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Research Article

A STUDY ON EPIPHYTIC LICHENS FROM *PRUNUS PERSICA*

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ABSTRACT

Objective: The objective of this study is to explore and identify the epiphytic lichens on the tree bark of *Prunus persica* from Kodaikanal area and analyze its phytochemical properties.

Methods: Samples were collected from Kodaikanal area, identified by morphological and chemical constituents. Macromolecules present were quantified by DNS method, Lowry's method, and lipid tests. The secondary metabolites present were analyzed by standard phytochemical tests and thin-layer chromatography.

Results and Conclusion: It was interesting to observe that species belonging to the lichen genera - *Parmotrema*, *Ramalina,* and *Usnea* dominated the area. The samples have been identified and deposited in LWG herbarium, NBRI, Lucknow. As lichens form an ideal model to study the humongous secondary metabolites present in it, a preliminary investigation was performed to understand the nutritive value as well as phytochemicals present in the lichens. The results indicate that these organisms can be of potential medicinal value with *Ramalina* and *Usnea* species contributing good amount of macronutrients present in them, while qualitative analysis of phytochemicals reports all the lichens for enormous metabolites.

Keywords: Epiphytic lichens, Kodaikanal, Thin-layer chromatography, Phytochemistry, *Prunus persica*.

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INTRODUCTION

As a worldwide spread consortium of self-supporting associations, lichens represent a symbiotic association between the photobiont (algae) and the mycobiont (fungi). Lichens grow anywhere and on anything covering approximately 8% of Earth's surface [1]. These lichens are slow-growing organisms that occupy harsh environments of life. This property of lichen marks it as a pollution indicating organisms and the best biomonitors of air quality [2]. Lichens carry thousands of secondary chemicals in them, and over the past two decades, there has been a growing interest in lichens as a source of novel and pharmacologically active biomolecules [3]. The use of lichens as folk medicine has been reported across the world since centuries, particularly in temperate and arctic regions due to its nutritive value and impressive medicinal properties [4].

Moderate temperature, sufficient sunlight, and moisture are the necessary conditions for growth of lichens, making it adaptable to survive varying climates and altitudes around the world. These species are found on rocks, soil, trees, and land surfaces. Epiphytic lichens are the lichens which grow on the tree surfaces, more often on healthy trees as well as in stressed and unhealthy trees. The physical parameters of the tree are very critical for the type of lichen growing on it; smoother surface of the bark allows a crustose form of lichens to grow while uneven surfaces facilitate attachment of foliose and fruticose type of lichens.

Before the 19th century, the lichens were grouped based only on the morphological parameters. With the advent of advanced microscopic techniques like electron microscope, it became possible for minute observation of the morphological structures of the species that led to a proper taxonomical identification. However, the important feature toward identifying lichens is based on its chemistry, which is widely used as a taxonomic tool for over a century [5].

While Lichen diversity shares 2.4% of global land surface, India shares the richness of lichen diversity contributing to nearly 15% among the species of lichens recorded by lichenologists worldwide [6,7]. However, there are many more untouched regions in India, especially mountains and forest canopies among the Eastern and Western Ghats that needs to be explored. Kodaikanal, which is located in the Palani hills, have a diversity of lichens with medicinal and ecosystem values that can be noticed throughout the area. In Tamil Nadu, a total of about 555 lichen species under 128 genera have been reported [8]. However, a majority of lichens reported in the study have not been included in the checklist which is very well indicative that the lichens of Kodaikanal have not been explored completely for its biodiversity.

To understand the diversity of lichens present, this has been a preliminary work in randomly collecting a few of the epiphytic lichens from the tree *Prunus persica* at Kodaikanal area which has been deposited in LWG herbarium, NBRI, Lucknow.

MATERIALS AND METHODS

Collection of the study material

All the lichen samples chosen for the study were collected manually from the peach tree, *P. persica.*

Identification of lichen species

The lichens collected were identified at NBRI, Lucknow, with the help of Dr. Sanjeeva Nayaka, Principal Scientist, Lichenology laboratory. The lichens were identified by studying the morphological, microscopical, and chemical characteristics [9].

Observation and categorization

The general characteristics of the fruiting bodies were observed under the compound microscope at a magnification of ×40. The color of the thallus, texture, presence of isidia, soredia, pruina, and pycnidia on the upper surface were observed. The presence of pores and rhizines as well as the color on the lower surface was also noted. Characteristic structures such as apothecia and perithecia were looked on the thallus. The presence of ascospores was observed by taking a thin hand section

of the thallus or the ascocarp. Further, the lichens were grouped based on the growth form, crustose, squamulose, foliose, and fruticose type.

Chemistry of lichens

To understand the nutritive value of lichens, the primary metabolites were analyzed. Reducing sugars present were estimated by DNS method [10], protein by Lowry's method [11], and lipids using cholesterol kit supplied by M/S Biosystems.

Phytochemical analyses were carried out for the acetone extracts of the lichen samples following standard protocols [12,13]. The preliminary identification of the chemical compounds present in lichens were analyzed by spot tests - K test, C test, KC test, and PD test [14,15]. Thin-layer chromatography (TLC) was performed to further confirm the lichen chemistry using acetone extracts of the lichens [9,16].

RESULTS

The samples sharing different niches of the tree, *P. persica,* was collected manually from Kodaikanal, shade dried, and segregated for identification. Macroscopic and microscopic features of the lichens were observed for the initial characterization at NBRI, Lucknow (Fig. 1 and Table 1). Based on the attachment of the thallus to the substratum, the lichens are categorized into different types: Crustose lichens - which attach tightly against the substrate, squamulose lichens - which are tightly clustered and slightly flattened pebble-like units, foliose lichens - leaf like which are not tightly bound, and fruticose lichens - which are free-standing branching tubes. The lichen species identified in the present study are epiphytic lichens which represent all the lichen categories. *Ramalina conduplicans* (Fig. 1a), *Ramalina subpusilla* (Fig. 1b), *Ramalina sinensis* (Fig. 1k), *Usnea cineraria* (Fig. 1h), and *Usnea* sp., (Fig. 1i) are fruticose type of lichens. *Parmotrema reticulatum* (Fig. 1c), *Parmotrema cristiferum* (Fig. 1d), and *Canoparmelia texana* (Fig. 1o) belong to foliose type of lichens. *Lecanora caesiorubella* (Fig. 1e), *Lecanora cenisia* (Fig. 1f), and *Lecanora helva* (Fig. 1n) represent crustose forms of lichens, while *Chrysothrix chlorina* (Fig. 1l), *Hafellia curatellae* (Fig. 1j), and *Phaeographis intricans* (Fig. 1m) belong to the squamulose type. It was observed that foliose and fruticose lichens were abundantly present all over the tree trunk and branches of *P. persica* while there were only few squamulose lichens. All the respective voucher specimens that were identified have been deposited with its respective accession numbers in LWG virtual herbarium, Lucknow (Table 2).

Considerable amount of the macromolecules were present in lichens (Table 3). *R. conduplicans* is reported to have the highest content of reducing sugar (900 µg/g of sample), protein (930 µg/g of sample), and lipid (26.07 µg/g of sample) (Table 3). *Parmotrema* species also showed a considerable amount of macromolecules with similar content of glucose, protein, and lipid. *R. subpusilla* showed a higher amount of protein content, while *U. cineraria* revealed higher lipid content.

Table 1: Categorization of lichens based on growth form, morphology, and microscopy

Sl. No.	Code No.	Growth form	Morphological and microscopical characteristics
1	KP001	Fruticose	Thallus shrubby, corticolous, forked branches ending with nodular structures, surface flat, pseudophyllae abundant on lower surface of the branches. Has dorsiventral main lobes with thin secondary branches that are tapering toward the apices. Grayish-green in color. Apothecia present. Ascospores are long and wide in size
2	KP001A	Fruticose	Thallus fragile, lobe cavity is open and tube-like. Grayish-green in color, smooth, and striately ridged. Apothecia present. 8 bicelled ascospores seen
3	KP002	Foliose	Thallus loosely held to the substratum which is 5–16 cm in diameter. The lobes were elongate, irregular, and plane with round margin. Cilia at the margin of the thallus observed. Upper surface was pale gray, dull, and smooth. Soredia found in the margins. Lower surface is black with rhizines
4	KP003	Foliose	Thallus loosely held to the substratum, 3–15 cm in diameter. Lobes were subirregular, elongate. Had round apices with cilia around it. The upper surface is gray, smooth, dull. Soredia is granular. Lower surface is black and brown with rhizines
5	KP004	Crustose	Thallus is areolate and dispersed throughout the substratum. Surface is whitish gray, smooth, epruinose with no distinct margin. Apothecia is sessile. Amphithecium and parathecium present with crystal-like structures. Simple ascospores observed. Pyncidia not seen
6	KP005	Crustose	Thallus is areolate and dispersed throughout the substratum. Surface is yellowish-gray with a distinct margin. Apothecia is sessile. Amphithecium and parathecium present with large crystalline structures. Ellipsoid ascospores observed. Pyncidia not seen
7	KP006	Fruticose	Thallus shrubby, corticolous, forked branches ending with nodular structures, surface flat, pseudophyllae abundant on lower surface of the branches. Has dorsiventral main lobes with thin secondary branches that are tapering toward the apices. Grayish-green in color. Apothecia present. Ascospores are long and wide in size
8	KP007	Fruticose	Thallus is erect and branching. Surface is dark brown with black-colored basal disc. The lateral branches are divergent that are dense toward apical region and perpendicular, simple to branched position. The surface is slightly shiny and densely papillated. Cilia found throughout the margins
9	KP008	Fruticose	Thallus is tufted. Surface is grayish-green blackened base that is attached to substratum. Secondary branches are crowded and densely covered with minute ciliated structures
10	KP009	Fruticose	Thallus is corticolous, erect, broad, flat, and reticulate. Upper surface is green and lower surface is whitish. Apothecia is numerous with ascospores
11	KP010	Squamulose	Thallus is granular and cracked in between, areolate. Surface of the entire thallus is powdery and yellowish
12	KP010A	Squamulose	Thallus is corticolous. Surface is grayish-white to pale yellow-brown with black disc-shaped structures. Apothecia is covex in shape with thick black margin
13	KP010B	Squamulose	Thallus is pale, thin, smooth, and whitish. Presence of fine white pruinose Apothecia are slit-like lirellae and immersed within the thallus. Ascocarps are densely stellate and the branches adjoining are separated by very narrow non- thalloid tissue
14	KP010C	Crustose	Thallus is continuous with blackish areoles. Surface is yellowish white, smooth, epruinose present with indistinct margin. Soredia is granular. Apothecia is seen subimmersed along the thallus
15	KP011	Foliose	Thallus has irregular and elongated lobes. Upper surface is pale greenish-gray and lower surface is brownish-black

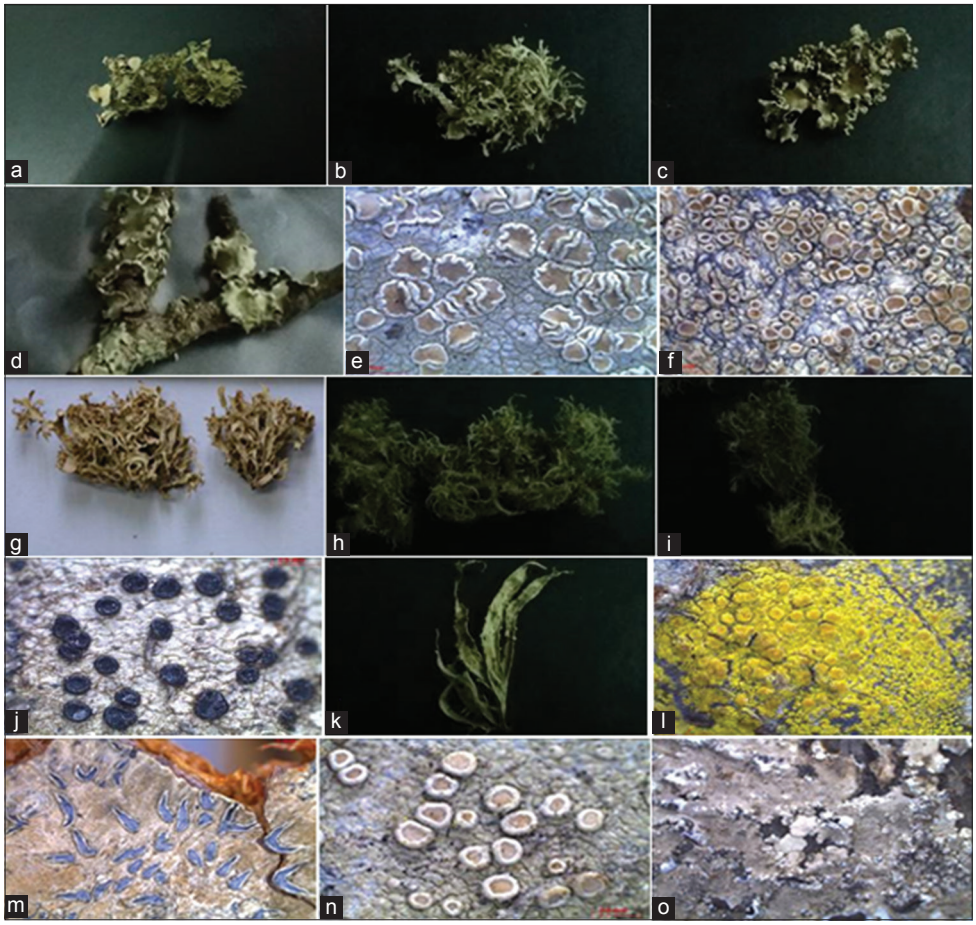

Figure 1: Identified lichens. (a) *Ramalina conduplicans* Vain. (b) *Ramalina subpusilla* (Nyl.) Krog and Swinsc. (c) *Parmotrema reticulatum* (Taylor) Choisy. (d) *Parmotrema cristiferum* (Taylor) Hale, (e) *Lecanora caesiorubella* Ach. (f) *Lecanora cenisia* Ach. (g) *R. conduplicans* Vain. (h) *Usnea cineraria* Mot. (i) *Usnea* sp. (j) *Hafellia curatellae* (Malme) Marbach. (k) *Ramalina sinensis* Jatta, (l) *Chrysothrix chlorina* (Ach.) J.R. Laundon. (m) *Phaeographis intricans* (Nyl.) Vain. (n) *Lecanora helva* Stizenb. (o) *Canoparmelia texana* (Tuck.) Elix and Hale

Table 2: List of lichens identified and deposited at LWG herbarium, NBRI, Lucknow

Code No.	Species	Accession no. of LWG
1 - KP001	*Ramalina conduplicans* Vain.	35125
1 - KP001A	*Ramalina subpusilla* (Nyl.) Krog and Swinsc.	35126
2 - KP002	*Parmotrema reticulatum* (Taylor) Choisy	35136
3 - KP003	*Parmotrema cristiferum* (Taylor) Hale	35128
4 - KP004	*Lecanora caesiorubella* Ach.	35130
5 - KP005	*Lecanora cenisia* Ach.	35127
6 - KP006	*Ramalina conduplicans* Vain.	35131
7 - KP007	*Usnea cineraria* Mot.	35133
8 - KP008	*Usnea* sp.	35132
9 - KP009	*Ramalina sinensis* Jatta	35129
10 - KP010	*Chrysothrix chlorina* (Ach.) J.R. Laundon, *Hafellia curatellae* (Malme) Marbach, Phaeographis intricans (Nyl.) Vain., *Lecanora helva* Stizenb.	35135
11 - KP011	*Canoparmelia texana* (Tuck.) Elix and Hale	35134

Preliminary analysis to understand the category of phytochemicals in lichens portrayed the presence of phenols, tannins, flavonoids, alkaloids, steroids, terpenes, glycosides, and saponins (Table 4). Apart from alkaloids and saponins, all the metabolites were present in *R. subpusilla,* while in *R. conduplicans*, steroids and saponins were not observed. Both the *Parmotrema* species indicated the presence of all the metabolites, while only saponins were absent. *Lecanora* species did not show the presence of terpenoids, glycosides, and saponins. In *Usnea* species, alkaloids and terpenoids were not found while other metabolites were prominent for the test.

Depsides and depsidones were the major compounds detected in most of the lichen tested followed by the presence of atranorin in spot analysis (Table 5). Presence of usnic acid was seen in all the species of *Ramalina*, *Parmotema,* and *Usnea* lichens. *Lecanora cenisia* indicated the presence of didymic acid and pannaric acid. Pulvinic acid derivatives were the result of spot test in *C. chlorina*.

TLC analysis of the acetone extracts of the lichens gave a picture about the presence of several compounds in lichens with reference to standards (Fig. 2 and Table 6). It was observed that majority of the lichens have atranorin and salazinic acids. *R. conduplicans* was identical with the presence of sekikaic acid. *Usnea* sp. reported the presence of stictic and norstictic acid along its characteristic usnic acid. It was interesting to note that no distinctive chromatogram developed for *R. sinensis* and *H. curatellae*. *L. cinesia* had only atranorin while *L. caesiorubella* also reported protocetraric acid. Divaricatic acid complex along with atranorin was found in *C. texana*.

DISCUSSION

Epiphytic lichens are known as environmental indicators. The lichen categories, particularly, the foliose and fruticose are

characteristic forms of epiphytic lichens. In this study, important lichen species were identified such as the *Ramalina*, *Parmotrema,* and *Usnea* species. It was noted that the lichen hyphae penetrated the bark of the tree, *P. persica* causing damaging effect on the tree. Hale [17] also reported that the lichen rhizines penetrate extensively throughout the cortex, bast, and the cambium of living wood. Lichens coexistence with plants is reported for a long time, and hence, it is understood that plants have developed various defense mechanism against the penetration of lichens. Hence, apart from its deleterious effects, healthy trees with numerous epiphytic lichens can also be seen [18].

Table 3: Quantity of macromolecules present in lichen samples

S. No.	Lichen code no	Reducing sugar µg/g of the sample	Proteins µg/g of the sample	Lipid µg/g of sample
1	KP001A	690	850	15.14
2	KP002	720	150	22.10
3	KP003	720	150	22.10
4	KP004	240	280	13.11
5	KP005	210	165	16.0
6	KP006	900	930	26.07
7	KP007	300	260	18.76

Traditional preparative methods such as boiling and steaming are used to make the macrolichens edible. This removes the lichen secondary compounds and hydrolyzes the lichen polysaccharides to yield glucose and other digestible simple sugars [19]. Macromolecular analysis of the lichens in this report specifies the presence of considerable quantity of carbohydrates, proteins, and lipids in the samples analyzed. *R. conduplicans* is reported to have a high content of the macromolecules (Table 3). This is evident with the fact that the lichen *R. conduplicans* is an edible lichen that has been used for cooking

Table 4: Secondary metabolites found in lichen samples

S. No.	Code No.	Phenols	Tannins	Flavonoids	Alkaloids	Steroids	Terpenoids	Glycosides	Saponins
01	KP001A	+	+	+	−	+	+	+	−
02	KP002	+	+	+	+	+	+	+	−
03	KP003	+	+	+	+	+	+	+	−
04	KP004	+	+	+	+	+	−	−	−
05	KP005	+	+	+	+	+	−	−	−
06	KP006	+	+	+	+	−	+	+	−
07	KP007	+	+	+	−	+	−	+	+

+Indicates presence of the metabolite, −Indicates absence of the metabolite

Table 5: Lichen phytochemicals - spot analysis

S. No.	Code No.	Color tests				Other test chloramine T	Probable compound
		C test	K test	KC test	PD test		
1	KP001A	-	-	-	-	+	Depsides, usnic acid
2	KP002	-	+	-	-	+	Depsides and depsidones, usnic acid
3	KP003	-	+	-	-	+	Depsides and depsidones
4	KP004	-	+	-	-	-	Depsides and depsidones
5	KP005	+	-	-	+	-	Didymic acid, pannaric acid
6	KP006	+	-	-	+	+	Depsides - atranorin, norstictic acid, salazinic acid, lecanoric acid
7	KP007	-	+	-	+	+	Usnic acid, depsidones
8	KP008	-	-	-	+	+	Usnic acid
9	KP009	-	-	+	-	+	Depsides - atranorin, usnic acid
10	KP010A	-	-	-	-	-	Pulvinic acid derivatives
11	KP010B	TLC analysis only					
12	KP010C						
13	KP010D	-	+	-	+	+	Atranorin, methylperlatolic acid
14	KP011	+	-	-	+	-	Atranorin, divaricatic acid

TLC: Thin-layer chromatography

Table 6: Chemistry of lichens based on TLC

S. No.	Lichen code	Species	Chemicals as per TLC plate
1	C	Control - *Parmelinella wallichiana* (Taylor) Elix and Hale	Salazinic acid, atranorin
2	KP001A	*Ramalina subpusilla* (Nyl.) Krog and Swinsc.	Salazinic acid
3	KP002	*Parmotrema reticulatum* (Taylor) Choisy	Salazinic acid, Atranorin
4	KP003	*Parmotrema cristiferum* (Taylor) Hale	Salazinic acid, Atranorin
5	KP004	*Lecanora caesiorubella* Ach.	Protocetraric acid, atranorin, triterpene
6	KP005	*Lecanora cenisia* Ach.	Atranorin
7	KP006	*Ramalina conduplicans* Vain.	Sekikaic acid
8	KP007	*Usnea cineraria* Mot.	Salazinic acid, atranorin
9	KP008	*Usnea* sp.	Norstictic acid, stictic acid, salazinic acid, usnic acid
10	KP009	*Ramalina sinensis* Jatta	Not visible
11	KP010A	*Chrysothrix chlorina* (Ach.) J.R. Laundon	Vulpinic acid
12	KP010B	*Hafellia curatellae* (Malme) Marbach	Not visible
13	KP010C	*Phaeographis intricans* (Nyl.) Vain.	Norstictic acid, stictic acid, constictic acid
14	KP010D	*Lecanora helva* Stizenb.	Atranorin
15	KP011	*Canoparmelia texana* (Tuck.) Elix and Hale	Divaricatic acid complex, atranorin

TLC: Thin-layer chromatography

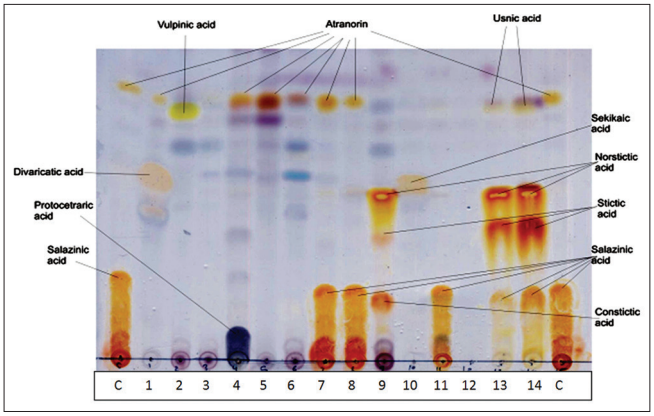

Figure 2: Compounds present in lichens based on thin-layer chromatography. (c) Control sample (*Parmelinella wallichiana*). (1) *Ramalina subpusilla* (Nyl.) Krog&Swinsc. (2) *Parmotrema reticulatum* (Taylor) Choisy. (3) *Parmotrema cristiferum* (Taylor) Hale. (4) *Lecanora caesiorubella* Ach. (5) *Lecanora cenisia* Ach. (6) *Ramalina conduplicans* Vain. (7) *Usnea cineraria* Mot. (8) *Usnea* sp. (9) *Ramalina sinensis* Jatta. (10) *Chrysothrix chlorina* (Ach.) J.R. Laundon. (11) *Hafellia curatellae* (Malme) Marbach. (12) *Phaeographis intricans* (Nyl.) Vain. (13) *Lecanora helva* Stizenb. (14) *Canoparmelia texana* (Tuck.) Elix and Hale

in Central and South Eastern Asian countries [20]. High protein and reducing sugar content in *R. conduplicans* has also been reported [21]. In this study, both the *Parmotrema* species have portrayed good amount of macromolecules in them. Foliose parmeloid lichens are the commonly used variety as spices [22]. However, the best species reported among lichens is known to be *Parmotrema perlatum*, known as "black stone flower," and has been used as a common spice in India. This lichen is also reported to have important therapeutic values [23]. The tribal community is using several lichens as foods as well as they are sold in markets of Himachal Pradesh [24]. Although lichens are used in food by many different cultures across the world, there are concerns about its edibility. This is because of the indigestible nature of the lichen polysaccharides by humans. Furthermore, certain secondary metabolites such as vulpinic acid and usnic acid are reported to be toxic at higher concentration [25].

The strong presence of phenols, terpenes, tannins, and flavonoids in all the lichen samples tested supports lichen's antioxidant activity and conveys the embodiment of several important metabolites of pharmaceutical interest. Several reports have analyzed similar results, thereby grounding lichen compounds as an alternative drug source for treating diseases caused due to free radicals [26,27].

Chemotaxonomic studies of several research works have indicated that lichens have the most unique secondary metabolites that represent chemical classes of depsides, depsidones, and dibenzofurans [28,29]. This is concomitant with the present study of the lichen species based on TLC and spot analysis. The lichen compounds analyzed have reported to have various medicinal values. Usnic acid, salazinic acid, and atranorin are the major compounds reported in this study which have several important biological roles. These compounds have strong antimicrobial property [30-32], anticancerous properties [33], antiherbivorous [34],; antioxidants [35,36], and immunomodulatory [37]. Almost all the metabolites identified in the TLC results of this study have also been reported as sources of commercial dyes [38].

CONCLUSION

This study provides evidence for a diversified category of epiphytic lichens from the same tree species, *P. persica*. The investigations on the lichen species with respect to its nutritive value and phytochemicals clearly merit their significant role for edibility as well as a stand for identifying potential molecules that can be of importance in the Pharma Industry.

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