally requires a mordant to improve the fastness of the fiber dye. The secondary metabolites known as "lichen acids" are the main source in lichen dyes (Veranja *et al*., 2005; Richardson, 1988a).

Natural dyes are usually derived from plants, invertebrates and minerals source, most of them are from plant and other organic sources such as fungi and lichens. Lichen dyes have a particular affinity for natural fibers (silk and cotton), other products can also be dyed with lichens such as leather, marble, wood, wine and food materials as well. Lichens are well-known organisms and have a long dye yielding history (Diadick, 2001). First documented dye produced by *Roccella spp.* was Orchil dye (purple color) through ammonia fermentation method (Margareta, 1981). Many dye colors are converted through the extraction process, not visible in the fresh lichen thallus. Indigenous knowledge, particularly associated with extraction of dyes from lichens is ancient (Shukla & Upreti 2015). Cow urine method (CUM) mostly used in ancient times, then ammonia fermentation method (AFM), dimethyl sulphoxide extraction method (DEM) and boiling water method (BWM) are most familiar extraction methods for lichen dyes. Mostly purple, pink, yellow, brown, orange, and green dyes can be extracted from lichens. A list of more than hundred dye yielding lichen species was given by Casselman (2001).

After the discovery of first synthetic dye in 1856 natural dyes colors were completely replaced by synthetic dyes due to easy extraction and cost-effectiveness (Margareta, 1981). Synthetic dyes have immense detrimental environmental impacts due to their non-biodegradable nature and noxious effects. The demand of dyes for textile, food and cosmetics industries from natural sources has increased in the recent years due to the high rate of pollution level (Bolton, 1960). Recently several attempts are made for the development of environment and users friendly pigments mostly from the natural sources.

Lichens contain characteristic compounds known as depsides and depsidones that are made up of two/three phenolic units derived from the acetate-polymelonate pathway by a fungal partner (Asahina & Shubata 1954). Depsides were first discovered in the early part of the 20th century; are small molecules consisting of a series of linked phenol carboxylic acids esters. They are derived from Ordellinic acid (Asahina & Shibata 1954). Both compounds are the main source of dye which can color natural fibers (Richardson 1988b).

Pakistan is a country with different vegetation zones (Collinson, 1977), with high mountains in the north (Himalayas, Hindukush, Karakorum), subtropical Indus valley (dominated by arable land), deserts in Baluchistan and Thar Desert in the east. Consequently, the lichen biota is probably very rich and varied. However, unfortunately the lichen biota of Pakistan is so far largely unknown, especially with regard to different properties of lichens e.g. antimicrobial activity, ethno lichenology, dye yielding potential etc. There are 20,000 lichens species described all over the world, and Pakistan represents 367 (0.5%) of known lichens (Aptroot & Iqbal, 2012). The Himalayan region of Pakistan has rich lichens biota, including of large number of parmelioid lichens species that provide

excellent source of lichens dyes (Upreti *et al.,* 2010). The dye yielding properties of Pakistani lichens are not known until now. Thus in the present study an attempt has been made to screen out the most common and abundantly growing Himalayan lichens for their potential of dye yielding properties and as well as their substances.

Materials and Methods

Collection and identification of lichen sample

Collection lichen sample: In the present study, the lichens samples collection was performed in (34°16"88 N, 73°22"15 E), Abbottabad District, Pakistan (Fig. 1a). Abbottabad, with a geographical area of about *1,969km*^{2} is situated between 34.1304° North latitudes and 73.1822° E. East longitudes and 1,260 meters (4,134 ft.) altitude. Few species of Lichens were reported from this district by Aptroot & Iqbal (2012), however most areas of the Abbottabad remained unexplored especially with respect to dye yielding potential of lichens. To bridge this gap, the studies on lichens of Abbottabad initiated in the year 2015. The lichens material was processed immediately in the Botany Department, Hazara University Mansehra Pakistan, to reduce the chance of contamination. Samples were carefully collected; dust, soil, and rock debris were removed, shade dried to a constant weight (dry weight) and were kept at room temperature until extraction in the sterile Petri plate.

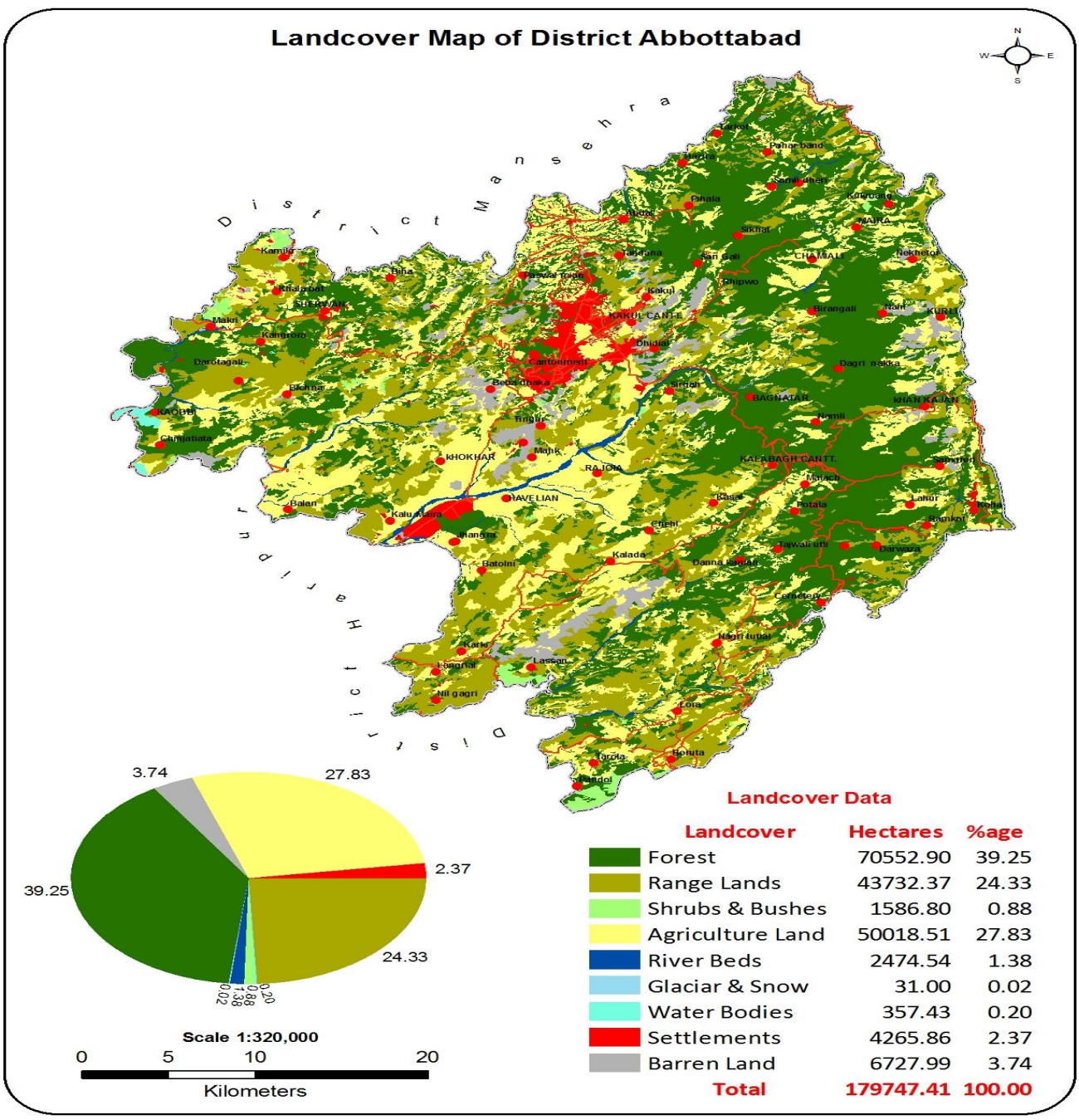

Fig. 1a. Map of district Abbottabad.

http://103.240.220.71/btt/repos/files/2015/12/District-Abbottabad-A4.jpg

Identification of lichen samples: Lichen specimens were collected from Mushakpuri, Nathia Gali, Namli Maira, Chairsajikot, Bagnotar, Chamnaka, Muslimabad, Harnoi, Banda Sapan, Salhad, Dhamtour, Taqia, Bodla, Chamhad and Havelian village areas (Fig. 1b & Table 5) of District Abbottabad during 2015. The samples collected were *Flavoparmelia caperata, Dermatocarpon miniatum, Flavopunctelia soredica, Hyperphyscia adglutinata, Hypogymnia physodes, Parmelia neodiscordans, Menegazzia terebrata, Parmelia saxatilis, Parmelia sulcate, Parmotrema reticulatum, Parmotrema tinctorum, Cladonia arbuscular, Cladonia furcata, Umbilicaria mammulata, Umbilicaria polyphylla, Umbilicaria vellea, Xanthoria elegans, Xanthoria parietina* and *Ochrolechia turneri.* The identification was done by morphology using relevant keys and monographs (Smith, 1918; Chopra, 1934; Culberson & Kristinsson, 1970; Culberson, 1972; Shyam *et al*., 2010; Aptroot & Iqbal, 2012; Tucker, 2014; Awasthi, 1988). Microchemical colour tests were also performed for identification of their secondary metabolites.

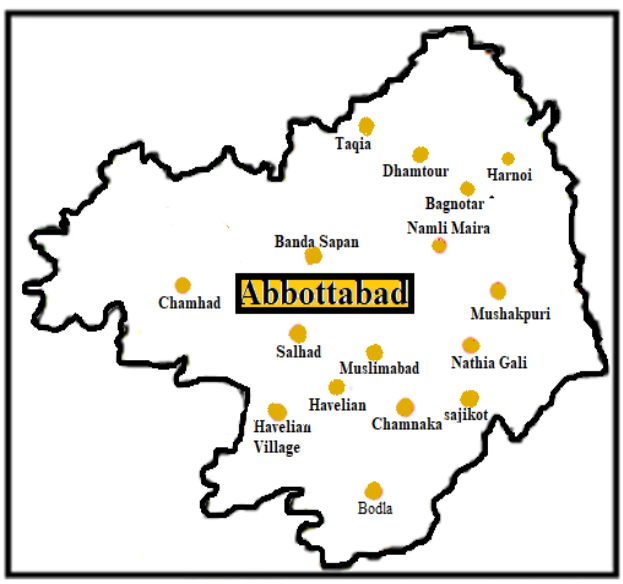

Fig 1b. Map of district Abbottabad showing lichens collection areas.

Spot tests: Colour tests were performed by chemical reagents; applying on the thallus, resulting change in colour. No change in colour was denoted by a negative (-) symbol and positive change is denoted by a positive (+) symbol followed by the colour produced. The chemical reagents used are as follows.

K test (Potassium): 10-25% aqueous solution of potassium hydroxide was applied to the thallus.

C test (Calcium hypochlorite): A freshly prepared aqueous solution of calcium hypochlorite or bleaching powder, it was prepared by dissolving calcium hypochlorite in distilled water in 2% ratio.

KC test (Potassium and Calcium hypochlorite): At a particular spot of the thallus potassium hydroxide was applied first and immediately followed by calcium hypochlorite.

Pd test (Paraphenylenediamine): Solution of paraphenylenediamine was prepared in ethanol in a small quantity for the use of a single day because it was unstable and could not be used for the next day. A more stable solution called Steiner's PD solution was prepared by dissolving 1 gm of paraphenylenediamine and 10 gm of sodium sulfite in 100 ml of distilled water with 1 ml of a liquid detergent.

I test (Iodide): Three gm of iodine was dissolved in water with 0.5 gm of potassium iodide.

These chemicals were applied to cortex and medulla of lichen thallus (Table 2).

Extraction of dyes from lichen samples: In history traditionally cow urine method (CUM) was employed for extraction of dyes then replaced with ammonia fermentation method (AFM) and later boiling water method (BWM) was introduced. In addition to the traditional methods, Dimethylsulphoxide Extraction Method (DEM) was used for extraction of lichen dyes. In this study the dyes were extracted with ammonia fermentation (AFM), boiling water (BWM) and Di-methyl sulphoxide extraction method (DEM).Lichen samples were segregated, cleaned off substratum, thoroughly washed and dried. Dried samples of lichens were crushed, powdered then weighted and used for extraction of dyes and dying experiments were carried out. Tussar silk fibers were obtained from local market. Fibers samples were weighted and thoroughly washed with distilled water before they were used for dying so that the dye penetrate and fix well into the fibers. Equal weights of dry lichens and silk fibers were used. Both dye extractions and dying were done in 250 ml flask at room temperature except BWM. No mordant was used because lichen dyes were unique in that they did not require any mordant. Three dying methods used were:

Ammonia fermentation method (AFM): Four grams of lichens were added to diluted ammonium hydroxide solution (1:10) thoroughly mixed and left for a month in a flask. Then the extract was filtered by using Whatman filter paper. Four grams of silk were added. After one month threads were removed, dried and the colour was noted.

Boiling water method (BWM): Four grams of powdered lichen was added to water and heated till boiling and filtered into a flask. The fibers were then immersed in a dye bath containing filtrate and were heated at 90°C for two hours. After cooling dye bath threads were removed, rinsed in water, dried and color was noted.

Di-methylsulphoxide Extraction Method (DEM): Four grams of crushed lichen was added to 50ml of Dimethyl sulphoxide solution in a flask**.** The content was stirred vigorously and maintained at room temperature for one month. After one month extract was filtered and threads were added for dying. After one-month threads were removed, washed in cold water, dried and colors were noted.

Stability of dyes against the light was tested following Sharma & Grover (2011). Dyed fibers were exposed to sunlight for 8 hours/day for a week. The fibers were also washed with detergent to observe the fastness of color. The colors were named with those matching Ridgway colors undyed colored fibers were used as control (Ridgway, 1912). COSMIN software was used to detect HEX Colour codes with RBG and HSL values.

Results

Although The Himalayan lichen biota of Pakistan is not fully explored no doubt it has a rich diversity of foliose lichens that are a potential source of natural dyes which can provide brilliant colors in different solvents. These can be used as a source for making dyes due to their unique chemistry and abundant biomass. A variety of colour dyes like yellow, brown, purple, green, pink and olive were obtained from lichens Fig. 2(a-s) in this study. All the three methods employed in this study have given different colors to the fibers. All the lichens were tested for dying tussar silk fiber. A bright and attractive color by at least one of the methods employed was obtained. Tussar silk fiber is used for dying tests, as it had buff color, so after dying the fiber appeared different from white silk and cotton fibers. Out of three methods employed dye colors extracted from Ammonia fermentation method (AFM) were much brighter as compared to boiling water method (BWM) and Dimethylsulphoxide Extraction Method (DEM) (Fig. 3). COSMIN software was used to detect HEX Colour codes with RBG and HSL values (Table 3).

Colour dyes obtained from AFM ranged from Olive Brown, Yellow, Buffy brown, Ivory Yellow, Lemon yellow, Purple, Purple Pink, Brown, Seal Brown, Deep cerise, cerise, red, Pink and Magenta, whereas DEM produced Isabella, Ivory Yellow, Purple, Cerise, Green, Brown, Deep cerise, Olive and Magenta. Yellow, Orange, Cerise, Brown, Deep cerise, Cerise and Fuchsia obtained through BWM (Table 1). Out of nineteen lichens selected for dye extraction 6 lichen species produced Brown shades, 6 Yellow, 2 Purple, 1 red, 1 Green, 3 Olive, 1 Orange and 8 cerise color shades (Fig. 4).

Colour fastness: On exposure to sunlight/washing dyes differed in the stability of colours. *Parmelia neodiscordans* Hale., *Menegazzia terebrata* (Hoffm.) A. Massal., *Parmotrema tinctorum* (Despr. ex Nyl.) Hale., *Umbilicaria polyphylla* (L.) Baumg., *Umbilicaria vellea* (L.) Ach. and *Ochrolechia turneri* (Sm.) Hasselrot showed more stable colours as compared to others. 15 AFM results gave stable colours while in case of DEM there were 14 stable colour results. BWM showed less effective results as compared to the other two methods. The change in colour is due to photo-oxidation chromophores (colour producing structure).

Correlation of dye colour with lichen secondary metabolites: We tried to find a correlation between the dye colours with the secondary metabolites of lichen species. Spot tests results were not only used for identification of lichens but also used for detection of major secondary metabolites (Table 2). Spot tests of *Parmelia saxatilis* (L.) Ach., *Parmelia sulcata* Taylor and *Parmotrema reticulatum* (Taylor) M. Choisy indicated the presence of salazinic acid due which these lichens produced shades of brown colour. Lichens containing only salazinic acid were responsible for orange and brown dyes while both salazinic acid and atranorin produced yellow colour. Parietin is responsible for Yellow, pink and Magenta shades of *Xanthoria elegans* and *Xanthoria parietina* dyes while atranorin produced yellow colour in *Hypogymnia physodes* (L.) Nyl. Orange colour dye of *Flavoparmelia caperata* (L.) Ach. is due to usnic acid while gray colour might be due to ceparatic acid. Usnic acid and lecanoric acid caused orange shades in *Flavopunctelia soredica* (Nyl.) Hale dye. *Parmotrema tinctorum* (Despr. ex Nyl.) Hale. imparted orange dye to fibers because it had both atranorin which caused yellow and lecanoric acid i.e. converted to orcein (red). Orange shades were also reported from *Flavopunctelia soredica* (Nyl.) Hale (Shukla *et al.,* 2014). Gyrophoric acid was detected from genus *Umbilicaria* that produced royal purple colour.

Spot test results (Table 2) revealed that most of the lichen species had lecanoric acid. Lecanoric acid is actually *p*-depside that hydrolysis to orsellic acid and undergoes a series of reactions to form colour producing chemical i.e. orcein having chemical formula $C_{28}H_{24}N_2O_7$. Orcein is not approved as a food dye. Most of the secondary metabolites detected in this study had ortho-hydroxy aldehyde group, which reacted with free amino acid group and formed stable Schiff base which ultimately imparted colours to fibers.

Chemistry of Dye producing Lichen's secondary metabolites: Lichens produced a variety of secondary metabolites. Eighty percent of these metabolites are specifically produced by lichens while 20% are commonly produced by plants or in higher fungi (Casselman, 2001). These secondary products of lichens undergo a series of chemical reactions in the presence of air, water and solvents to produce colour compounds used for dyeing purpose. These secondary products in lichens commonly called lichen acids are of fungal origin. More than thousand secondary metabolites are known worldwide with lichens reference (Dean *et al.,* 2012). Majority of these compounds are phenolic in nature dibenzofuranes (usnic acid), (b-orcinol derivatives and orcinol), depsidones (salazinic acid), depsides (barbatic acid), lactones (protolichesterinic & nephrosterinic acid), depsones (picrolichenic acid), quinones (parietin) and pulvinic acid derivatives (Upreti *et al.,* 2010). The depsidones and depsides are aromatic in nature formed by two or three phenolic units. Lichens have diverse biosynthetic pathways (shikimic acid, polymalonate and mevalonic acid pathways) to produce these different compounds depsides, depsidones and esters are precursors of orcein (coloured compound) in lichen dyes (Upreti *et al.,* 2012). These chemicals hydrolyze and are converted into orsellic acid which further undergoes decarboxylation reaction to produce orcein. Then after condensation reactions orcein give rise to various derivatives. Orcein derivatives in different concentration actually impart dye colour (Shukla & Upreti 2015). The coloring of any substrate is due to the chemical reaction between chemical constituents and Orcein derivatives of dye substrate (Upreti *et al.,* 2010). The ortho-hydroxyl aldehyde group of lichen dyes reacts with free amino group of natural protein fibers and converted into Schiff base (compounds having C=N function) (White *et al.,* 2014). Orcein as large colorless crystals can be extracted by ethanol extraction. Orcein contains a variety of phenazones. Orcein is a mixture of hydroxy-orecins, amino-orceins and amino-orceinimines (Veranja *et al.,* 2005).

Table 1. List of dyes extracted from lichens by 3 different methods.

S. No.	Lichen names	Dye extraction by different methods			Stability of colors on exposure to sunlight/washing
		AFM	DEM	BWM	
1.	*Flavoparmelia caperata* (L.) Ach.	Olive Brown	Isabella	No colour.	AFM & DEM colors slightly fade up in sunlight
2.	*Dermatocarpon miniatum* (L.) Mann.	Ivory Yellow	Ivory Yellow	Ivory Yellow	AFM & BWM colors slightly fade, DEM color stable.
3.	*Flavopunctelia soredica* (Nyl.) Hale.	Buffy brown	Yellow	Orange cinnamon	Only DEM colour stable.
4.	*Hyperphyscia adglutinata* (Florke) H. Mayrh. & Poelt.	Ivory Yellow	Ivory Yellow	No color.	Both colors stable.
5.	*Hypogymnia physodes* (L.) Nyl.	Lemon yellow	Lemon yellow	No colour.	Only DEM color stable.
6.	*Parmelia neodiscordans* Hale.	Purple	Purple	No colour.	All colors stable.
7.	*Menegazzia terebrata* (Hoffm.) A. Massal.	Purple Pink	Cerise	Cerise	All colours stable.
8.	*Parmelia saxatilis* (L.) Ach.	Brown	Green	Light Brown	Only DEM color stable.
9.	*Parmelia sulcata* Taylor.	Brown	Brown	Light Brown	AFM & BWM colors stable.
10.	*Parmotrema reticulatum* (Taylor) M. Choisy.	Olive Brown	Brown	Mikado Brown	BMW color fading on washing, AFM & DEM colors were stable.
11.	*Parmotrema tinctorum* (Despr. Ex Nyl.) Hale.	Seal Brown	Warm Sepia	Verona brown	All colours stable.
12.	*Cladonia arbuscula* (Wall.) Rabenh.	Deep cerise	Deep cerise	Deep cerise	BMW colour fading on washing.
13.	*Cladonia furcata* (Hoffm.) Florke.	Deep cerise	Deep cerise	No color	DEM color fading on washing.
14.	*Umbilicaria mammulata* (Ach.) Tuck.	Cerise	cerise	cerise	BMW color fading on washing
15.	*Umbilicaria polyphylla* (L.) Baumg.	cerise	cerise	cerise	All colours stable.
16.	*Umbilicaria vellea* (L.) Ach.	Deep cerise	cerise	Fuchsia	All colours stable.
17.	*Xanthoria elegans* (Link) Th. Fr.	Corinthian red	Olive	Ivory Yellow	BMW colour fading on washing, AFM & DEM colours were stable.
18.	*Xanthoria parietina* (L.) Beltr.	Congo pink	Yellow	Marguerite Yellow	BMW color fading on washing, AFM & DEM colors were stable.
19.	*Ochrolechia turneri* (Sm.) Hasselrot. Purpal	Purple	Purple	Purple	All colours stable.

AFM = Ammonia fermentation method, **DEM** = Di-methylsulphoxide Extraction Method, **BWM** = Boiling water method.

Table 2. Spot test results and detected compounds from investigated species.

S. No.	Lichen names	Spot test								Compound detected
		K test		C test		KC test		P test		
1.		**Cor.**	**Med.**	**Cor.**	**Med.**	**Cor.**	**Med.**	**Cor.**	**Med**	
2.	*Flavoparmelia caperata*	–	–	–	–	+	–	+	–	Usnic acid
3.	*Dermatocarpon miniatum*	–	–	–	–	–	–	–	–	none detected
4.	*Flavopunctelia soredica*	Y	–	R	–	R	–	–	–	Usnic & lecanoric acid
5.	*Hyperphyscia adglutinata*	–	–	–	–	–	–	–	–	none detected
6.	*Hypogymnia physodes*	Y	–	–	–	O- R	Y	O-R	–	Atranorin
7.	*Parmelia neodiscordans*	–	–	–	–	–	–	–	–	Atranorin, orcinol
8.	*Menegazzia terebrata*	–	–	–	–	–	–	–	–	none detected
9.	*Parmelia saxatilis*	Y	–	–	–	–	–	Y	O	Atranorin, salazinic acid,chloroatranorin
10.	*Menegazzia terebrata*	Y	Y	–	–	–	–	Y	O	Atranorin, salazinic acid, chlorotranorin
11.	*Parmotrema reticulatum*	Y	–	–	–	–	–	Y	O	Atranorin,salazinic acid,chloroatranorin
12.	*Parmotrema tinctorum*	Y	–	R	–	R	–	–	–	Atranorin & lecanoric acid
13.	*Cladonia arbuscula*	–	–	–	–	–	–	R	–	Fumarprotocetraric, protocetraric & usnic acid.
14.	*Cladonia furcata*	Y	–	–	–	–	–	R	–	Fumarprotocetraric acid, atranorin
15.	*Umbilicaria mammulata*	–	–	R	–	_	–	–	–	Gyrophoric acid
16.	*Umbilicaria polyphylla*	–	–	R	–	R	–	–	–	Gyrophoric,lecanoric, umbilicaric acids.
17.	*Umbilicaria vellea*	–	–	R	–	R	–	–	–	Gyrophoric,lecanoric acids.
18.	*Xanthoria elegans*	P	–	–	–	–	–	–	–	Parietin
19.	*Xanthoria parietina*	P	–	–	–	–	–	–	–	Parietin
20.	*Ochrolechia turneri*	–	–	–	–	–	–	–	–	Orcinol

Cor= Cortex; **Med**=Medulla; **Y**=Yellow; **R**=Red; **P**=Purple; **O**=Orange

K test = Potassium, **C test =** Calcium hypochlorite, **KC test =** Potassium and Calcium hypochlorite, **P test =** Paraphenylenediamine

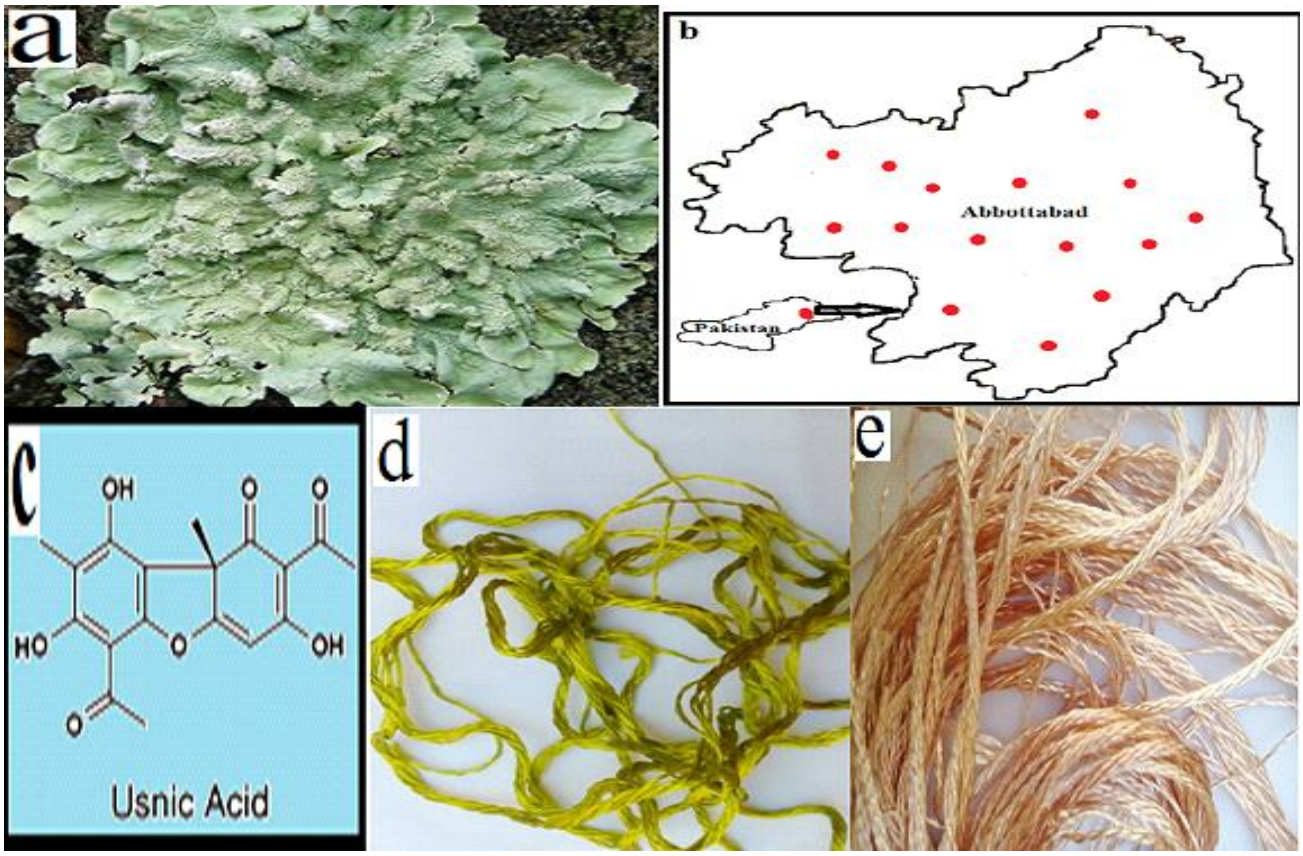

Fig. 2(a). a Natural thallus of *Flavoparmelia caperata*, b map showing distribution, c chemical structure of Usnic acid (White *et al.,* 2014), d silk fiber dyed through AFM, e silk fiber dyed through DEM.

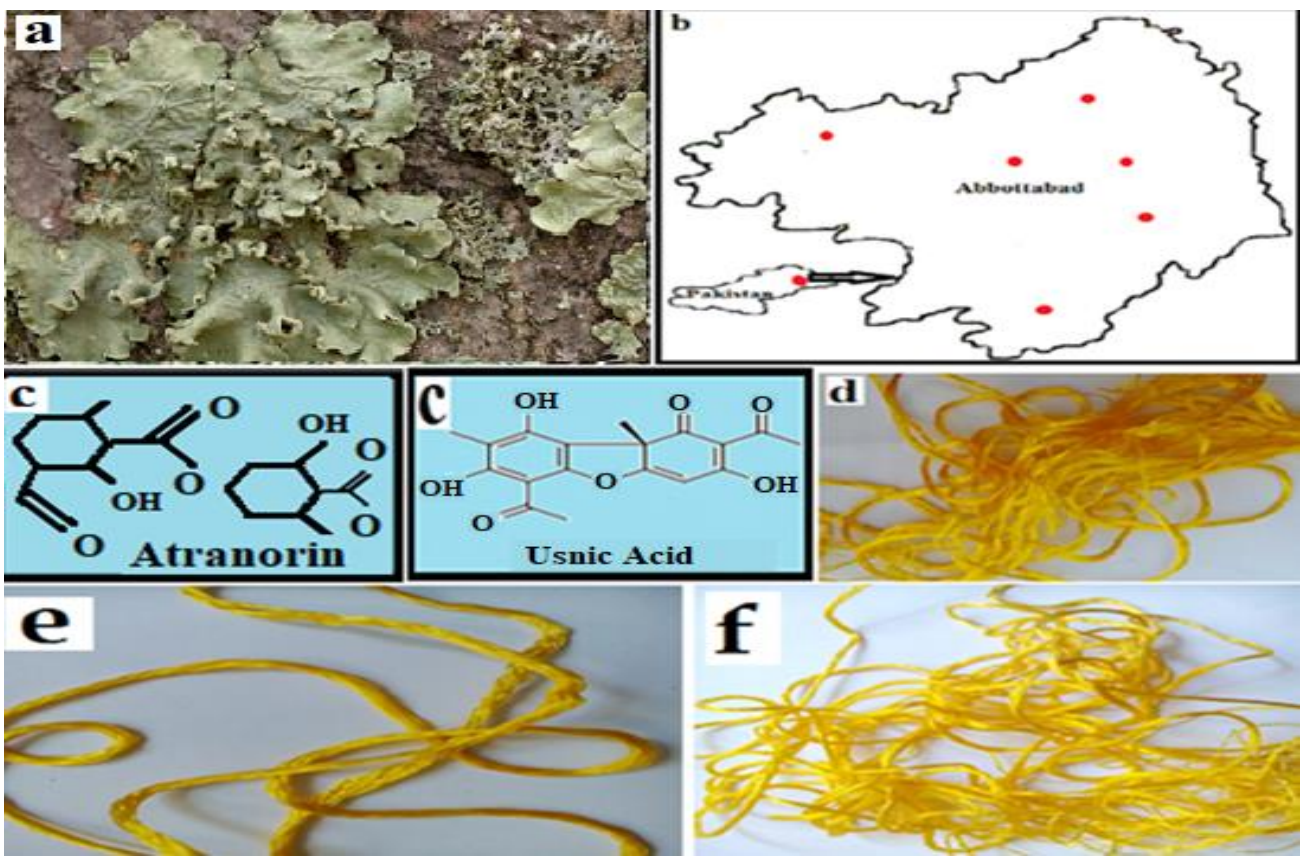

Fig. 2(b). a Natural thallus of *Flavopuntelia soredica*, b map showing distribution, c chemical structure of Atronin, Usnic acid (White *et al.,* 2014), d silk fiber dyed through AFM, e silk fiber dyed through DEM, f silk fiber dyed through BWM.

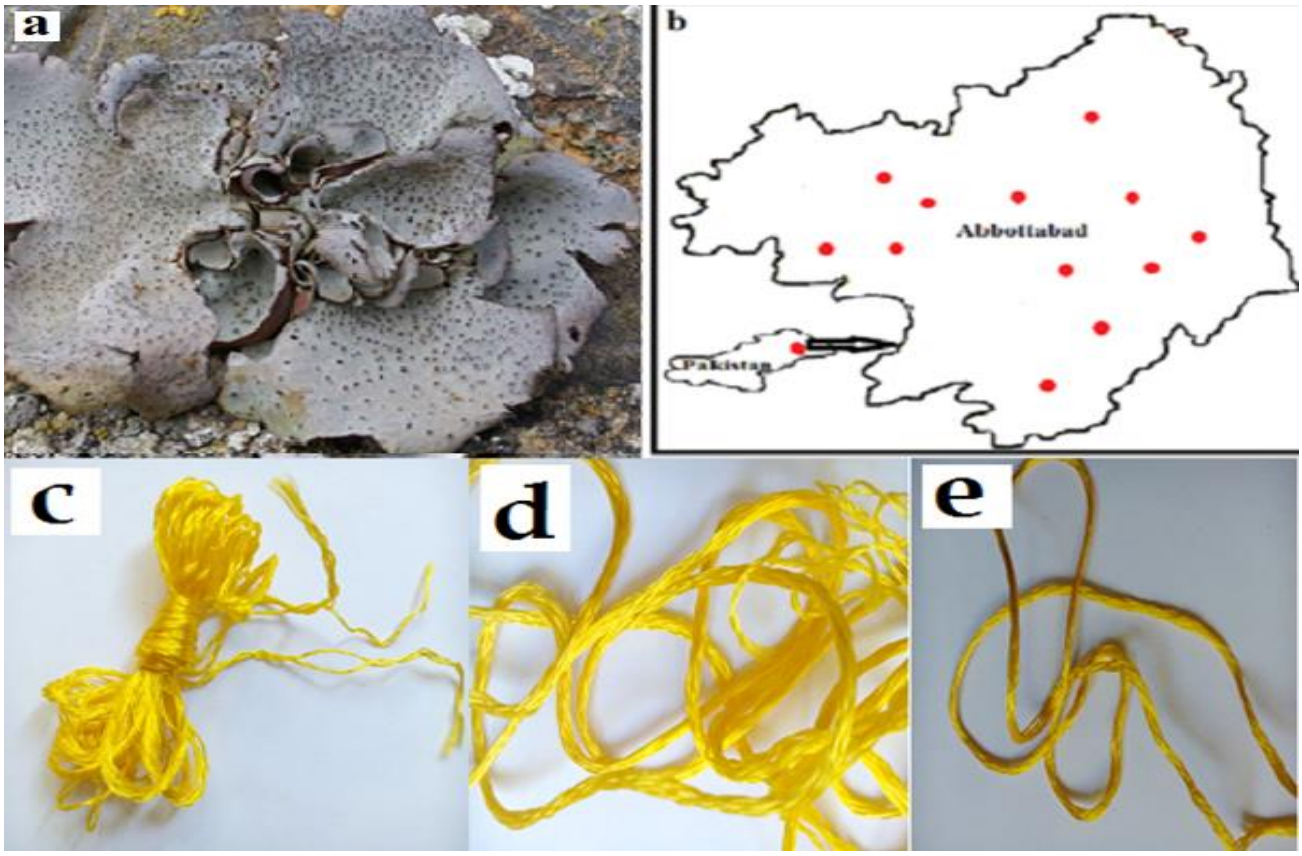

Fig. 2(c). a Natural thallus of *Dermatocarpon miniatum,* b map showing distribution, c silk fiber dyed through AFM, d silk fiber dyed through DEM, f silk fiber dyed through BWM.

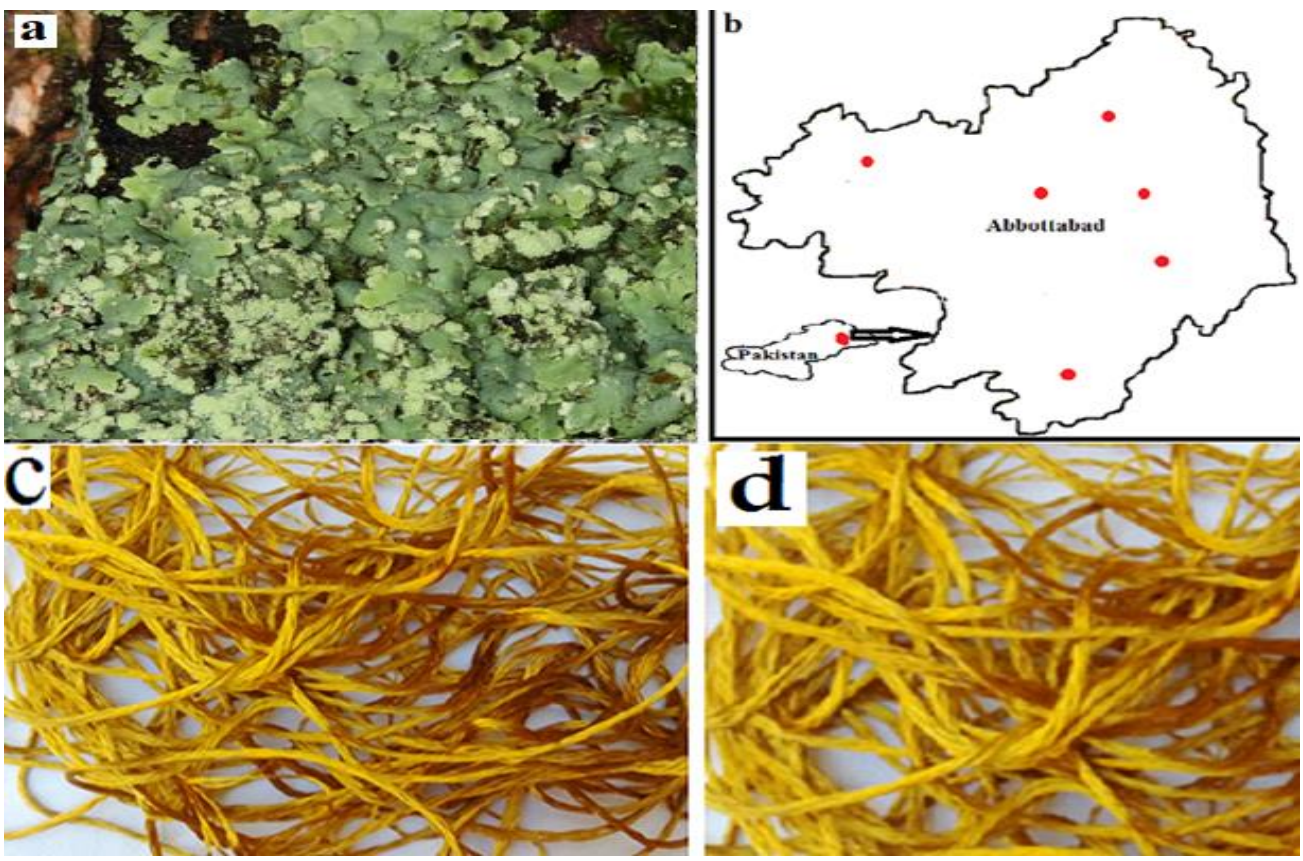

Fig. 2(d). a Natural thallus of *Hyperphyscia adglutinata*, b map showing distribution, c silk fiber dyed through AFM, d silk fiber dyed through DEM, e silk fiber dyed through BWM.

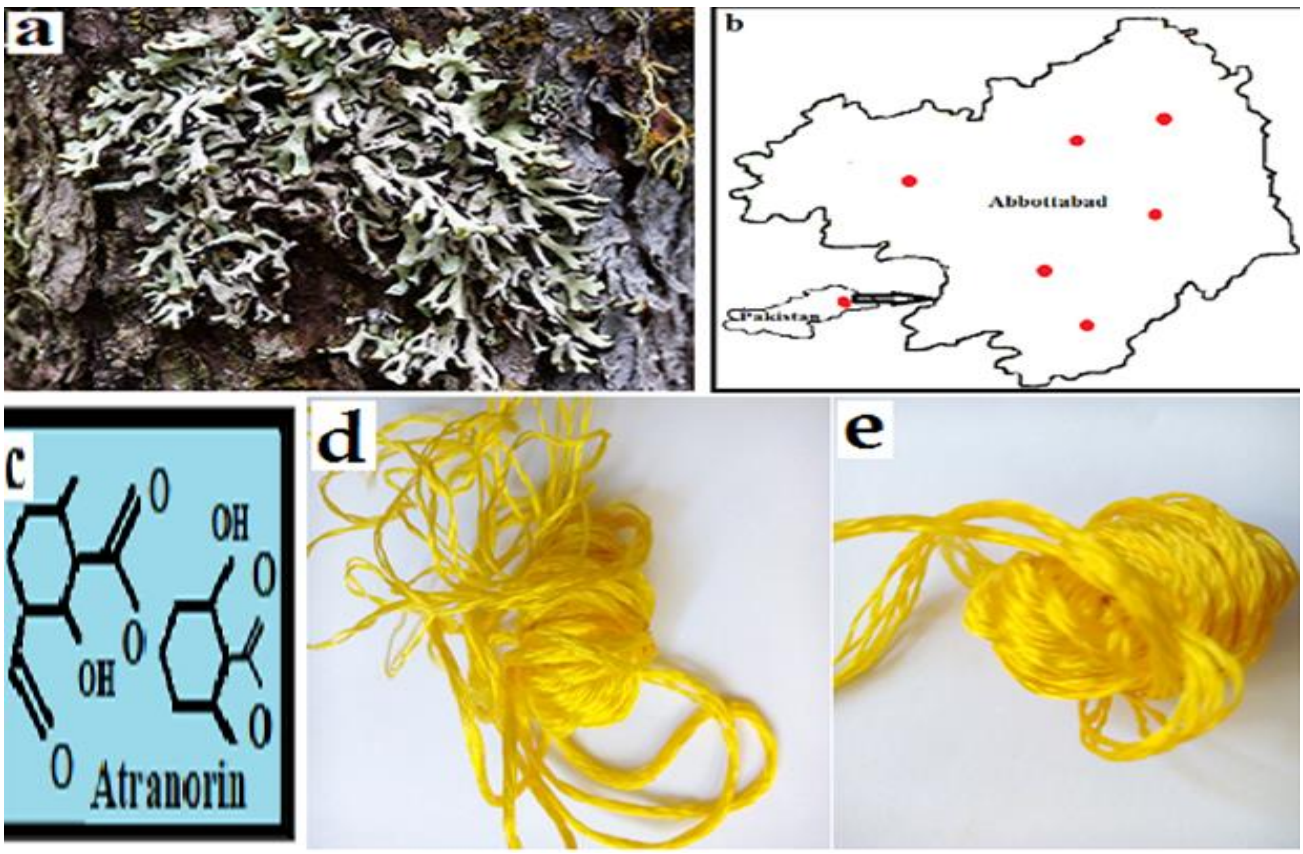

Fig. 2(e). a Natural thallus of *Hypogymnia physodes* Hale., b map showing distribution, c chemical structure of Atranorin (White *et al.,* 2014), d silk fiber dyed through AFM, e silk fiber dyed through DEM.

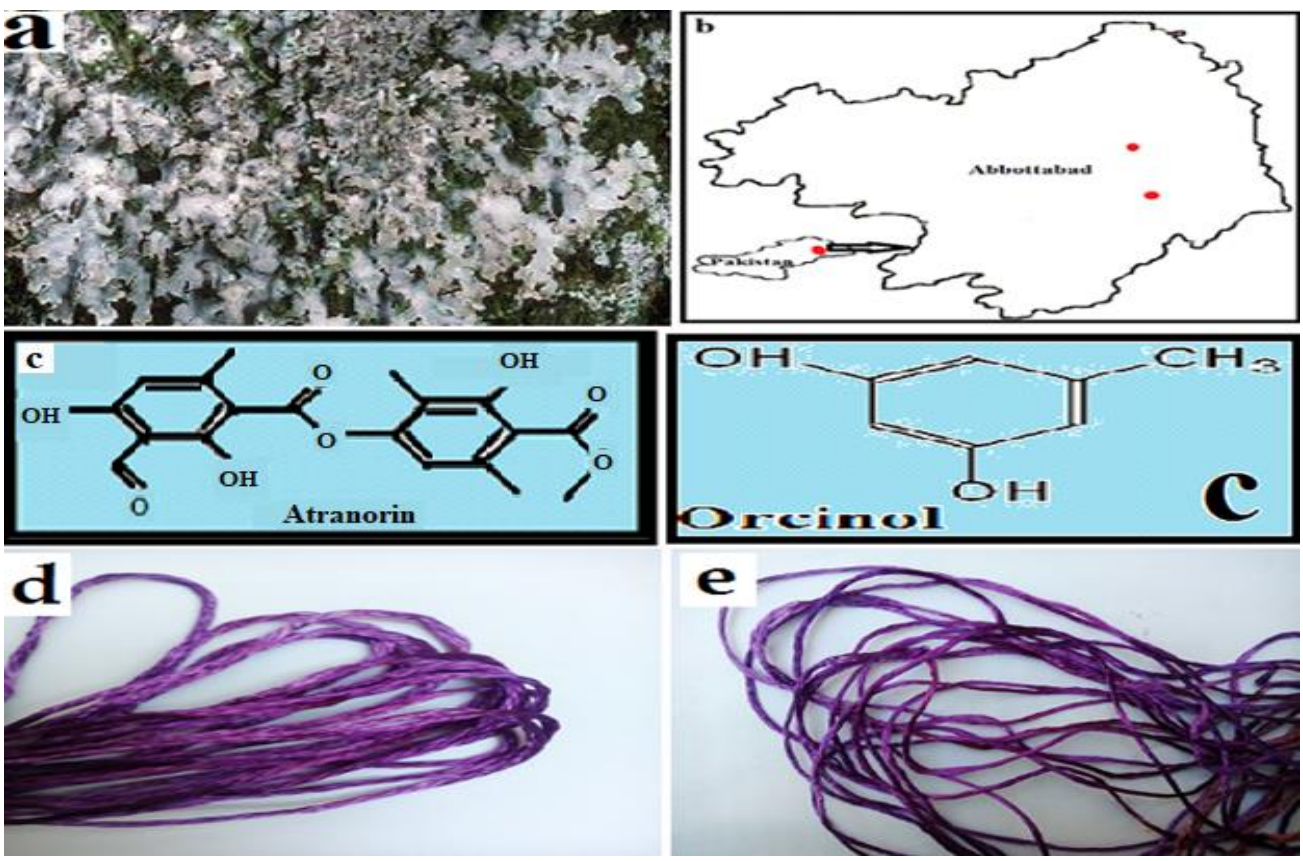

Fig. 2(f). a Natural thallus of *Parmelia neodiscordans*, b map showing distribution, c chemical structure of Atranorin & Orcinol (White *et al.,* 2014), d silk fiber dyed through AFM, e silk fiber dyed through DEM.

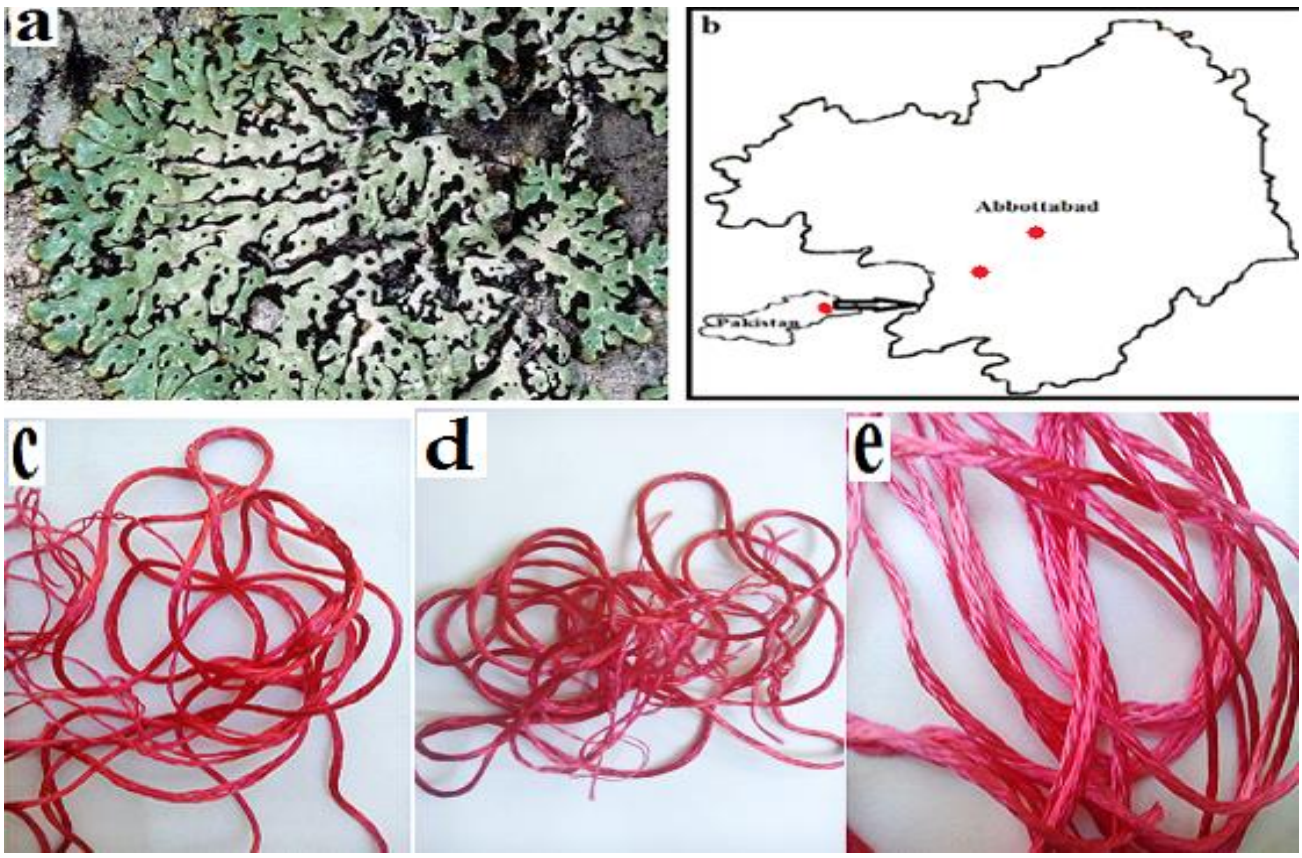

Fig. 2(g). a Natural thallus of *Menegazzia terebrata*, b map showing distribution, c silk fiber dyed through AFM, d silk fiber dyed through DEM, e silk fiber dyed through BWM.

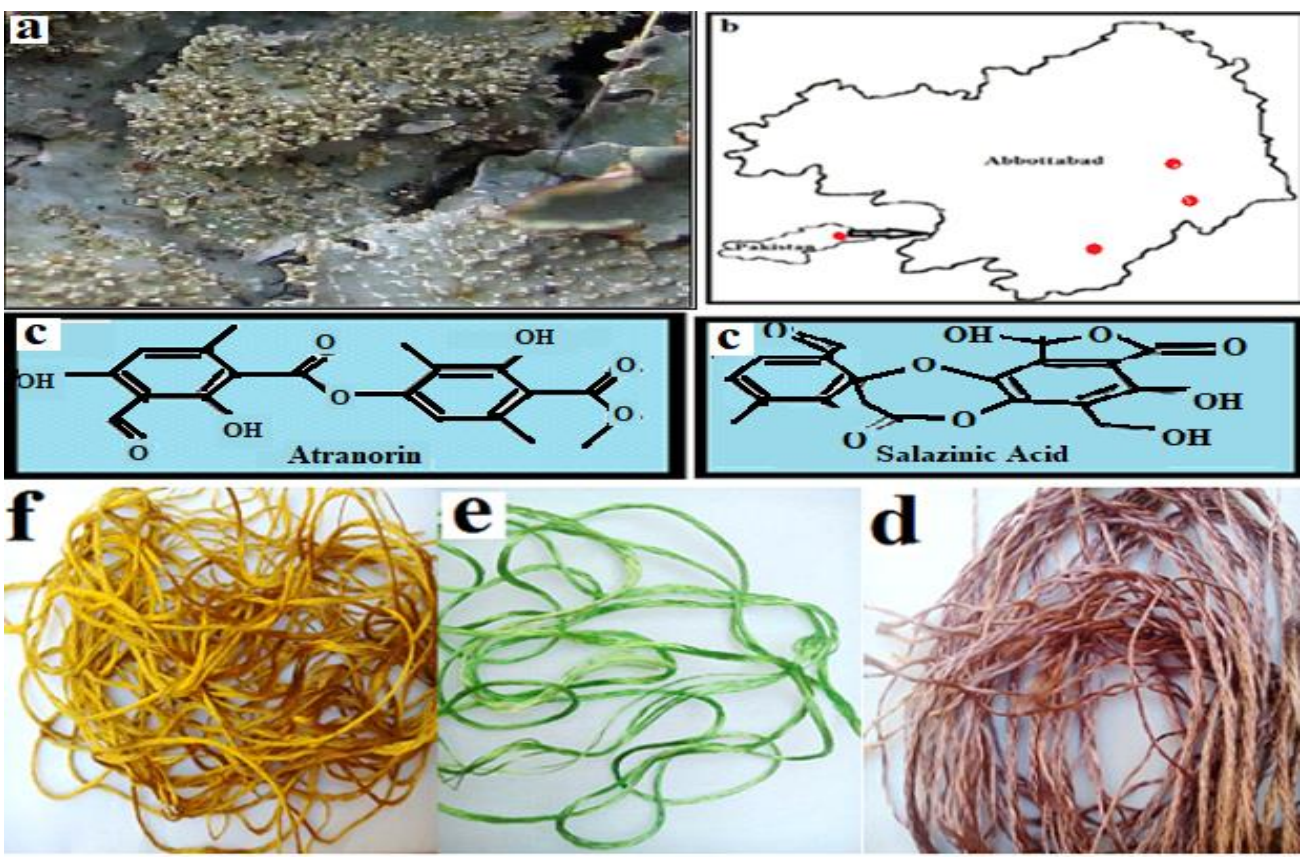

Fig. 2(h). a Natural thallus of *Parmelia saxatilis*, b map showing distribution, c chemical structure of Atronin and Salazinic acid (White *et al.,* 2014), d silk fiber dyed through AFM, e silk fiber dyed through DEM, f silk fiber dyed through BWM.

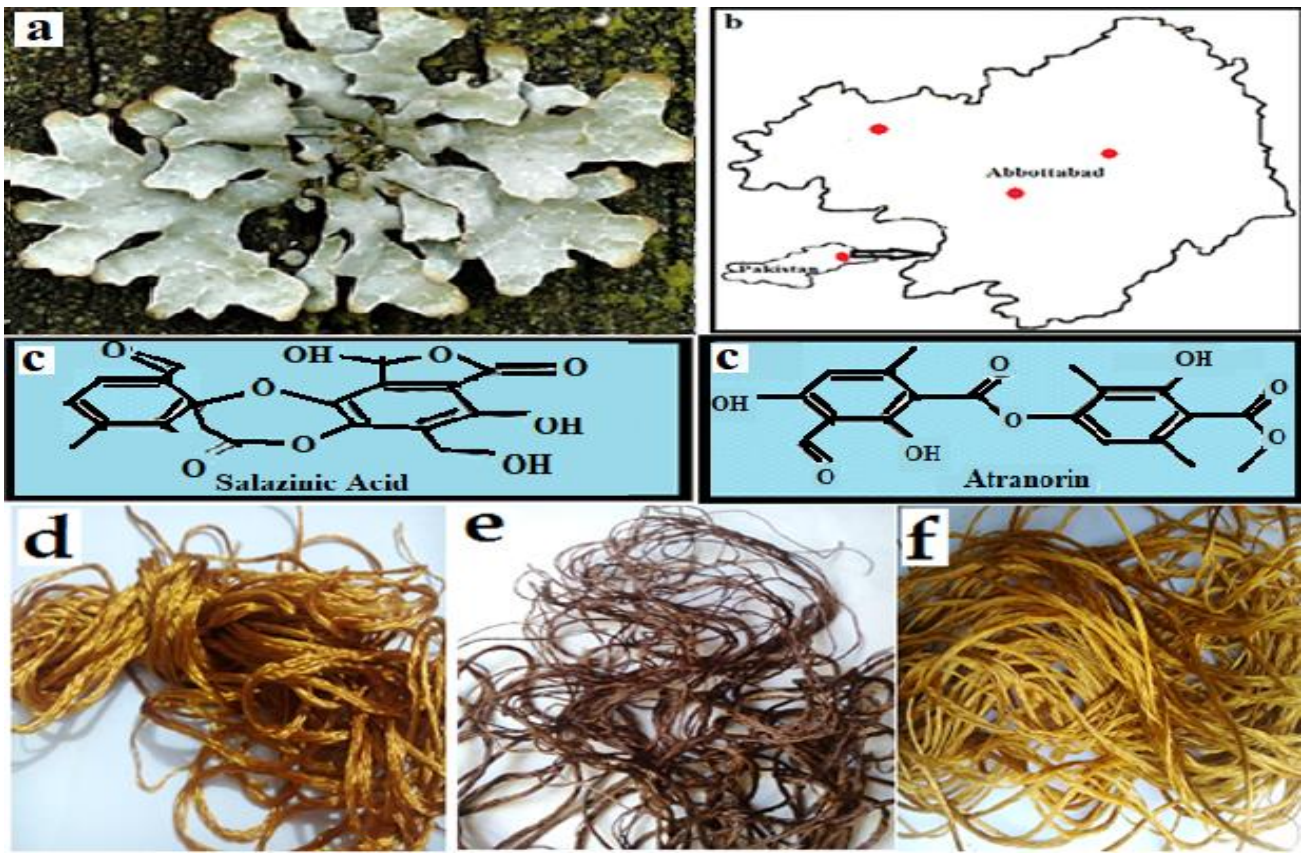

Fig. 2(i). a Natural thallus of *Parmelia sulcata*, b map showing distribution, c chemical structure of Atronin and Salazinic acid (White *et al.,* 2014), d silk fiber dyed through AFM, e silk fiber dyed through DEM, f silk fiber dyed through BWM.

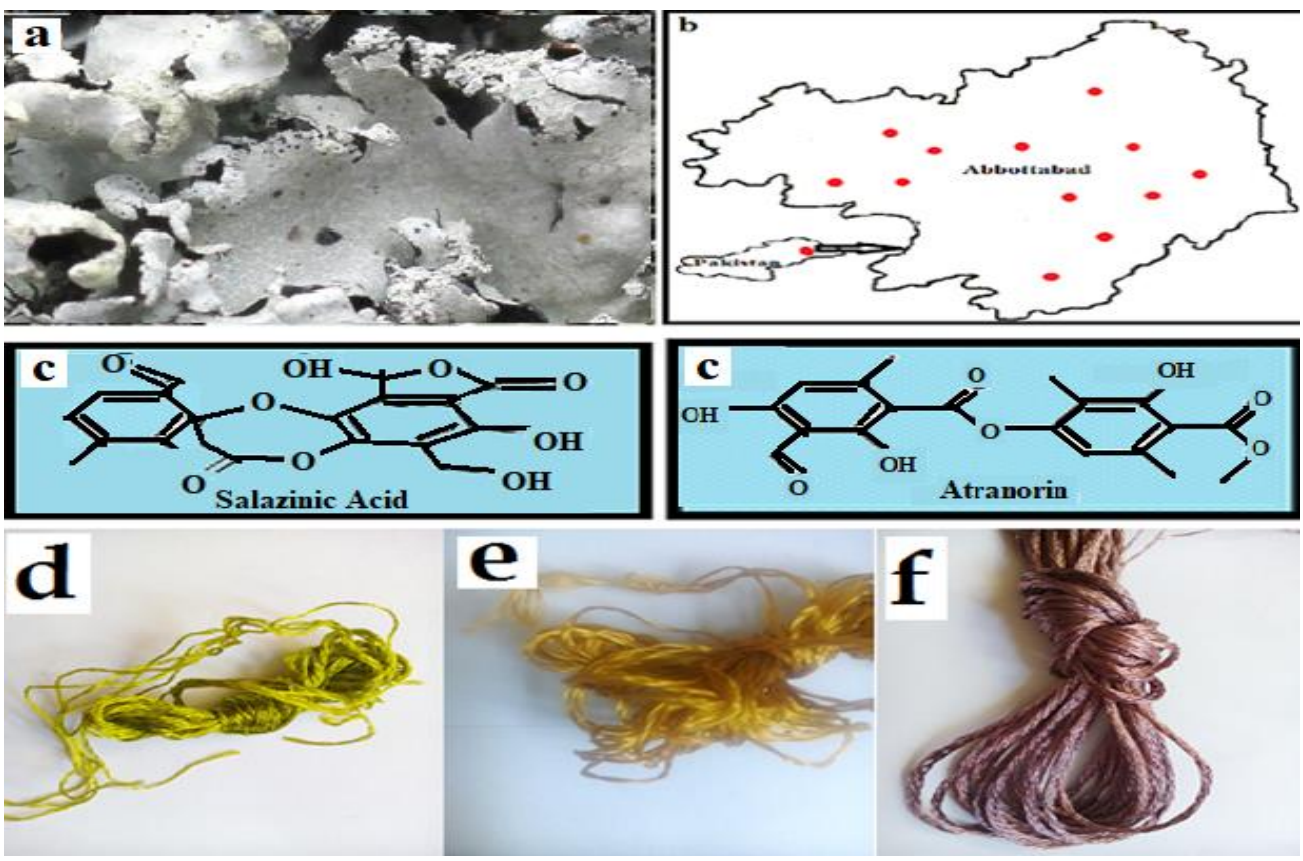

Fig. 2(j). a Natural thallus of *Parmotrema reticulatum*, b map showing distribution, c chemical structure of Atronin and Salazinic acid (White *et al.,* 2014), d silk fiber dyed through AFM, e silk fiber dyed through DEM, f silk fiber dyed through BWM.

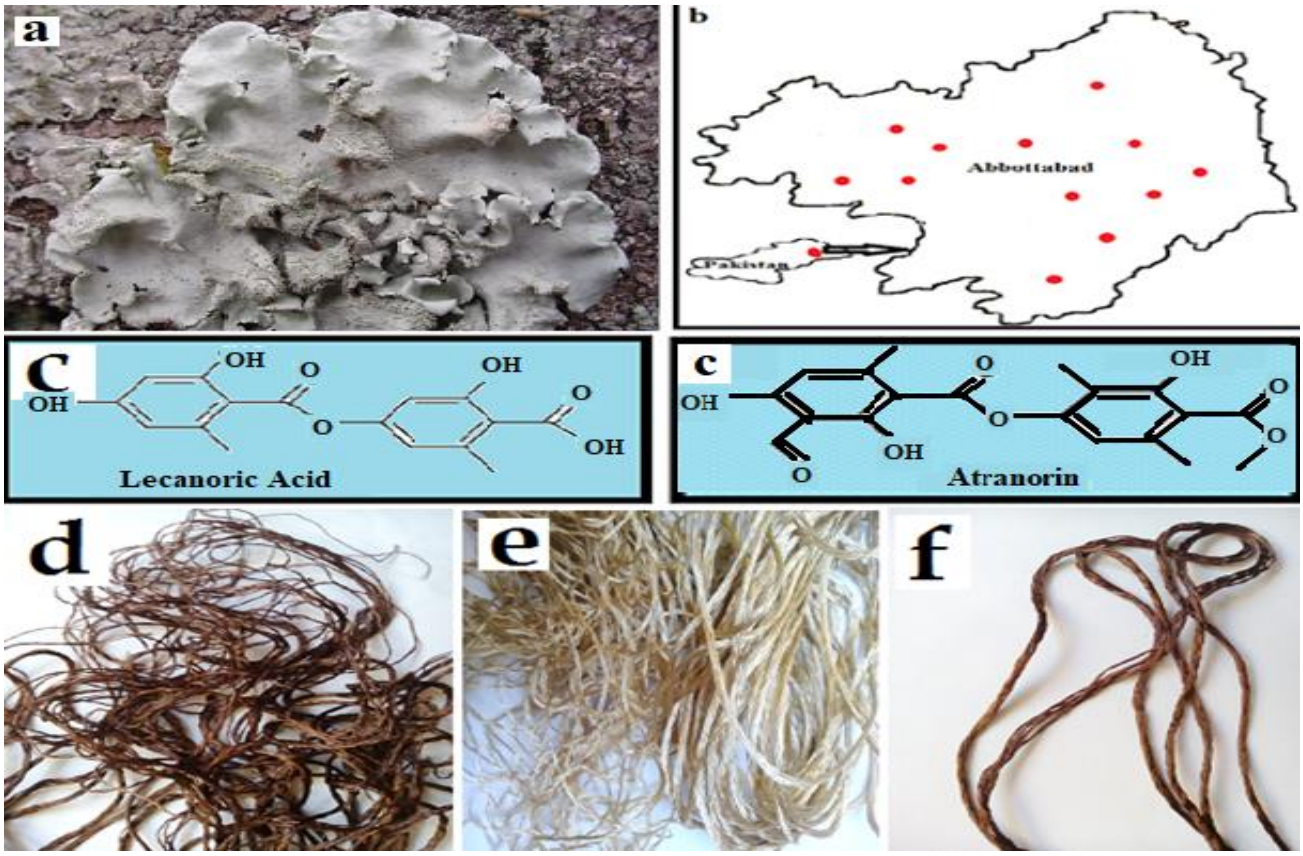

Fig. 2(k). a Natural thallus of *Parmotrema tinctorum*, b map showing distribution, c chemical structure of Atranorin & Lecanoric acid (White *et al.,* 2014), d silk fiber dyed through AFM, e silk fiber dyed through DEM, f silk fiber dyed through BWM.

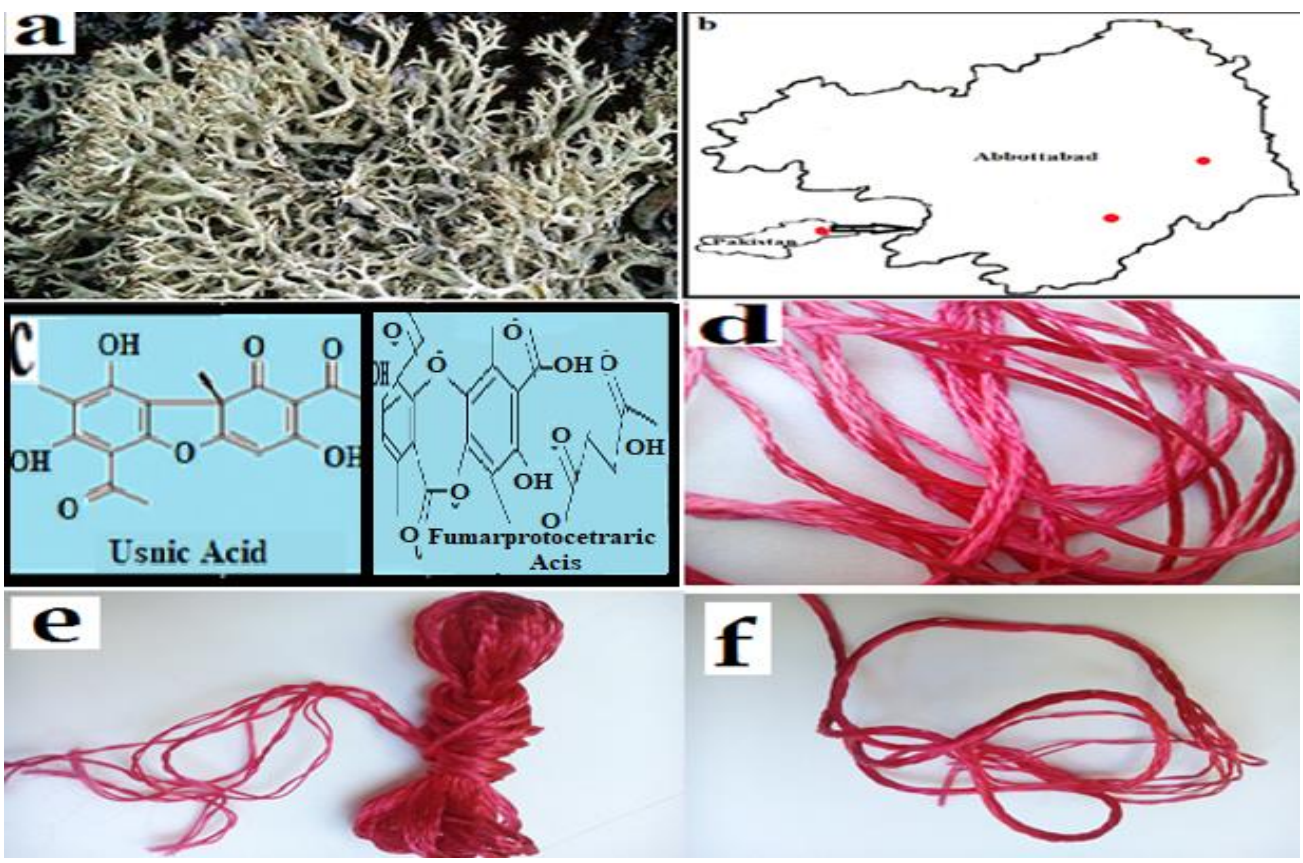

Fig. 2(l). a Natural thallus of *Cladonia arbuscula*, b map showing distribution, c chemical structure of Fumarprotocetraric, protocetraric & usnic acid (White *et al.*, 2014), d silk fiber dyed through AFM, e silk fiber dyed through DEM, f silk fiber dyed through BWM.

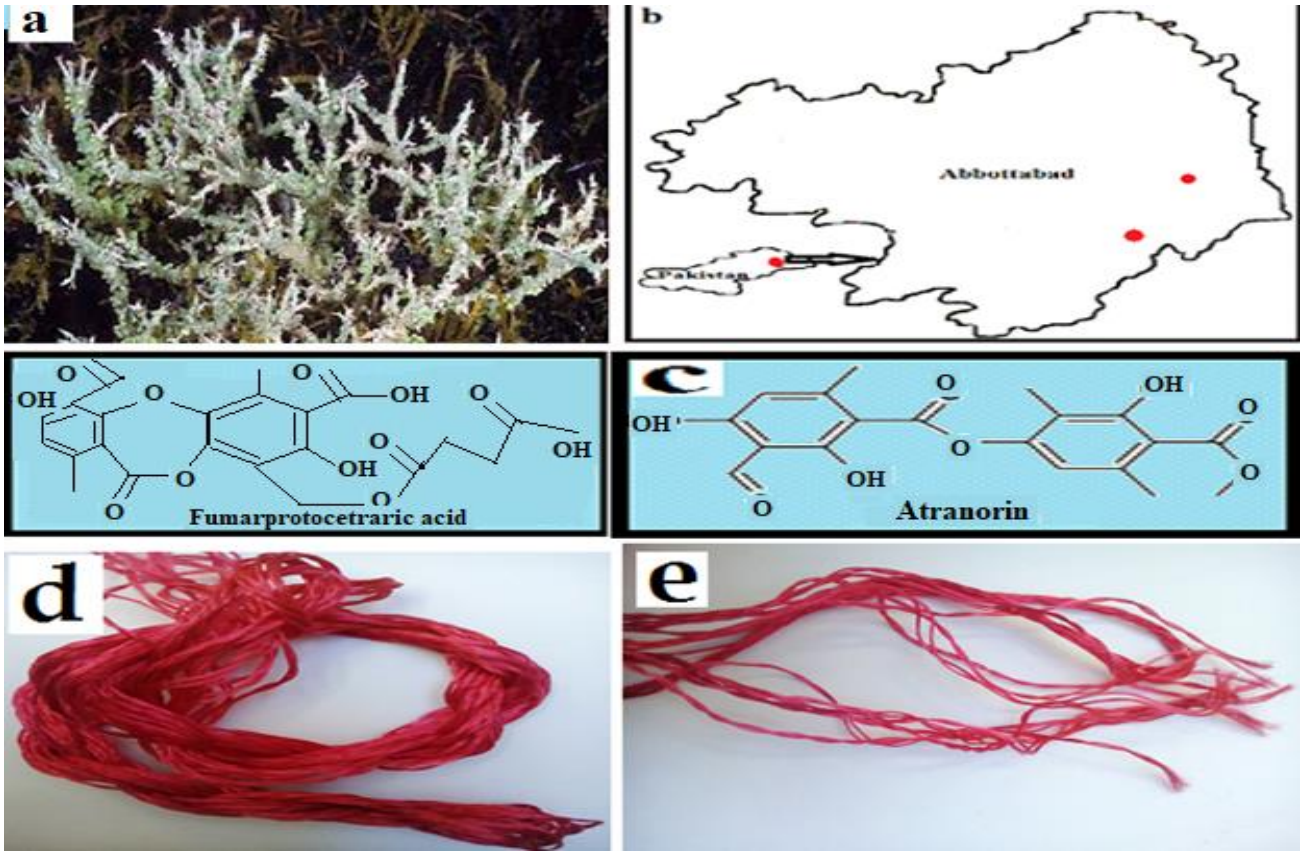

Fig. 2(m). a Natural thallus of *Cladonia furcata*, b map showing distribution, c chemical structure of Fumarprotocetraric acid & Atranorin (White *et al.,* 2014), d silk fiber dyed through AFM, e silk fiber dyed through DEM.

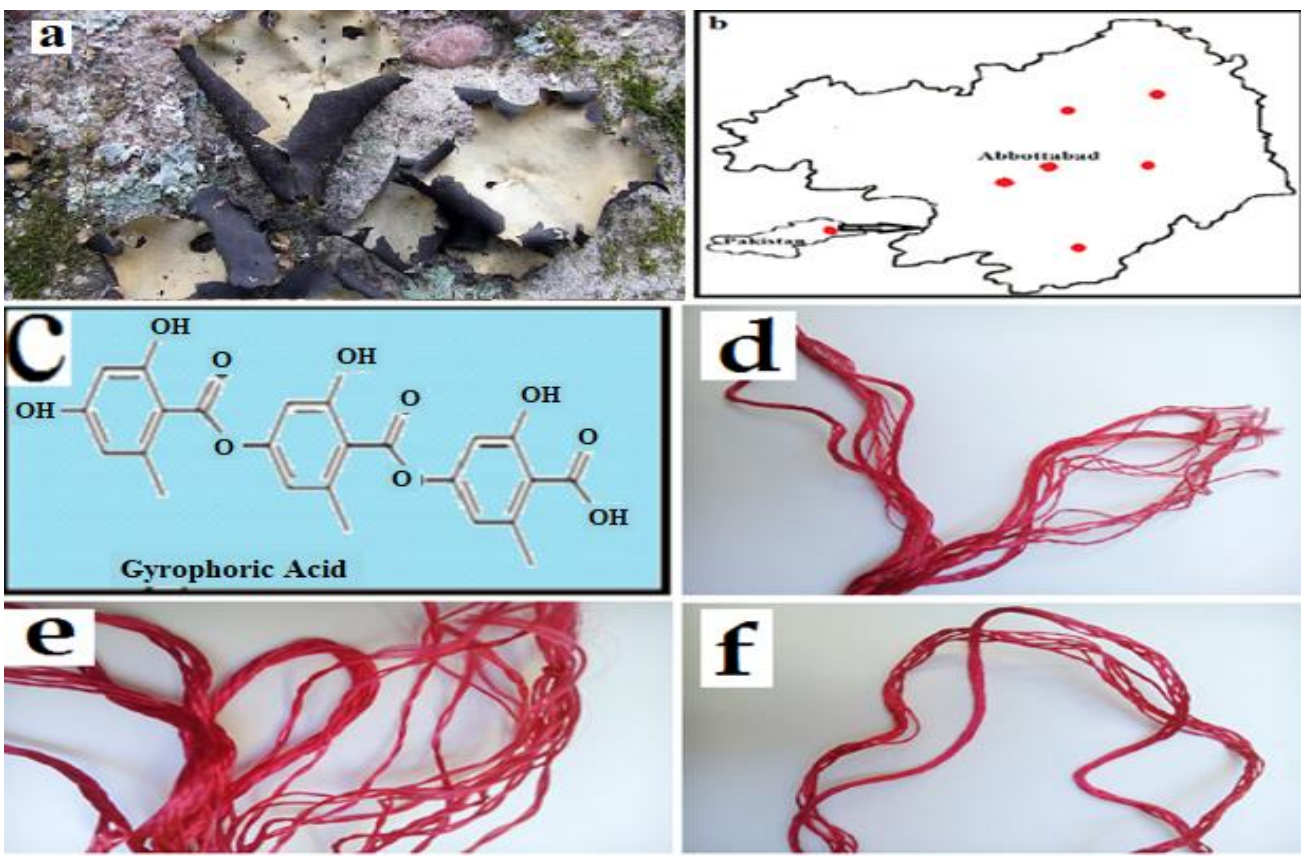

Fig. 2(n). a Natural thallus of *Umbilicaria mammulata*, b map showing distribution, c chemical structure of Gyrophoric acid (Kosanic *et al.,* 2014), d silk fiber dyed through AFM, e silk fiber dyed through DEM, f silk fiber dyed through BWM.

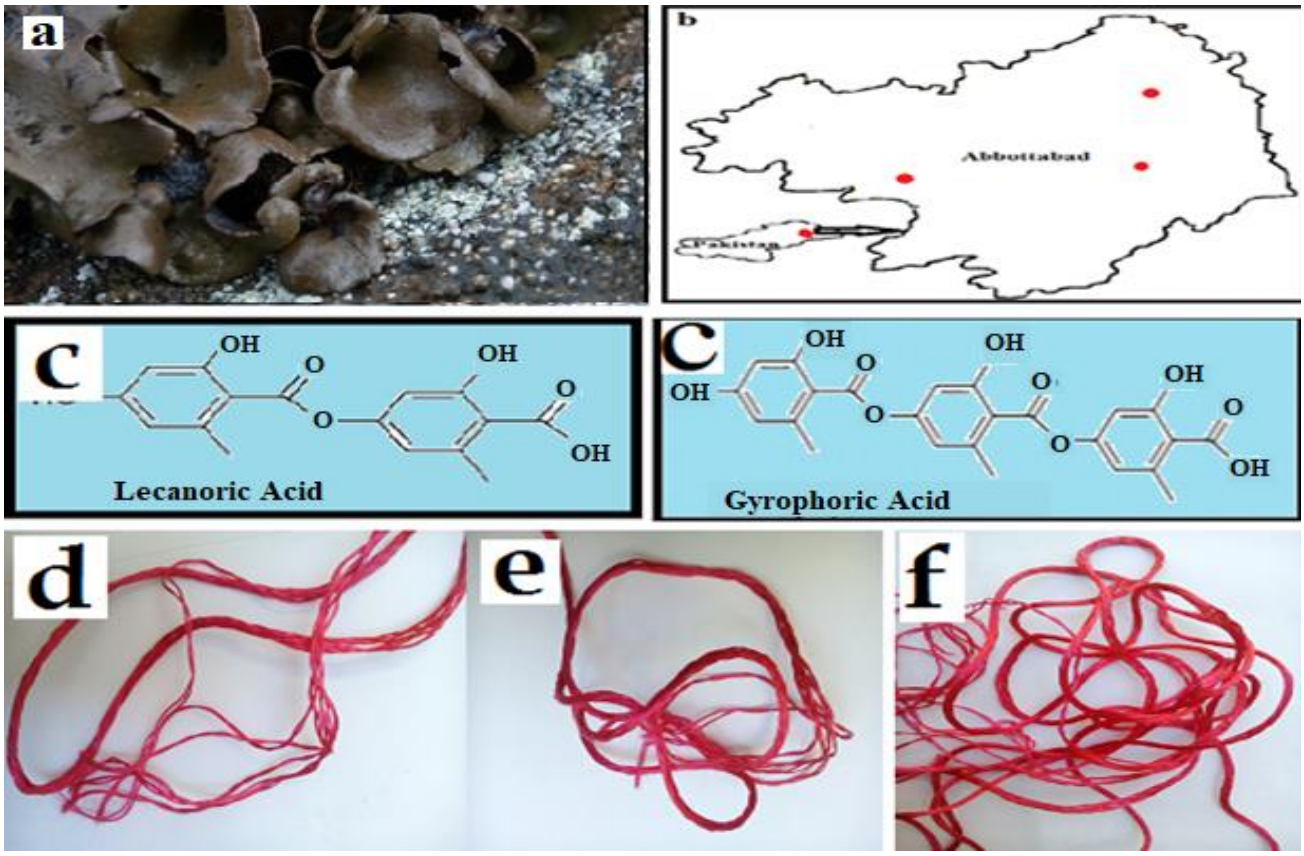

Fig. 2(o). a Natural thallus of *Umbilicaria polyphylla*, b map showing distribution, c chemical structure of Gyrophoric, Lecanoric & Umbilicaric acid (Podterob *et al*., 2008) , d silk fiber dyed through AFM, e silk fiber dyed through DEM, f silk fiber dyed through BWM.

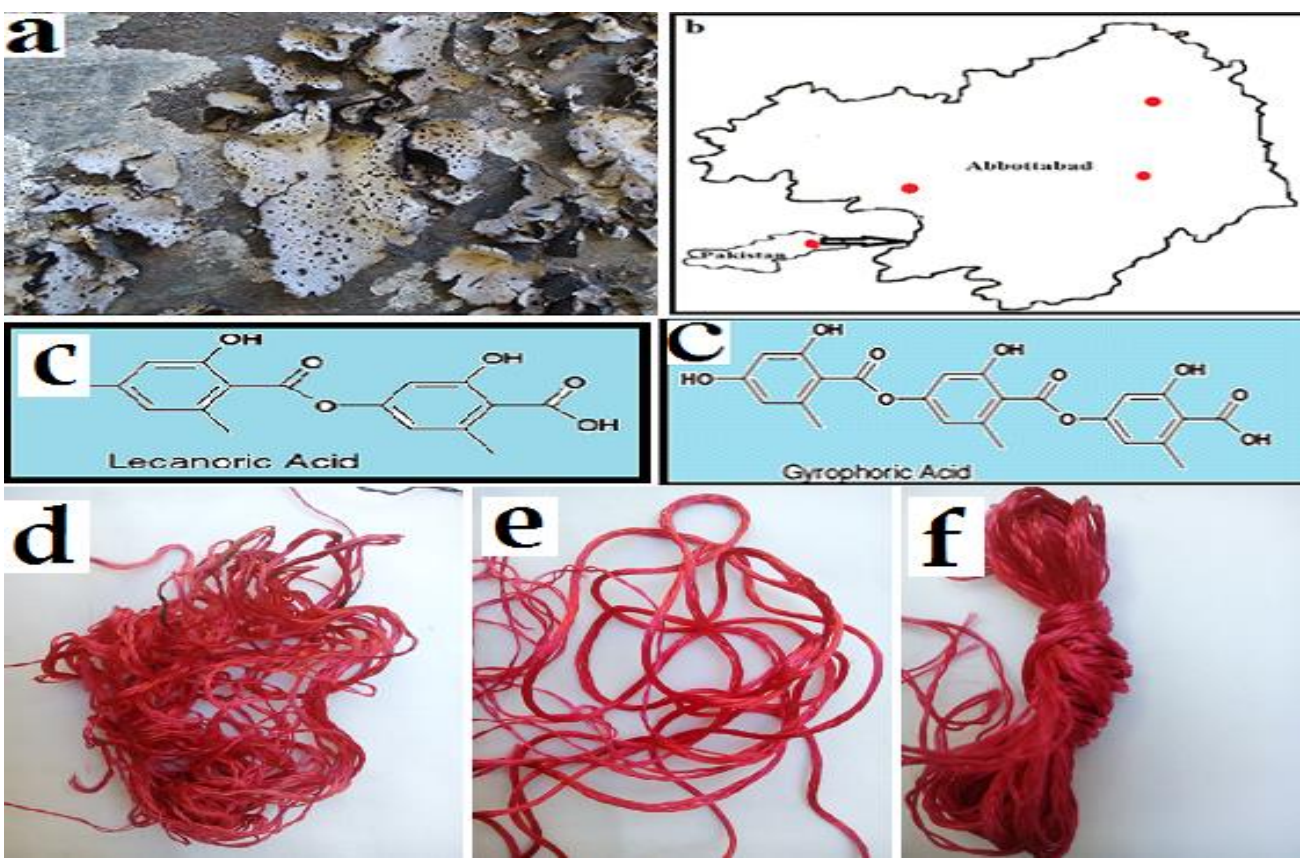

Fig. 2(p). a Natural thallus of *Umbilicaria vellea*, b map showing distribution, c chemical structure of Gyrophoric & Lecanoric (Podterob *et al*., 2008), d silk fiber dyed through AFM, e silk fiber dyed through DEM, f silk fiber dyed through BWM.

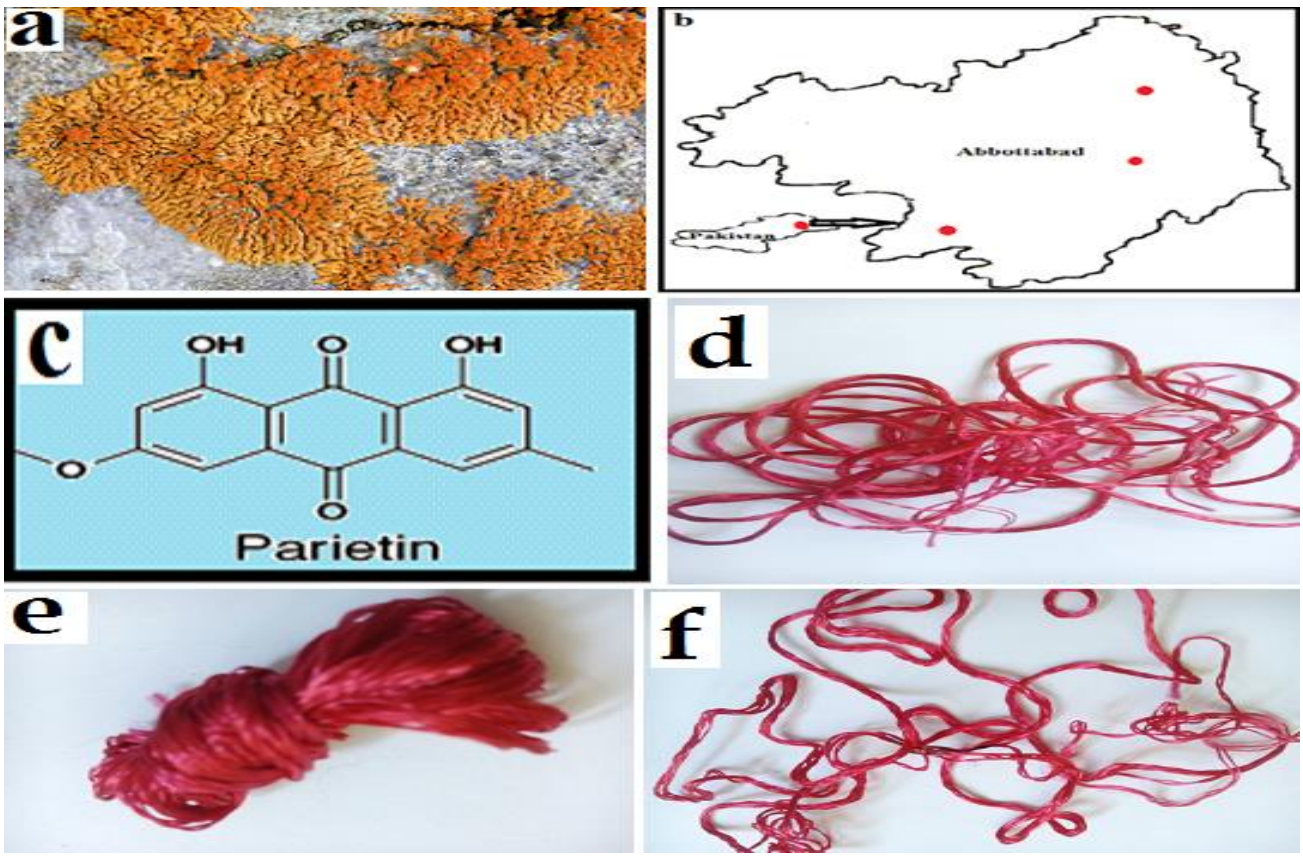

Fig. 2(q). a Natural thallus *Xanthoria elegans*, b map showing distribution, c chemical structure of Parietin (White *et al.,* 2014), d silk fiber dyed through AFM, e silk fiber dyed through DEM, f silk fiber dyed through BWM.

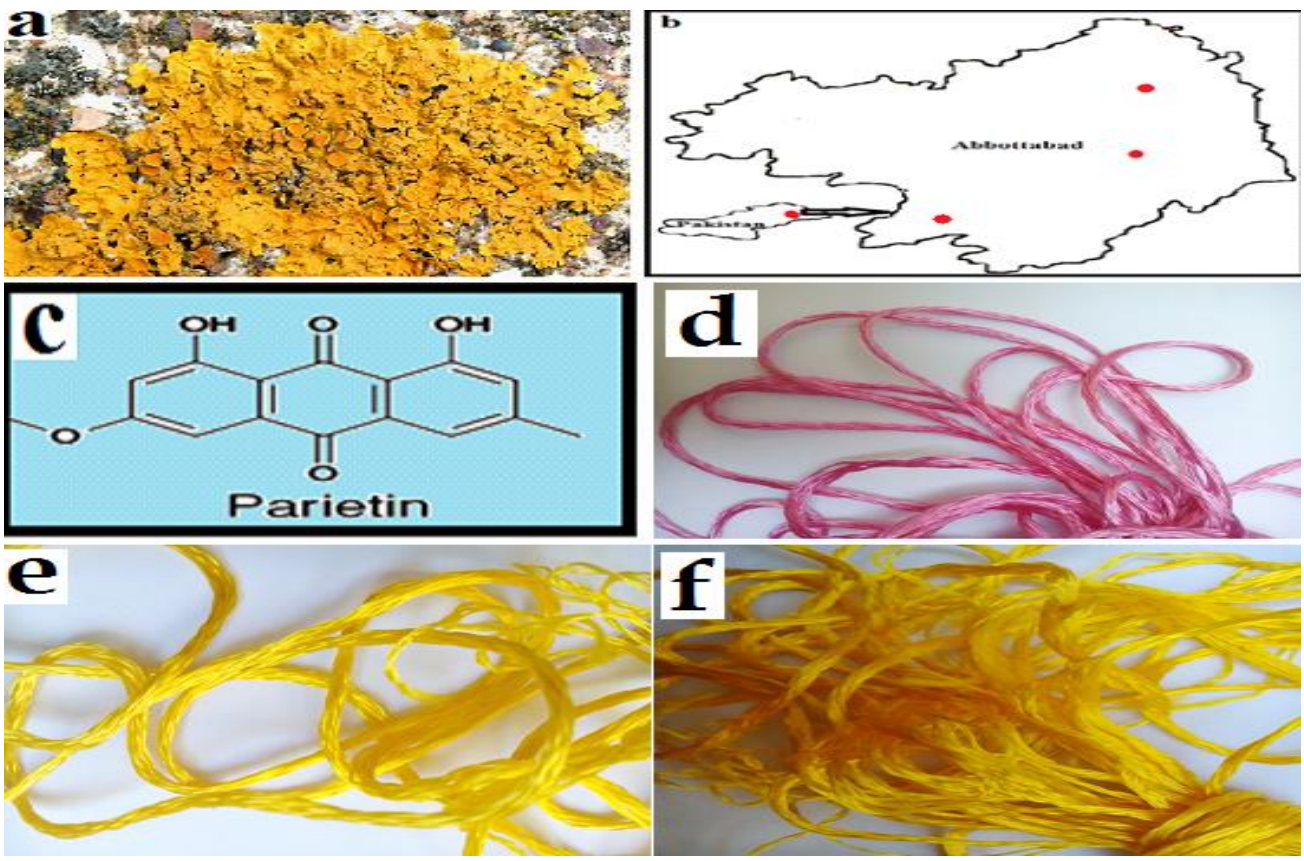

Fig. 2(r). a Natural thallus *Xanthoria parietina*, b map showing distribution, c chemical structure of Parietin (White *et al.,* 2014), d silk fiber dyed through AFM, e silk fiber dyed through DEM, f silk fiber dyed through BWM.

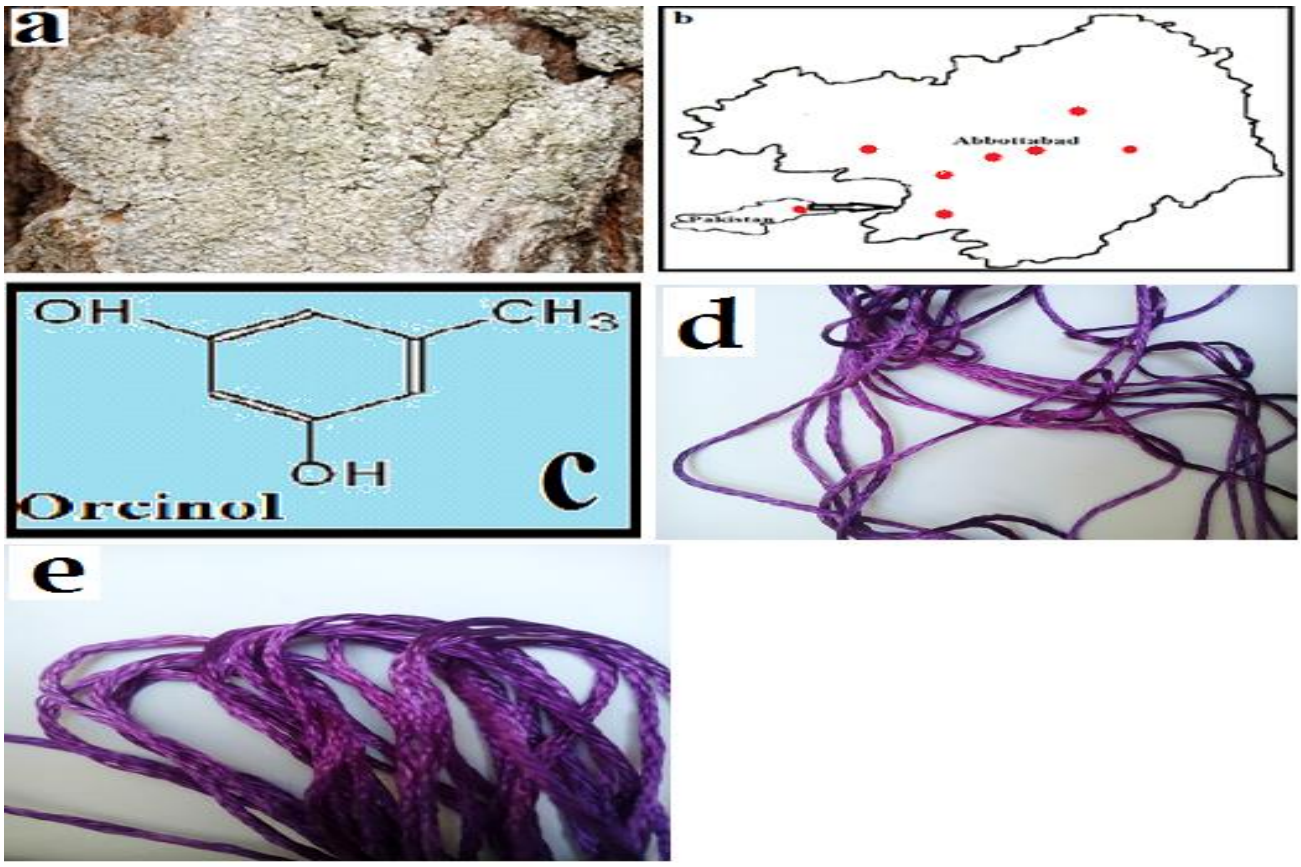

Fig. 2(s). a Natural thallus *Ochrolechia turneri*, b map showing distribution, c chemical structure of Orcinol (White *et al.,* 2014), d silk fiber dyed through AFM, e silk fiber dyed through DEM.

Fig. 3. Dye extraction methods (AFM, BWM & DEM).

Table 3. HEX color code, RGB color model and HSL (hue, saturation, and lightness) values for each dye color.

S. No.	Colours	HEX	*RGB*	*HSL*
1.	Isabella	#D2B48C	210, 180, 140	34, 44%, 69%
2.	Ivory Yellow	#F0E68C	240, 230, 140	54, 77%, 75%
3.	Buffy brown	#A0522D	160, 82, 45	19, 56%, 40%
4.	Lemon yellow	#FFFF00	255, 255, 0	60, 100%, 50%
5.	Yellow	#FFD700	255, 215, 0	51, 100%, 50%
6.	Purple	#800080	128, 0, 128	300, 100%, 25%
7.	Brown	#8B4513	139, 69, 19	25, 76%, 31%
8.	Light Brown	#A0522D	160, 82, 45	19, 56%, 40%
9.	Green	#9ACD32	154, 205, 50	80, 61%, 50%
10.	Olive Brown	#556B2F	85, 107, 47	82, 39%, 30%
11.	Seal Brown	#8B4513	139, 69, 19	25, 76%, 31%
12.	Deep cerise	#DB7093	219, 112, 147	340, 60%, 65%
13.	cerise	#FF69B4	255, 105, 180	330, 100%, 71%
14.	Corinthian red	#CD5C5C	205, 92, 92	0, 53%, 58%
15.	Congo pink	#FA8072	250, 128, 114	6, 93%, 71%
16.	Magenta	#FF1493	255, 20, 147	328, 100%, 54%
17.	Olive	#808000	128, 128, 0	60, 100%, 25%
18.	Warm Sepia	#D2B48C	210, 180, 140	34, 44%, 69%

Discussion

Lichen dyes by a variety of vernacular names have a long history, dyes from lichens (orchil) were discovered before 1500 BCE, in the Roman period and they were considered synonymous with wealth and power, wearing purple colour was an indicative of status and privilege, and the dye industry was also politicized (Diadick, 2001). Fruticose and foliose lichens were tested for dyeing yielding potential because of their bigger size and shape. Different studies were conducted for dye extraction all over the world (Shukla *et al.*, 2015; Fazioa *et al.*, 2007; Diadick, 2001; Casselman, 2001; Dean *et al.*, 2012; Upreti *et al.*, 2012).

The dye colors vary with the harvesting season of lichens thallus, soil properties and fermentation time. Lichen dyes are much better then synthetic dyes as these are friendly to our environment, imparts musky odor to fibers, dyed products are insect-proof because lichen secondary compounds make the fibers distasteful for insects, have limited colour stability against sunlight and as no mordents have been used in the study and without any mordents these are bright and beautiful colours. Probably more colorfast dyes could be obtained if mordents are used during extraction. On the other side, the tedious extraction and long dying time increase the cost of lichen dyes.

Table 4. Dye yielding Lichen species and their chemistry.

S. No.	Species name	Dye color produced	Compound detected	References
1.	*Flavoparmelia caperata*	Brown	Usnic acid	Shukla *et al*., (2014)
2.	*Dermatocarpon miniatum*	Brown to grey	None detected	Brough (1988), Upreti *et al*., (2010).
3.	*Flavopunctelia soredica*	Brown to Yellow	Usnic & lecanoric acid	Casselman (2011), Shukla and Upreti (2014a).
4.	*Hyperphyscia adglutinata*	Yellow	none detected	
5.	*Hypogymnia physodes*	Yellow	Atranorin	
6.	*Parmelia neodiscordans*	Purple	Atranorin, orcinol	Brough (1988).
7.	*Menegazzia terebrata*	Pink shades		Brough (1988).
8.	*Parmelia saxatilis*	Brown to green	Atranorin, salazinic acid,chloroatranorin	Upreti *et al.,* (2010).
9.	*Parmelia sulcata*	Brown to yellow	Atranorin, salazinic acid, chlorotranorin	Kok (1966), Brough (1988), Casselman (1994), Hodge (2006)
10.	*Parmotrema reticulatum*	Brown	Atranorin,salazinic acid,chloroatranorin	Shukla and Upreti (2014a)
11.	*Parmotrema tinctorum*	Brown	Atranorin & lecanoric acid	Upreti *et al.* (2010), Shukla and Upreti (2014a)
12.	*Cladonia arbuscula*	Pink shades	Fumarprotocetraric, protocetraric & usnic acid.	
13.	*Cladonia furcata*	Pink shades	Fumarprotocetraric acid, atranorin	
14.	*Umbilicaria mammulata*	cerise	Gyrophoric acid	Upreti *et al.* (2010), Casselman (1994)
15.	*Umbilicaria polyphylla*	cerise	Gyrophoric,lecanoric, umbilicaric acids.	Upreti *et al.* (2010), Casselman (1994)
16.	*Umbilicaria vellea*	cerise	Gyrophoric,lecanoric acids.	Upreti *et al.* (2010), Casselman (1994).
17.	*Xanthoria elegans*	Red to yellow	Parietin	Shukla and Upreti (2014b)
18.	*Xanthoria parietina*	Pink to yellow	Parietin	Upreti et al. (2010), Shukla and Upreti (2014b)
19.	*Ochrolechia turneri*	Purple	Orcinol	Kok (1966), Casselman (1994)

Table 5. Occurrences of species in different localities.

S.No.	Specie	M.P	N.G	N.M	C.S	B	C	M	H	BS	S	D	T	B	C	H.V
1.	*Flavoparmelia caperata* (L.) Ach.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2.	*Dermatocarpon miniatum* (L.) Mann.	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+
3.	*Flavopunctelia soredica* (Nyl.) Hale.	–	–	+	–	–	–	+	–	–	+	+	+	+	+	–
4.	*Hyperphyscia adglutinata* (Florke)	–	–	+	–	–	–	+	–	–	+	+	+	+	–	–
5.	*Hypogymnia physodes* (L.)	–	–	+	–	–	+	+	–	–	–	+	+	+	–	–
6.	*Parmelia neodiscordans* Hale.	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–
7.	*Menegazzia terebrata* (Hoffm.) A. Massal.	–	–	–	–	–	–	–	–	–	+	–	–	–	–	+
8.	*Parmelia saxatilis*	–	+	+	+	–	–	–	+	–	+	–	–	–	–	–
9.	*Parmelia sulcata* Taylor.	–	–	–	–	–	–	–	–	+	–	–	+	–	+	–
10.	*Parmotrema reticulatum* (Taylor) M. Choisy.	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–
11.	*Parmotrema tinctorum* (Despr. Ex Nyl.)	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–
12.	*Cladonia arbuscula* (Wallr.) Rabenh.	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–
13.	*Cladonia furcata* (Hoffm.) Florke.	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–
14.	*Umbilicaria mammulata* (Ach.) Tuck.	–	+	–	–	–	–	–	+	–	+	–	–	+	–	–
15.	*Umbilicaria polyphylla* (L.) Baumg.	+	+	–	–	+	–	–	+	–	–	–	–	–	–	–
16.	*Umbilicaria vellea* (L.) Ach.	+	+	–	–	+	–	–	+	–	–	–	–	–	–	–
17.	*Xanthoria elegans* (Link) Th. Fr.	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–
18.	*Xanthoria parietina* (L.) Beltr.	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–
19.	*Ochrolechia turneri* (Sm.) Hasselrot.	–	+	–	+	–	–	–	–	+	+	–	+	+	–	+

+ = Presence, -- =**M.P=** Mushakpuri, **N.G=** Nathia Gali, **N.M=** Namli Maira, **C.S=** Chairsajikot, **B=** Bagnotar, **C=** Chamnaka, **M=** Muslimabad, **H=** Harnoi, **BS=** Banda Sapan, **S=** Salhad, **D=** Dhamtour, **T=** Taqia, **B=** Bodla, **C=** Chamhad, **H.V=** Havelian village

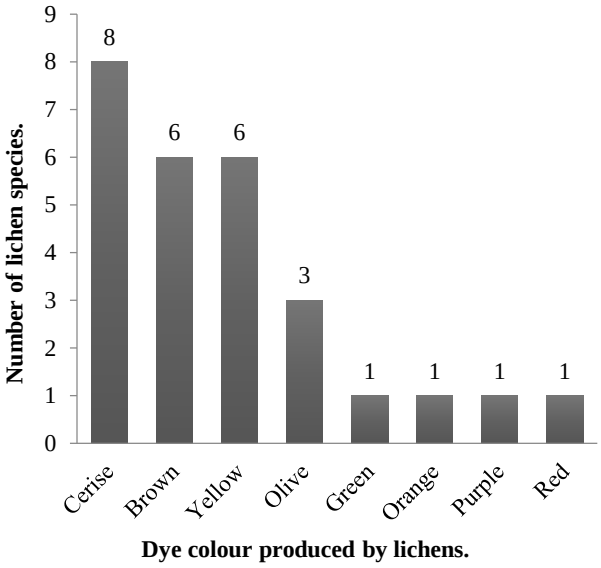

Fig. 4. Number of lichen species and their dye color.

Lichen dyes on wool and silk are fast to washing and exposure to sunlight. According to Perkins (1986), many writers believed that lichen dyes were not fast on any fiber but our results were in disagreement with Perkin and are in agreement with Shukla *et al*., (2015) and Brough (1988) who believe that lichen dyes are fast. Mostly mordant dyes need a mordant to improve the fastness of the dye against washing and exposure to sunlight. Most natural dyes are mordant dyes but lichen dyes are unique in this respect that it does not require any mordant. Many mordants especially associated with the heavy metal category are hazardous to environment and health, in this respect lichen dyes are safe to use and friendly to the environment.

Lichen substances form the 19 lichens used in this study have also been reported (Culberson, 1970; Culberson *et al.,* 1977; Hale, 1979; Leuckert, 1977; Thomson, 1979 & 1984). In this study Spot tests of *Parmelia saxatilis* (L.) Ach., *Parmelia sulcata*Taylor. and *Parmotrema reticulatum* (Taylor) M. Choisy. are P positive (showed orange color) i.e. an indication of salazinic acid. Orange shades are due to salazinic acid but in the present investigation, it produced brown dye as reported by Shukla *et al*., (2014), according to him due to increased fermentation time orange shades were changed to dark shades of brown dye color. Brough (1988) also justified the brown shades for these lichens for the same reason. Similarly, Parietin which is responsible for Yellow, pink and Magenta shades of *Xanthoria elegans* and *Xanthoria parietina* dyes is also reported by Fazioa *et al*., (2007). Gyrophoric acid (that produced royal purple color) was detected from genus *Umbilicaria* by spot tests results in our investigation as well as by Bolton (1960). *Parmotrema tinctorum* and two species of *Umbilicaria* which imparted purple dye color have lecanoric acid (p-depside) that hydrolyzed and produced orsellic acid color-producing substance orcein. These results validate the historical references (Table 4).

These precious and beautiful creatures of nature like trees should not be destroyed, it is strongly recommended that only large sizedd and naturally detached lichens should be used for dying; the whole thallus must not be harvested. These cannot be used in textile industries on the commercially large scale because of their small size as compared to higher plants and extremely slow growth rate, but certainly, they can serve on small-scale handloom industries to offer employment to local people.

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