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Preliminary phytochemical screening of different solvent extracts of lichens from Kodagu district, Karnataka

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Abstract
Lichens are fungi that live in intimate symbiotic association with green algae or cyanobacteria. Nine lichen species were collected and identified as *Flavoparmelia caperata* (L.) Ach., *Roccella montagnei* Bèl. Emend. Awas., *Teloschistes flavicans* (Swartz) Norm., *Physcia aipolia* (Ehrh. ex Humb.) Furnr., *Parmotrema austrosinensis* (Zahlbr.) Hale, *P. grayanum* (Hue) Hale, *P. tinctorum* Nyl., *P. reticulatum* (Taylor) Choisy and *Usnea subflorida* (Zahlbr.) Motyka. From which 45 extracts were prepared using Petroleum ether, Chloroform, Ethyl acetate, Acetone and Methanol solvents. Preliminary phytochemical screening for the presence of Tannins, Alkaloids, Saponins, Glycosides, Flavonoids, Proteins, Triterpenes, Carbohydrates and Steroids was carried out on aforesaid extracts. Important phytochemicals like Tannins, Proteins, Carbohydrates and Steroids were present in most of the lichen extracts tested. Petroleum ether and Chloroform extracts did not show any phytochemicals. Saponins were present only in *U. subflorida.* Methanolic extract was more active and showed almost all tested phytochemicals in all the lichen samples.

Keywords: lichens, phytochemicals, tannins, alkaloids, proteins, carbohydrates.

1. Introduction
A lichen is a stable, ecologically obligate, self supporting mutualism between an exhabitant fungus (the mycobiont) and one or more inhabitant, extracellularly located unicellular or filamentous photoautotrophic partners (the photobiont: alga or cyanobacterium) [1]. Lichens are able to survive in extreme environmental conditions; they can adapt to extreme temperatures, drought, inundation, salinity, high concentrations of air pollutants, and nutrient-poor, highly nitrified environments [2], and they are the first colonizers of terrestrial habitats.
Lichens synthesize a great variety of metabolites, many of which are unique. Lichen substances can arbitrarily be divided into two groups: primary and secondary compounds. Primary lichen substances have structural functions and roles in cellular metabolism. These are mainly the same substances as in other plants. The roles of secondary lichen substances ultimately remain unclear [3]. Most of the lichen secondary metabolites are derived from the polyketide biosynthetic pathway (also called acetylpolymalonyl pathway), with a few originating from the shikimate and mevalonate biosynthetic pathways. These chemically diverse (aliphatic and aromatic) lichen substances have relatively low molecular weight [4]. They are produced by the mycobiont [5], and accumulate in the cortex (such as atranorin, parietin, usnic acid, fungal melanins) or in the medullary layer (such as physodic acid, physodalic acid, protocetraric acid) as extracellular tiny crystals on the outer surfaces of the hyphae.
Lichen substances exhibit a great diversity of biological effects, including antimicrobial, antiinflammatory, analgesic, antipyretic and antiproliferative and cytotoxic activities, and there has been a growing interest in the pharmaceutical properties of compounds derived from lichens [6]. Lichens are valuable plant resources and are used as medicine, food, fodder, perfume, spice, dyes and for miscellaneous purposes throughout the world [7]. The use of lichens in medicine is based on the fact that they contain unique and varied biologically active substances, mainly with antimicrobial actions. The aim of this work is to identify and analyse the phytochemistry of lichens with different solvent extracts.

2. Materials and methods

2.1 Collection of Lichen Material

Samples of lichens were collected from Madikeri district, Karnataka. The lichen samples were identified by morphological, anatomical, chemical tests [8]. The collected lichen materials were identified as *Flavoparmelia caperata* (L.) Ach., *Parmotrema austrosinensis* (Zahlbr.) Hale, *Roccella montagnei* Bèl. Emend. Awas., *Teloschistes flavicans* (Swartz) Norm., *Physcia aipolia* (Ehrh. ex Humb.) Furnr., *Parmotrema grayanum* (Hue) Hale, *Parmotrema tinctorum* Nyl., *Parmotrema reticulatum* (Taylor) Choisy and *Usnea subflorida* (Zahlbr.) Motyka. These lichens were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles at 4 °C.

2.2 Preparation of lichen extracts

The lichen extracts were prepared by cold maceration method using Petroleum ether, Chloroform, Ethyl acetate, Acetone and Methanol. Approximately 5 gm of lichen material was soaked in 25 ml of respective solvent and kept on a rotary shaker for 24 hours at room temperature. This solution was filtered with the help of Whatman No. 1 filter paper. The filtrate was used for the phytochemical screening.

2.3 Qualitative chemical evaluation

The different extracts thus obtained were qualitatively tested for the presence of various phytochemical constituents [9, 10, 11].

Test for Tannins

Ferric chloride Test; 2 ml Crude extract was mixed with a few drops of 5% ferric chloride solution. Formation of blue colour indicated the presence of hydrolysable tannins.

Test for Alkaloids

Dragondroff's test; 2 ml of crude extract is added to 1% HCl, steam for 10 minutes. To this add 6 drops of Dragondroff's reagent; Reddish brown precipitate indicates the presence of alkaloids.

Test for Saponins

Frothing test; 2 ml of Crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for glycosides

Keller-kilani test; 2 ml of Crude extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2 ml of concentrated H_2SO_4. A brown ring at the interphase indicated the presence of cardiac glycosides.

Test for Flavonoids

NaOH solution test; 2 ml of crude extract is added to 2 ml of 10% NaOH solution. Yellow to orange colour indicates the presence of flavanoids.

Test for Proteins

Xanthoproteic test; 2 ml of crude extract is added to 2 ml of HNO_3, boil in a water bath. Orange co-lour indicates the presence of proteins.

Test for Triterpenoids: Salkowski Test; 2 ml of crude extract is shaken with 1 ml of chloroform and a few drops of concentrated sulphuric acid were added along the side of the test tube. A red brown colour formed at the interface indicated the test as positive for triterpenoids.

Test for carbohydrates

Benedict's test; 2 ml of Crude extract when mixed with 2 ml of Benedict's reagent and boiled, a reddish brown precipitate formed which indicated the presence of the carbohydrates

Test for Steroids

Liebermann-Burchard reaction; 2 ml of crude extract is added to 2 ml acetic anhydride and a few drops of conc. H_2SO_4 is added. Blue-green ring indicates the presence of steroids.

2.4 Colour test

The colour is also known as spot test. Presence of certain lichen substances in tissues of lichen thallus produces a change in their colour when certain chemicals are applied on the surface of thallus. A positive change is denoted by a positive (+) symbol, followed by the colour produced, and no change in colour is denoted by a negative (-) symbol. The chemicals are routinely used as follows:

K- test: 10-25% aqueous solution of KOH, applied to the cortex, medulla, parts of apothecium.

C-test: A freshly prepared aqueous solution of Calcium hypochlorite, applied to the cortex, medulla, parts of apothecium.

KC- test: At a particular spot of thallus, K is applied first and immediately followed by C.

P- test: 1-5% solution of paraphenylenediamine is prepared in ethanol, and spotted on thallus.

P- test: 1-5% solution of paraphenylenediamine is prepared in ethanol, and spotted on thallus.

3. Results

In the present investigation, collection, identification, extraction and phytochemical evaluation of various extracts were derived from commonly occurring lichens growing in Kodagu district. In this study, 9 lichen species were collected from which 45 extracts were prepared using Petroleum ether, Chloroform, Ethyl acetate, Acetone and Methanol solvents. The preliminary phytochemical screening for the presence of Tannins, Alkaloids, Saponins, Glycosides, Flavonoids, Proteins, Triterpenes, Carbohydrates and Steroids was carried out on aforesaid extracts. Important phytochemicals like Tannins, Proteins, Carbohydrates and Steroids were present in most of the lichen extracts tested. Petroleum ether and Chloroform extracts did not show any phytochemicals. The extracts from *Flavoparmelia caperata* showed most of the constituents like Tannins from chloroform and methanolic extract, Flavonoids from Ethyl acetate, acetone and Methanolic extract, Proteins from Chloroform, Ethyl acetate, chloroform, acetone and methanolic extract, Triterpenes from acetone and ethyl acetate extract, Carbohydrates from ethyl acetate and methanolic extract and Steroids from Chloroform, ethyl acetate, acetone and methanolic extracts (Table 1).

Table 1: Preliminary phytochemical constituents of lichens with various solvent extracts

Sl no.	Lichen samples	Tannins					Alkaloids					Saponins					Glycosides					Flavonoids					Proteins					Triterpenes					Carbohydrates					Steroids				
		Petroleum ether	Chloroform	Ethyl acetate	Acetone	Methanol	Petroleum ether	Chloroform	Ethyl acetate	Acetone	Methanol	Petroleum ether	Chloroform	Ethyl acetate	Acetone	Methanol	Petroleum Ether	Chloroform	Ethyl acetate	Acetone	Methanol	Petroleum ether	Chloroform	Ethyl acetate	Acetone	Methanol	Petroleum ether	Chloroform	Ethyl acetate	Acetone	Methanol	Petroleum ether	Chloroform	Ethyl acetate	Acetone	Methanol	Petroleum ether	Chloroform	Ethyl acetate	Acetone	Methanol	Petroleum ether	Chloroform	Ethyl acetate	Acetone	Methanol
1	*Flavoparmelia caperata*	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	+	+	+	-	-	+	+	-	-	-	+	-	+	-	+	+	+	+
2	*Parmotrema austrosinensis*	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	+	+	-	+	+	-	-	-	-	-	-	-	+	+	+
3	*Parmotrema grayanum*	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	+	+	-	-	+	-	-	-	-	-	-
4	*Parmotrema reticulatum*	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-
5	*Parmotrema tinctorum*	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-
6	*Physcia aipolia*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-	-	-	-	+	-	-	-	+	+	+
7	*Roccella montagnei*	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	+
8	*Teloschistes flavicans*	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	+	-	-	-	+	+	+
9	*Usnea sulflorida*	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-

+ : Presence of compound
- : Absence of compound

Saponins were present only in *Usnea subflorida*. Glycosides were absent in all the tested lichen extracts. Methanolic extract was more active and showed almost all the tested phytochemicals in all the lichen samples. Alkaloids were present only in the methanolic extracts of *Parmotrema grayanum, P. reticulatum, P. tinctorum* and *Teloschistes flavicans.*

Flavonoids were present only in *Flavoparmelia caperata, Parmotrema austrosinensis* and *Usnea sulflorida*. Spot test showed the presence of Atranorin in most of the lichen tested like *Flavoparmelia caperata, Parmotrema austrosinensis, P grayanum, P reticulatum, P. tinctorum* and *Physcia aipolia* (Table 2).

Table 2: Spot test of collected lichens

Sl no.	Lichens	K-test	C-test	KC-test	PD-test	Secondary compounds
1	*Flavoparmelia caperata*	-	-	+ red	-	Atranorin, Usnic acid, Protocetraric acid
2	*Parmotrema austrosinensis*	-	+ red	+ red	-	Lecanoric acid
3	*Parmotrema grayanum*	-	-	-	-	Atranorin
4	*Parmotrema reticulatum*	+ yellow	-	-	+ orange	Atranorin, Salazinic acid
5	*Parmotrema tinctorum*	+ yellow	+ red	-	-	Lecanoric acid, Atranorin, Chloroatranorin
6	*Physcia aipolia*	+ yellow	-	-	+ Yellow	Atranorin, Zeorin
7	*Roccella montagnei*	+ red	+ Red	-	-	Lecanoric acid, Erythrin
8	*Teloschistes flavicans*	+ Red	-	-	-	Parietin
9	*Usnea sulflorida*	+ yellow	-	-	+ Red	Protocetraric acid and Barbatic acid.

4. Discussion

Phytochemical analysis conducted on the lichen extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities. The utility of lichens is due of range of secondary compounds produced by them. Lichen substances exhibit a great diversity of biological effects, including antimicrobial, antiinflammatory, analgesic, antipyretic and antiproliferative and cytotoxic activities, and there has been a growing interest in the pharmaceutical properties of compounds derived from lichens [6]. Barbatic acid, atranorin and lecanoric acids are lichen depsides reported as an analgesic, antipyretic and fungitoxic agent and commonly found in many lichens [12]. Our present study showed the presence of Tannins, Proteins, Carbohydrates and Steroids were present in most of the lichen extracts tested. Whereas Preliminary phytochemical analysis of *Ramalina hossei* H. Magn & G. Awasthi revealed the presence of Tannins and triterpenoids [13], *Everniastrum cirrhatum* (Fr.) Hale revealed the presence of alkaloids, saponins, tannins and terpenoids [14] and methanol extract of macrolichen *Ramalina conduplicans* Vain. Showed the presence of tannins and steroids [15]. The different solvents of different polarity is used to separate compounds based on their solubility in the extraction solvent. This extraction method was performed under continuous stirring and short term, is used to extract the maximum amount of bioactive components and prevent their modification or probable denaturation.

5. Conclusion

The present study may be useful to supplement the information with regard to its standardization and identification and in carrying out further research as a significant new source for novel bioactive substances. These compounds could be Eco friendly, environmentally safe and be replaced by fungicides or insecticides, also be used as a biodegradable product and also be safe alternative to treat infectious diseases.

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