



E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(5): 2611-2621
Received: 03-07-2017
Accepted: 04-08-2017

Hengameh Parizadeh
Department of Studies in
Microbiology, Manasagangotri,
University of Mysore, Mysore,
India

Rajkumar H Garampalli
Department of studies in botany,
Manasagangotri, University of
Mysore, Mysore, India

Physiological and chemical analysis for identification of some lichen extracts

Hengameh Parizadeh and Rajkumar H Garampalli

Abstract

Identification of lichen species is an interesting area that use morphological and chemical analysis. In the presented study, eight lichen species were collected and identified based on different procedures. Anatomically, they were examined for their growth type and thallus color, presence or absence of vegetative parts (Rhizines and cilia), and sexual reproductive parts (Their types of Apothecia and Perithecia, if any). Spot tests, TLC with different visualization method and micro-crystallography were chemical analysis applied to lichen fragment and their extract to help the identification of species. Finally, all studied species were identified as; *Heterodermia leucomelos* (L.) Poelt., *Cladonia subradiata* (Vainio) Sandst., *Parmotrema tinctorum* (Delise ex Nyl.) Hale, *Leptogium* sp. (Ach.) Gray., *Parmotrema crinitum* Choisy., *Herpothallon* sp. Tobler, *Parmotrema reticulatum* (Taylor) M. Choisy and *Ramalina celastri* (Sprengel) Krog & Swinscow. This is a rare organized report on identification of lichens based on both morphological and chemical investigations.

Keywords: Lichens, TLC, Crystallography, Identification, Microscopy

Introduction

Lichens are a classic example of symbiotic associations with multicomponent composition as their principal feature^[1]. They consist of three symbiotic partners: an ascomycetous fungus, a photosynthetic alga, and a basidiomycetous yeast^[43]. According to Hale (1974), lichens are basically no more difficult to study than any other group of cryptogams^[2]. Lichen species usually identifies using different methods, which together can reveal the species identity from identification keys available in the literatures^[3].

Morphology: Study of lichen morphology began with the work of Erik Acharius who is regarded as father of lichenology^[2]. Study on lichen structures began over a century ago when the light microscope became readily available. By 1860, Schwendener was able to present an accurate account of the internal structure of several fruticose lichens^[3, 4]. For lichen identification by morphology different parameters need to be noted:

Growth form: Morphology of lichen is usually determined by the organization of the fungal filaments^[5]. Their vegetative part is called thallus. Since the thallus is usually the most visually prominent part of the lichen, they are grouped by thallus type as: Crustose (crusts that are strongly attached to substrate), Squamulose (having scale-like lobes), Foliose (leafy structure), Umbilicate (attached at single point), Fruticose (shrubby) and Gelatinous (its mucousy-gelly type and its photobiont is cyanobacterium), Leprose (powdery). A macro-lichen is lichen that is either bush-like or leafy; all other lichens are termed micro-lichens. Here, "macro" and "micro" do not refer to size, but to the growth form^[6, 7] (Fig-1).

Despite the wide diversity of lichens growth forms, they all have similar internal morphology: Lichen's body is formed from filaments of the fungal partner. Filaments of its outer surface, where it comes in contact with the environment, are packed tightly together to form the "cortex". The dense cortex serves to keep out other organisms, and helps to reduce the intensity of light, which may damage the alga cells. The algal cells are distributed just below the cortex in a layer. Medulla (a loosely woven layer of fungal filaments) is below algal layer. In foliose lichens, there is a second cortex below the medulla, but in crustose and squamulose lichens, the medulla is in direct contact with the substrate^[8] (Fig-2).

Vegetative part: Lichens are characterized by variety of vegetative structures; rhizines, tomentum and cilia are also known among fungi. But soredia, isidia, hormocysts, lobules, cyphellae and pseudocyphellae and cephalodia are special to lichenized fungi. Pycnidia and conidia are non-symbiotic reproduction parts^[2].

Correspondence

Rajkumar H Garampalli
Department of studies in botany,
Manasagangotri, University of
Mysore, Mysore, India

These projections on surface of lichens can be seen by eye and stereomicroscope and are important elements for their identification.

Sexual reproduction: It is depend on sexual life cycle of Ascomycota and according to different projections on the surface of thallus for sexual reproduction, lichens can be identified. These non-vegetative bodies includes Mazaedia and Apothecia: Lecanorine, Lecideine, Biatorine, Zeorin and Perithecia: Hysterothecia and Pseudothecia [2, 4, 9, 10, 11].

Chemical characteristics

Spot test: These reactions are quick and inexpensive way to screen lichen substances and to help identification of lichen species [196]. Four tests are routinely used in lichenology; the C, K, KC (discovered by Nylander 1866) and the PD test introduced by Asahina 1934. Color test performs by applying specific reagent to small lichen fragment. Cortex and medulla should be tested separately. Change in color of spotted area by each solvent is assumed as a positive result, which is due to presence of certain phytochemicals. In reference literature of identification keys [9, 10, 11, 12, 13, 14, 15, 16], result of spot test is one of main characters to achieve identification [4].

Micro-crystallography: In 1936, Asahina introduced microcrystallization [17] as the first generally applicable method for tentative identification of lichens on a micro scale. The method has been used extensively for chemical studies in connection with taxonomic work. It is controlled re-crystallization of extracted lichen substances and observation of these crystals under stereo microscope and can distinguish substances more accurately [4].

TLC analysis: The first separation of lichen substances was reported by Stahl and Schorn (1961) [18]. It is one of the best micro-chemical methods for the systematic botanist due to its sensitivity, rapidity, general applicability and simplicity. The procedure is base on spotting lichen extracts on a TLC plates and running it into an appropriate solvent, then visualizing separated bands which are different classes of secondary metabolites. By calculating R_f (retention factor) of each separated band and compare it with reference literature, identification of lichen substances and subsequently the species, becomes easier [4].

Identification keys: Keys are used to identify unknown lichens. There are different literatures by various lichenologists with classified keys to identify lichen species. These references are mostly specified to each region. Some examples of these types of reference literatures are as follow; Keys to Lichens of North America by Brodo and Sharnoff (2001) [19], Macrolichens of the Pacific Northwest by B. McCune and Geiser (1997) [20], key to the genera of Australian macrolichens by McCarthy and Malcolm (2004) [21], Lichen flora of the United States by B. Fink (1935) [9], and most important lichen key books in India: A key to the Macrolichens of India and Nepal by Awasthi (1988) [12], "A Compendium of the Macrolichens from India, Nepal and Sri Lanka" By Awasthi (2007) [13] and "A key to michrolichens of India, Nepal and Sri Lanka" by Awasthi (1991) [16]. Some online keys like website of Botanischer Garten und Botanisches Museum, Germany were also used.

Materials and methods

Collection of lichen samples: Lichen species were collected

during March 2013 from Ooty, Tamil Nadu, India mainly at coordinates of 11.4064°N to 11.436160°N and 76.6032° E to 76.696408° E with average altitude of 2,240 meters. No crustose lichen from bark of tree was collected so most of lichens were collected as they were fallen from the tree since they were of foliose and fruticose types. Collected lichens were soaked in water and washed, then were dried and certain quantity of each species was powdered by grinder for the extraction process. Remaining parts were preserved in acid-free packets for further use.

1.2. Identification: Lichen samples were identified on the base of their morphology and chemical characteristics.

A) Lichens were screened for their morphology based on their growth type, presence or absence of vegetative parts (Rhizines and cilia), and sexual reproductive parts (Their types of Apothecia and Perithecia, if any) and color of thallus [22, 23].

B) Spot tests, TLC and micro-crystallography were chemical methods applied to lichen fragment and their extract to help the identification of species.

B-1) Spot test: for 'C' test, a drop of freshly prepared $\text{Ca}(\text{OCl})_2$ or NaOCl solution was applied on lichen fragment. Aromatic compounds with two free-OH- *meta* groups react to this solution by a red color on thallus of lichen [4]. For 'K' test, 10-25% aqueous solution of potassium hydroxide was used. Quinonoid lichen pigments react to this solution as dark red color [4]. For 'PD' test, 1-5% ethanolic solution of *p*-phenyl-enediamine was used, which reacts with aromatic aldehydes and gives yellow to red color on tested fragment. For KC test, first K solution applies followed by immediate use of C. Some depsides and depsidpnes can give red color after applying this procedure [4]. 'I' test also was used by a 0.5% potassium iodide solution which reacts with certain polysaccharides in lichen.

B-2) Micro-crystallography: A small piece of each lichen thallus was placed on a slide and lichen substances were extracted by drop wise adding crystallizing solution: GAW; Glycerol: ethanol: water (1:1:1) [4]. After light heating of slide, they were observed under microscope and captured images were compared with reference in literature like; Culberson, 1969 [26] and 1970 [27, 28], Huneck and Yoshimura (1996) [29] and Hale (1974) [2].

B-3) TLC-Visualization: Methanolic lichen extracts were spotted on heat-activated silica coated TLC Aluminum sheets (Silica gel 60 F₂₅₄, Merck, Germany) and were run by TEF solvent (Toluene: Ethyl acetate: Formic acid; 65.5 ml: 41.5 ml: 4 ml) [28]. Developed bands were visualized under UV chamber. The developed bands were also visualized by different chemical reagents in order to have accurate identification by their present phytochemicals and secondary compounds. Firstly, developed plates were sprayed with 10% Sulphuric acid solution, which is the most classic and useful method to identify lichen substances. Thereafter, to analyze phenolic nature of lichens secondary compounds, plates were sprayed with 1% ferric chloride solution in 50% methanol [29]. Moreover, a solution of Vanillin-sulfuric acid was used to identify steroidal lichen substances [30]. Another reagent used in this regard was *p*-Anisaldehyde solution with 97% sulfuric acid to reveal phenol, terpenes, sugars, and steroidal nature of available compounds [30]. Iodine granules were used to make iodine-vaporized plates, which cause the phenolic compounds

to turn brown in color.

Finally, all the gathered information were matched with reference keys in various literatures [12, 13, 16, 17]; Awasthi (1988) [12], (1991) [16], (2007) [13] had mentioned different result of spot test in lichen species of India. Huneck (1999) [32] and Elix (2014) [31] had described the color of each lichen substances under both UV conditions.

After identification, a part of each lichen species was deposited as dried herbarium specimen in National Lichen Herbarium, NBRI, Lucknow, India.

Results

Characterization of collected lichen species was done based on morphology and chemical characteristics. Identification of lichens from morphology was done by their structure, presence or absence of vegetative and reproductive parts, and color and texture of thallus. Cross section of each lichen sample also was prepared on slide and observed under Lawrence & Mayo-Stereo Microscope to study their structures. Inner section of lichen species was covered with hyphae and at the middle of them algal cells was sandwiched (plate-1).

Determination of chemical characteristics of lichen samples is another important method for their identification, which was achieved by identification of their secondary metabolites and their chemical nature using micro-crystallization (Plate-2), spot test (Table-1) and visualization of TLC (Fig-4 to 7) with different reagents. Culberson (1972) [33] had described colors of each prominent compound type after spraying with sulfuric acid solution. Likewise many references, which had been already mentioned, were used to analyze the results and based on them eight lichen species as:

***Cladonia subradiata* (Vain.) Sandst:** Morphologically, it was a corticolous, squamules lichen type with horizontal primary thallus and simple greenish white podetia (stalklike outgrowth of the thallus), subulate at base, upward micro-squamules and with granulose soredia (powdery propagules). Its thallus color was greenish brown [16, 17, 34]. Micro-crystallography showed presence of Fumarprotocetraric acid, which is common in this species. TLC visualization also confirmed presence of fumarprotocetraric acid.

***Heterodermia leucomelos* (L.) Poelt:** Collected as corticolous, subfruticose type (loosely attached, pendulous), had long black marginal rhizines (hair structure), corticated only at upper side [16, 17, 21, 35]. Crystallography showed presence of zeorin, atranorin, norstictic acid and salazinic acid. Presence of Norstictic acid, zeorin and atranorin were confirmed by TLC visualization. Spraying plates with anisaldehyde solution showed an intense bluish gray band in Rf: 5 of *H. leucomelos* (zeorin) confirming its alicyclic nature.

***Parmotrema crinitum* (Ach.) Choisy:** Found as corticolous, foliose lichen, lobes were in crenate form, with upper thallus of grey green, lower side black, marginal zone black and medulla in white color, simple to coralloid isidia (outgrowths vegetative part on thallus surface) [327]. Presence of stictic acid, constictic acid and atranorin was seen in micro-crystallography. Constictic acid, stictic acid, norstictic acid and atranorin were visible in TLC test. Visualization tests confirmed the phenolic characteristic of constictic acid at Rf: 1 and Norstictic acid at Rf:5. Constictic acid with brown and stictic acid with yellow band in *p. crinitum* illustrated presence of depsidones.

***Parmotrema reticulatum* (Taylor) Choisy:** A corticolous –

foliose lichen with ciliate lobes (ribbon-like lobe) [36]. Upper part looked dark gray, lower side centrally black, with white maculate (spots) and reticulately fissured structure (split branches), its soredia was in form of phylidia (small leaf-like outgrowths) [37] and rounded lobes. Rhizines were available at lower side [16, 17]. Presence of salazinic acid, protocetraric and protolichesterinic acids was confirmed by crystallography. Protolichesteric acid, protocetraric acid and atranorin were observed by TLC visualization.

Herpothallon Tobler: It was collected as terricolous, squamules type, with greenish-grey thallus color and hydrophobic texture [16, 17]. Crystallography showed the presence of psoromic acid, which is common in case of this genus. Presence of this secondary metabolite was also confirmed by TLC visualization.

***Parmotrema tinctorum* (Despr. ex Nyl.) Hale:** Seen as corticolous foliose type, eciliate lobes, upper side grey or darker color with emaculate, flattened to slightly granular filiform (tread-like) isidia [21]. Lecanoric and orsellinic acids were observed by crystallography [201, 204]. *P. tinctorum* showed orsellinic acid, lecanoric acid and atranorin.

***Leptogium* (Ach.) Gray:** Collected as corticolous foliose type, it is gelatinous when wet, its upper side is grey color [38], Thallus is fully wrinkled [16, 17]. An unknown compound was visible in crystallization and TLC visualization. Visualization showed that this species is rich in aromatic compounds.

***Ramalina celastri* (Sprengel) Krog & Swinscow:** Collected as corticolous fruticose lichen, shrubby to subpendulous and had lanceolate branches (lance head-like), with greenish gray to yellow thallus, obtained pseudocyphellae (tiny pores on the outer surface of lichen) [39, 40, 17]. Usnic acid was seen in crystallography and TLC test.

A part of each lichen species were deposited in National Lichen Herbarium, NBRI, Lucknow, India and their accession numbers are as follow: *Heterodermia leucomelos* (L.) Poelt. (Voucher No. 34755), *Cladonia subradiata* (Vainio) Sandst. (Voucher No. 34756), *Parmotrema tinctorum* (Delise ex Nyl.) Hale (Voucher No. 34757), *Leptogium* sp. (Ach.) Gray. (Voucher No. 34758), *Parmotrema crinitum* Choisy. (Voucher No. 34759), *Herpothallon* sp. Tobler (Voucher No. 34760), *Parmotrema reticulatum* (Taylor) M. Choisy (Voucher No. 34761) and *Ramalina celastri* (Sprengel) Krog & Swinscow (Voucher No. 34762).

Discussion

Collected lichens were identified as; *Cladonia subradiata*, *Heterodermia leucomelos*, *Parmotrema crinitum*, *Parmotrema reticulatum*, *Herpothallon* sp., *Leptogium* sp. and *Ramalina celastri*. Ferric chloride sprayed TLC plate, showed that presence of phenolic compounds in *P. crinitum* is more than rest of extracts. *R. celastri* followed by *P. reticulatum*, *P. crinitum* and *P. tinctorum* had showed steroid components in their substances by Vanillin-Sulfuric acid sprayed TLC plates. Among all sprayed plates with different visualizing solution, spraying plates with 10% sulphuric acid solution followed by baking at 120°C, found to be best visualizing way to illustrate all present lichen substances. Renner and Gerstner (1978b) described the TLC of lichen substances; also White and James (1985) gave much advice for TLC identification of lichen metabolites [41]. Arup *et al.* (1993) [42] used HPTLC to identify some lichen substances. Huneck (1999) [41] had gathered information regarding

identification of lichen substances by TLC, micro-crystallization and spot test. In this study, we had combined

all these chemical methods in order to have an accurate identification.

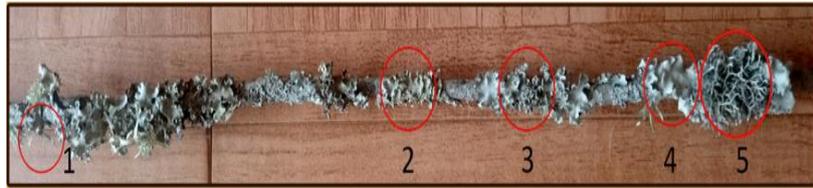


Fig 1: Two different lichen growth forms on a bark of tree: 1: *R. sinensis* (Fruticose), 2: *P. crinitum*, 3: *P. reticulatum*, 4: *P. tinctorum* (foliose), 5: *H. leucomelos* (Fruticose).

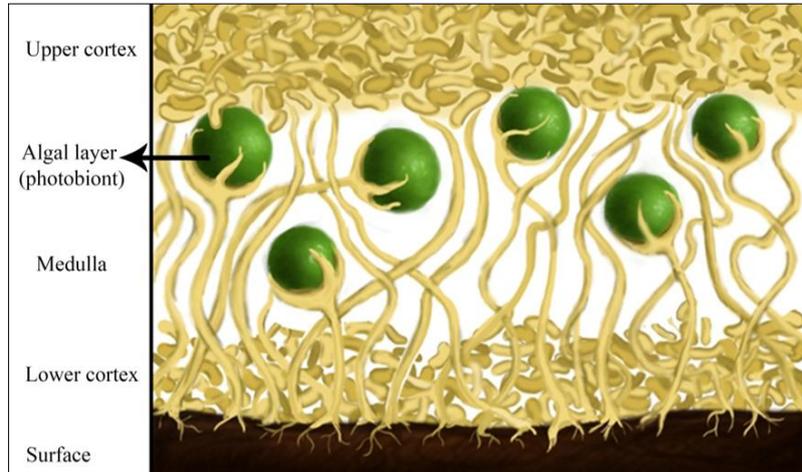


Fig 2: Internal structure of lichen in cross section view.



Fig-3: Lichen species used in this study. 1) *R.celastri*, 2) *C.subradiata*, 3) *Herpothallon* sp., 4) *P.crinatum*, 5) *P.tinctorum*, 6) *H. leucomelos*, 7) *P. reticulatum*, 8) *Leptogium* sp.

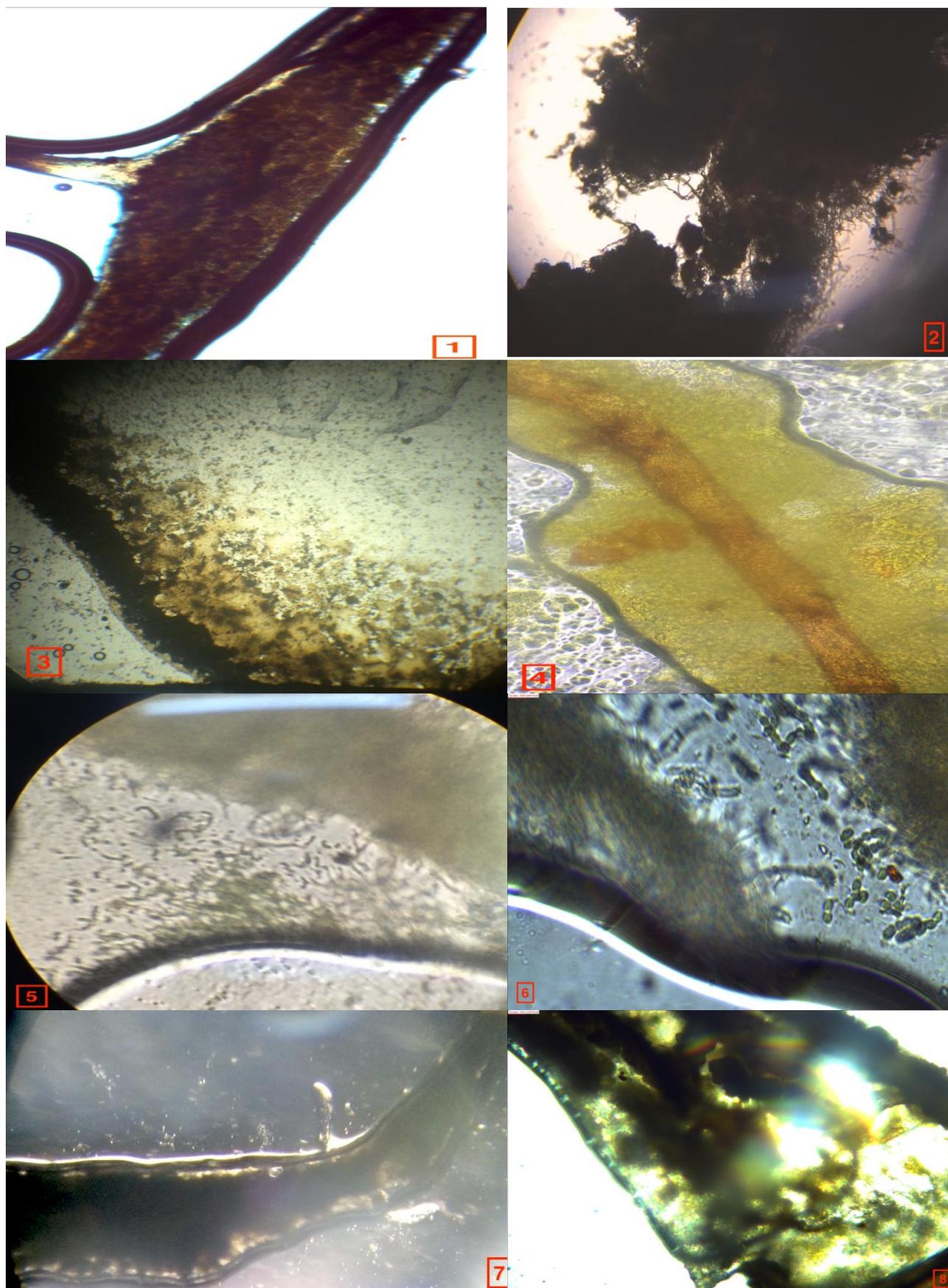


Plate 1: Cross section images of lichen species. 1) *H.leucomelos*, 2) *Herpothallon* sp., 3) *P.crinatum*, 4) *P. reticulatum*, 5) *R.celastri*, 6) *Leptogium* sp., 7) *C.subradiata*, 8) *P.tinctorum*.

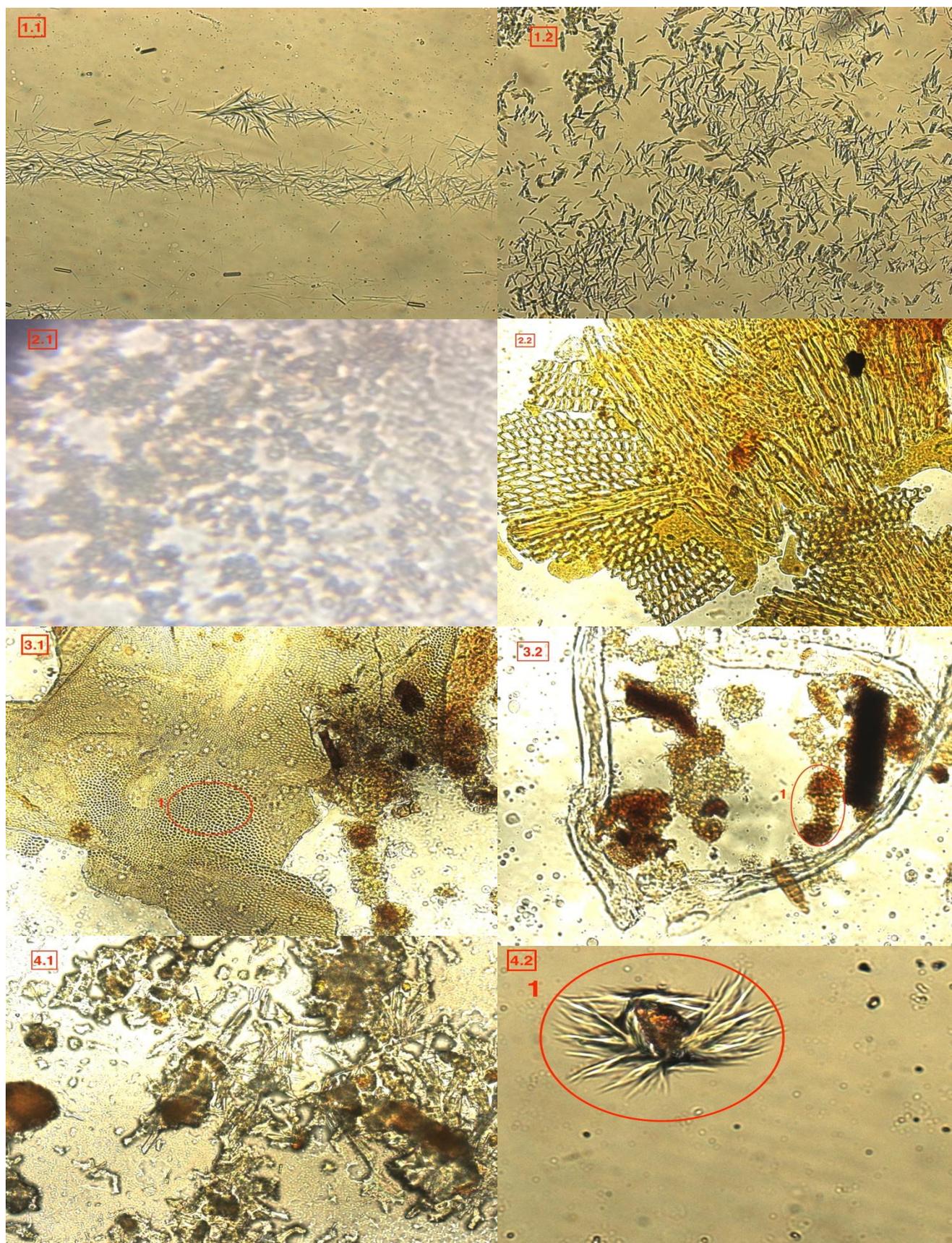
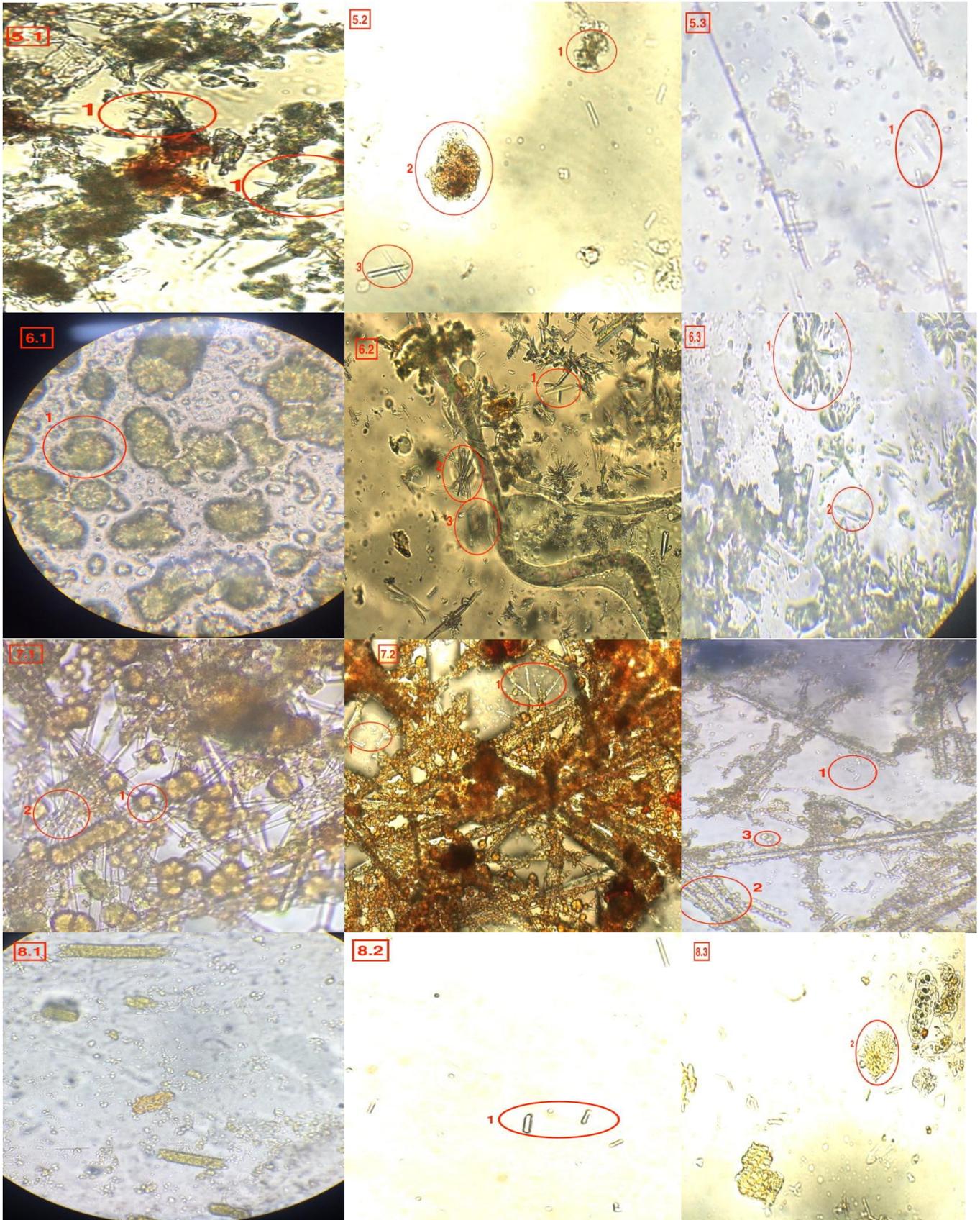


Plate 2: Micro-crystals of lichen substances: 1.1, 1.2) Fumarprotocetraric in *C.subradiata*, 2.1, 2.2) Unknown compound in *Leptogium* sp., 3.1) protolichesterinic, 3.2) Salazinic acid in *P. reticulatum*, 4.1) unknown, 4.2) Psoromic acid in *Herpothallon* sp.



5.1) Zeorin, 5.2) 1: salazinic acid, 2: Norstictic acid, 3: Atranorin in *H. leucomelos*, 6.1) Constictic acid, 6.2) 1: Atranorin, 2: Constictic acid, 3: Stictic acid in *P. crinitum*, 7.1) 1: Orsellinic acid, 2: Lecanoric acid, 7.2) Atranorin, 7.3) 1: Atranorin, 2: Lecanoric acid, 3: Orsellinic acid in *P. tinctorum*, 8.1) Usnic acid, 8.2 and 8.3) Unknown in *R. celastris*.

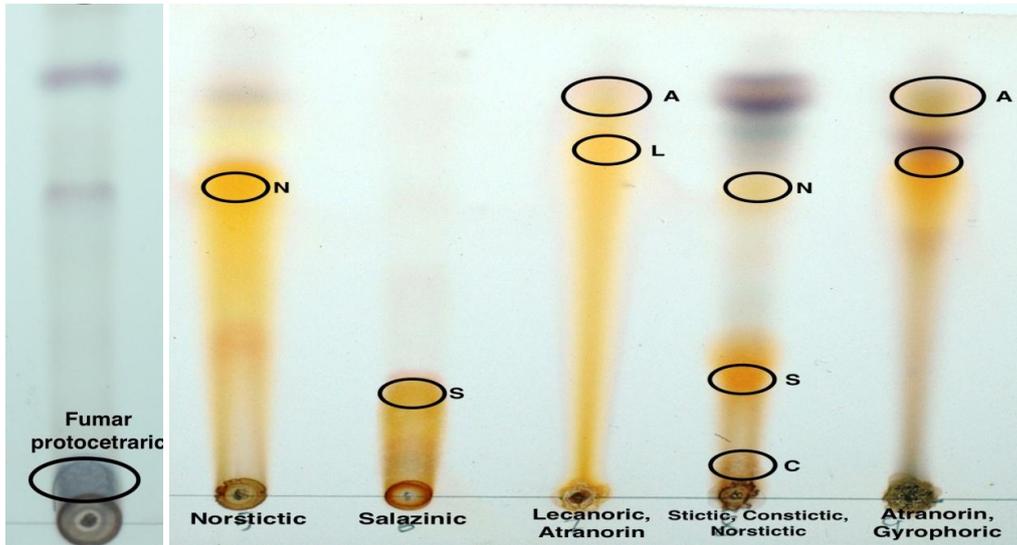


Fig 4: Known lichen compounds used as standard for identification of lichen substances.

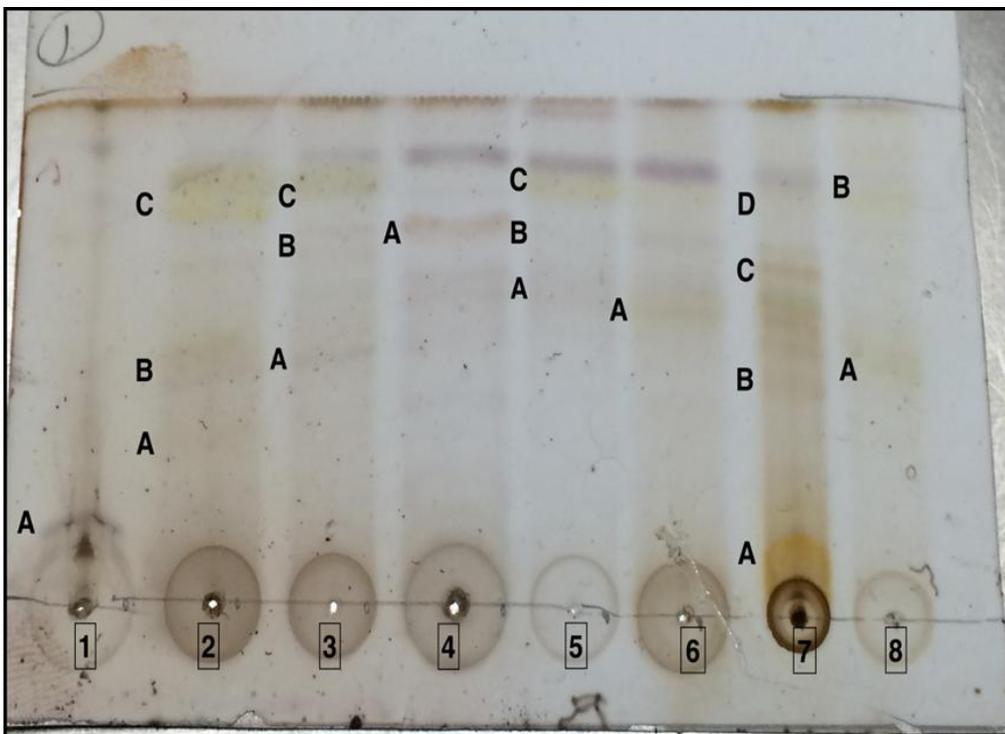
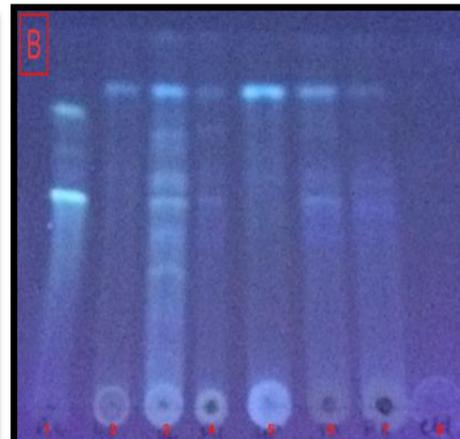
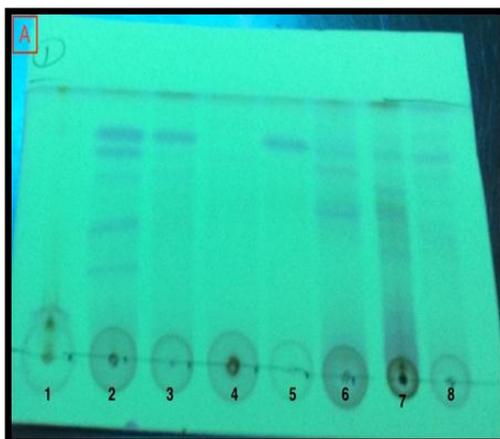


Fig 5: Visualized bands of lichens crude extracts sprayed by sulfuric acid: 1) *C. subradiata*: A. Fumarprotocetraric acid (R_f: 1), 2) *P. reticulatum*: A. Protolichestic acid (R_f: 2), B. Protocetraric acid (R_f: 3), C. Atranorin (R_f: 7), 3) *P. tinctorum*: A. Orsellinic acid (R_f: 3), B. Lecanoric acid (R_f: 5), C. Atranorin (R_f: 7), 4) *Leptogium*: A. Unknown compound (R_f: 5), 5) *H. leucomelos*: A. Norstictic acid (R_f: 4), B. Zeorin (R_f: 5), C. Atranorin (R_f: 7), 6) *Herpothallon*: A. Psoromic acid (R_f: 5), 7) *P. crinitum*: A. Constictic acid (R_f: 1), B. Stictic acid (R_f: 3), C. Norstictic acid (R_f: 4), D. Atranorin (R_f: 7), 8) *R. celsi*: A. unknown compound (R_f: 3), B) Usnic acid (R_f: 6).



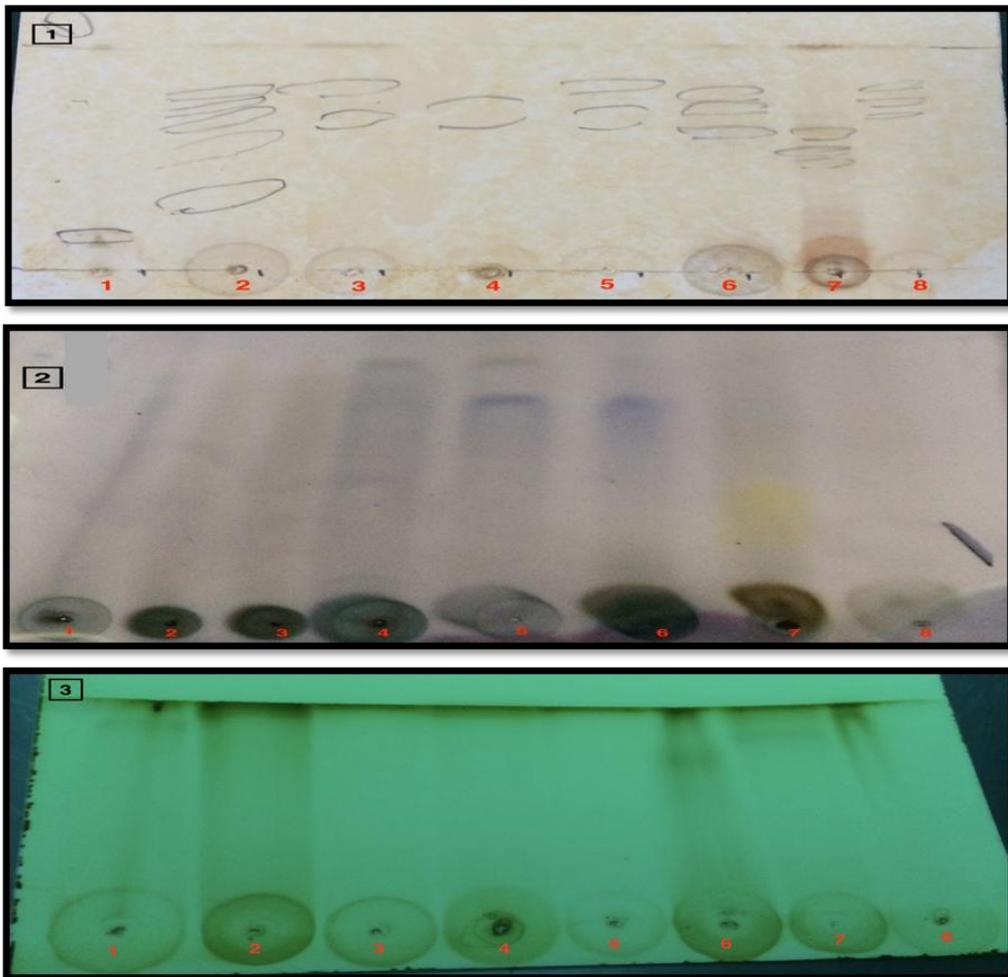


Fig 6: Chemically visualized plates. 1: Ferric chloride visualized plates after marking bands under UV chamber. 2. Anisaldehyde visualized plate. 3. Iodine vapourized plate. 1: 1) *C. subradiata*, 2) *P. reticulatum*, 3) *P. tinctorum*, 4) *Leptogium* sp., 5) *H. leucomelos*, 6) *Herpothallon* sp., 7) *P. crinitum*, 8) *R. celsatri*.



Fig 7: Vanillin-Sulfuric acid visualized plate:1) *P. tinctorum*, 2) *P. reticulatum*, 3) *P. crinitum*, 4) *R. celsatri*, 5) *Leptogium*, 6) *Herpothallon*, 7) *C. subradiata*, 8) *H. leucomelos*.

Table 1: Spot test on lichen species. All C⁺ were observed Red in color.

Lichen species	K	C	KC	I	Pd
<i>H. leucomelos</i>	+	-	-	-	+(Yellow)
<i>R. celsatri</i>	-	-	+	+	-
<i>Leptogium</i> sp.	+	+	+	-	-
<i>Herpothallon</i>	-	-	-	-	+
<i>P. tinctorum</i>	-	++	+	-	-
<i>P. crinitum</i>	+	-	-	+	+(Orange)
<i>P. reticulatum</i>	+	-	-	-	+(Red)
<i>C. subradiata</i>	+	+	-	-	+(Orange)

Table 2: Lichen compounds identified using TLC visualization and crystallography.

Lichens	Compounds
<i>H. leucomelos</i>	Salazinic acid, Norstictic acid, Zeorin, Atranorin
<i>R. celsatri</i>	Usnic acid
<i>Leptogium</i> sp.	-
<i>Herpothallon</i>	Psoromic acid
<i>P. tinctorum</i>	Orsellinic acid, Lecanoric acid, Atranorin
<i>P. crinitum</i>	Stictic acid, Norstictic acid, Constictic acid, Atranorin
<i>P. reticulatum</i>	Protolichestiric acid, Salazinic acid, Protocetraric acid, Atranorin
<i>C. subradiata</i>	Fumarprotocetraric acid

Table 3: Taxonomic classification of lichens that were used in this study.

Kingdom	Phylum	Class	Order	Family	Genus	Species
Fungi	Ascomycota	Lecanoromycete	Lecanorales	Physciaceae	<i>Heterodermia</i>	<i>leucomelos</i>
Fungi	Ascomycota	Lecanoromycete	Lecanorineae	Parmeliaceae	<i>Parmotrema</i>	<i>Reticulatum</i>
Fungi	Ascomycota	Lecanoromycete	Peltigerales	Collembataceae	<i>Leptogium</i>	-
Fungi	Ascomycota	Lecanoromycete	Lecanorineae	Ramalinaceae	<i>Ramalina</i>	<i>celsatri</i>
Fungi	Ascomycota	Arthoniomycete	Arthoniales	Arthoniaceae	<i>Herpothallon</i>	-
Fungi	Ascomycota	Lecanoromycete	Lecanorineae	Parmeliaceae	<i>Parmotrema</i>	<i>crinitum</i>
Fungi	Ascomycota	Lecanoromycete	Lecanorineae	Parmeliaceae	<i>Parmotrema</i>	<i>tinctorum</i>
Fungi	Ascomycota	Lecanoromycete	Lecanorales	Cladoniaceae	<i>Cladonia</i>	<i>subradiata</i>

Acknowledgment

We would like to acknowledge Dr. D.K. Upreti, chief scientist in lichenology lab of National Botanical Research Institute (NBRI), Lucknow India my research work in India for authenticating lichen samples used in this study and for all his kind suggestions in this study.

References

- Lobaova ES, Smirnov IA. Experimental Lichenology, *InTechOpen*, Advances in Applied Biotechnology. Prof. Marian Petre (Ed.), Russia, 2012.
- Hale ME. The biology of lichen, 2nd edition, Edward Arnold Publishers, 1974.
- <http://cber.bio.waikato.ac.nz/courses/226/Lichens/Lichens.html> Retrieved on: 19-04-2013
- Ahmadjian V, Hale ME. The lichen, *The Lichenologist* 6:2, Academic Press, INC., New York and London, 1974.
- Lichens and Bryophytes, Michigan State University, 10-25-99. Retrieved 10 October 2014.
- https://en.wikipedia.org/wiki/Lichen#cite_note-MSULB-26 Retrieved on: 19-04-2013
- "What is a lichen?, Australian National Botanical Garden". Retrieved 10-10-2014.
- <http://www.ucmp.berkeley.edu/fungi/lichens/lichenmm.html> Retrieved on: 19-04-2013
- Fink B. The lichen flora of the United States, University of Michigan Press, USA, 1935.
- Orange A, James PW, White FJ. Microchemical methods for the identification of lichens, British Lichen Society, London, UK, 2001.
- Thomas H. Nash III Lichen Biology, Cambridge University Press; 2 editions, 2008.
- Awasthi DD. A key to the macrolichens of India and Nepal. *J Hattori Bot Lab.* 1988; 65:207-302.
- Awasthi DD. A Compendium of the Macrolichens from India, Nepal and Sri Lanka. Bishen Singh Mahendra Pal Singh. India, 2007.
- Awasthi DD. A handbook of lichens, Bishen Singh Mahendra Pal Singh. India, 2000.
- Awasthi DD. Lichenology in Indian subcontinent, Bishen Singh Mahendra Pal Singh. India, 2000.
- Awasthi DD. A key to Microlichens of India. Nepal and Sri Lanka. *Bibliotheca lichenological*, 1991, 40.
- Rai H, Upreti DK. Terricolous Lichens in India: Morphotaxonomic Studies. Springer. India, 2014, 9.
- Stahl E, Schorm PJ. Thin layer chromatography of hydrophilic medicinal plant extracts. VIII. Coumarins, flavone derivatives, hydroxy acids, tannins, anthracene derivatives and lichens, *Hoppe Seylers Z Physiol Chem.* 1961; 20:325:263-74.
- Brodo IM, Sharnoff SD, Sharnoff S. Lichens of North America. Yale University Press USA, 2001.
- McCune B, Geiser L. Macrolichens of the Pacific Northwest, Oregon State University, USA, 1997.
- McCarthy PM, Malcolm W. key to the genera of Australian macrolichens, *Australian biological resources study*, Canberra, 2004.
- http://www.ces.iisc.ernet.in/biodiversity/sahyadri_ews/newsletter/issue16/identify.html Retrieved on: 23-09-2014
- Sudarshan PB, Sumesh ND, Subhash MD. *Sahyadri Shilapushpa*, sahyadri_ews/newsletter/issue3
- Culberson CF. Chemical and botanical guide to lichen products, University North Carolina Press. Chapel Hill. 1969, 141.
- Culberson CF. Supplement to Chemical and botanical guide to lichen products. *Bryologist*, 1970a; 73:177-377.
- Culberson CF. *Phytochemistry*, 1970b; 9:841.
- Huneck S, Yoshimura I. Identification of Lichen Substances. Springer-Verlag, Berlin, Heidelberg. New York. 1996, 238.
- Verma N. Studies on antioxidant activities of some lichen metabolites developed *in vitro*, University of Pune, India, 2011.
- http://lco.epfl.ch/files/content/sites/lco/files/load/TLC_Stains.pdf Retrieved on 15-02-2016
- Stains for Developing TLC Plates, McMaster University, http://www.chemistry.mcmaster.ca/adronov/resources/Stains_for_Developing_TLC_Plates.pdf
- Elix JA. A catalogue of standardized chromatographic data and biosynthetic relationships for lichen substances, Third Edition, Canberra, 2014.
- Huneck S, Yoshimura I. Identification of Lichen Substances, Springer-Verlag, Berlin, Heidelberg, New York, 1996.
- Culberson CF. Improved conditions and new data for the

identification of lichen products by a standardized thin-layer chromatographic method. *Journal of Chromatography*, 1972; 72:113-125.

34. <http://www.bgbm.org/sipman/keys/Ecuclad.htm>
35. Muktesh KMS. Lichen (macrolichen) flora of Kerala part of Western Ghats, KFRI Research Report 194, Kerala forest research Institute Peechi. Thrissur, India, 2000.
36. <https://en.wikipedia.org/wiki/Isidium> Retrived on 01-02-2016
37. http://www.lichens.lastdragon.org/faq/lichen_asexual_dispersal.html Retrived: 01-02-2016
38. Otálora M, Jørgensen P, Wedin M. A revised generic classification of the jelly lichens, Collemaaceae, Fungal diversity. 2014; 64:275-293.
39. <https://en.wikipedia.org/wiki/Pseudocyphella> Retrived on 01-02-2016.
40. Consortium of North American lichen herbaria: <http://lichenportal.org/portal/taxa/index.php?taxon=55418>, Retrived on 01-02-2016
41. Huneck S. The significance of lichens and their metabolites. *Naturwissenschaften*, 1999; 86:559-570.
42. Arup U, Ekman S, Lindblom L *et al.* High performance thin layer chromatography, an improved technique for screening lichen substances. *Lichenologist*. 1993; 25(1):61-71.
43. Toby S, Veera T, Philipp R, Dan V *et al.* Basidiomycete yeasts in the cortex of ascomycete macrolichens. *Science* JUL. 2016, 488-492.