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Phytochemical Screening of Secondary Metabolites by using different Solvents for Lichen: Parmotrema sp.

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Abstract: Lichens are composite species made up of a symbiotic relationship between a phycobiont and a mycobiont. Lichens usually occur in extreme environments and it can be useful to many commercial applications. Phytochemical study of extract of *Parmotrema* sp. was carried out in order to detect the presence of different secondary metabolites. These are commonly used in South Indian cuisine, as well as Hyderabad Biryani. It is also known as the Black Stone Flower. *Parmotrema* has a long history in ethanopharmaceutics. It revealed the presence of Alkaloids, Tannins, Phenols, Glycoside, etc. The present study reveals about its phytochemical analysis.

Keywords: Lichen, Kalpasi, Phytochemical screening, Secondary metabolites.

I. INTRODUCTION

Lichen is a fungus-algae symbiotic relationship that results in the formation of organisms. Since it serves two functions: algae and fungi, lichen is also classified as a hybrid or dual organism. The mycobiont is the fungal component of lichen, while the phycobiont is the algal component. They are so similar together that they appear to be a single plant. In all suitable climatic conditions, lichens can be found on rocks, barks, trees, soil, and leaves. More than 17,000 species and over 800 lichen products are utilized by human purposes; for eg: floral decorations, pollution monitoring, perfumery, dying, medicinal purposes. This symbiosis results in the development of lichen compounds, which are extracellular secondary metabolites. These compounds are found in the thalli and usually form crystals on the fungal hyphae's surface. More than 800 lichen secondary metabolites have been discovered so far, the majority of which are only found in lichens. Lichens have recently been used in a number of studies relating to phytochemical and pharmaceutical applications. Lichens and their secondary metabolites serve a variety of medicinal functions. *Parmotrema* sp. is used as spice all over the world. It has many ethanobotanical uses. It is also known as Kalpasi in south India.[1],[2]

A. Taxonomic Position

- 1) Kingdom: Fungi
- 2) Division: Ascomycota
- 3) Sub Division: Eumycotina
- 4) Class: Lecanoromycetes
- 5) Subclass: Ascolichens
- 6) Order: Lecanorales
- 7) Family: Parmeliaceae
- 8) Genus: *Parmotrema*

II. MATERIALS AND METHODOLOGY:

Thalli of lichens was purchased from the local herbal market of Ahmedabad, Gujarat. It is mainly used as a spice. It was authenticated by Indian Lichenological Society, India.

A. Preparation of plant extract.

Dried flowers of *Parmotrema* sp. were collected and homogenized into a fine powder using mixer grinder.

B. Preparation of Extracts

Powdered material was subjected to successive solvent extract using polar solvents like Distilled water and Methanol. 20g of powdered sample was added to 200 ml of suitable solvents and was covered with aluminium foil and kept in shaker for 24 hrs. The crude was extracted, filtered using whatman filter paper no.1 and evaporated. Phytochemical analysis were carried out from the crude.[3]

C. Phytochemical Analysis

Phytochemical analysis were done by certain reagents to test the Alkaloid, Flavonoids, Phenol, Tannin, Glycoside, Carbohydrates and Proteins. The phytochemical study was carried out by standard method[4].

1) Test for Alkaloids

a) *Mayer's Test*: Few ml extract was treated with Mayer's Reagent . Formation of white precipitate indicates the presence of alkaloids.

b) *Dragendorff's Test*: Few ml extract was treated with Dragendorff's Reagent . Formation of orange-red precipitate

2) *Test for Flavonoids*: Few ml extract was treated with Sodium hydroxide and dil. Hydrochloric acid. Yellow colour changes to colourless indicates the presence of flavonoids.

3) Test for Phenols

a) *Ferric chloride Test*: Few ml extract was treated with few ml distilled water and then with 5% of ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

b) *Lead Acetate Test*: Few ml extract was treated with few ml 10% Lead acetate solution. Formation of Bulky White precipitate indicates the presence of phenol.

4) Test for Tannins

a) *Lead Acetate Test*: Few ml extract was treated with few ml Lead acetate solution. Formation of Bulky White precipitate indicates the presence of tannin.

b) *Potassium Dichromate Test*: Few ml extract was treated with few ml Potassium dichromate solution. Precipitation indicates the presence of tannin.

5) *Test for Glycosides*: Keller-Killani Test Few ml extract were mixed with few ml distilled water .2ml of glacial acetic acid containing 1 to 2 drops of ferric chloride solution was added. The mixture was then poured into another test tube containing 2 ml of conc. Sulphuric acid. A brown ring at the interface indicates the presence of glycosides.

6) Test for Carbohydrates

a) *Molisch's Test*: Few ml extract was mixed with a-Naphthol .1 ml conc. Sulphuric acid. Reddish violet ring indicates the presence of carbohydrates.

b) *Fehling's Test*: Equal volume of Fehling A and Fehling B reagents were mixed together and 2 ml of it was added to the extract and gently boiled. A brick red colour indicates the presence of carbohydrates.

c) *Iodine Test*: Few ml extract was mixed with 2 ml of Iodine solution. A dark blue or purple colour indicates the presence of Carbohydrates.

7) Test for Proteins

a) *Ninhydrin Test*: 2ml of Extract was treated with 2 drops of ninhydrin solution. Appearance of violet colour indicates the presence of proteins.

b) *Millon's Test*: Few ml extract was treated with few drops of millon's reagent. Formation of white precipitate indicates the presence of proteins.

III. RESULTS AND DISCUSSION:

Table 1: Phytochemical screening of Parmotrema sp.

SR.NO	PHYTOCHEMICAL TEST	DISTILLED WATER	METHANOL
1.	Alkaloid		
	Mayer's Test	-	+
	Dragendorff's Test	+	++
2.	Flavanoid		
	Alkaline Reagent Test	-	-
3.	Phenol		
	Ferric chloride Test	-	-

	Lead Acetate Test	+	++
4.	Tannins		
	Lead Acetate Test	+	++
	Potassium Dichromate Test	+	++
5.	Glycosides		
	Keller Killiani Test	+	+
6.	Carbohydrate		
	Molish's Test	+	+
	Fehling's Test	-	-
	Iodine Test	-	-
7.	Proteins		
	Ninhydrin Test	-	-
	Millon's Test	-	+

The phytochemical active compounds of Parmotrema sp. were qualitatively analyzed and the results are presented. The phytochemical studies of two solvents shows the presence of tannins, proteins, phenols, alkaloids, glycosides and carbohydrates in almost all the two extracts. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. Results obtained from phytochemical screening were shown in Table.1. Thus the present investigation revealed that Parmotrema sp. appears to be rich in secondary metabolites, the preliminary study shows the presence of bioactive compounds. Further studies are in progress for quantification of particular bioactive constituents.

IV. CONCLUSION

The potential of recent developments in lichens and lichen-forming fungi to promote the commercialization of lichen-based products was investigated. Lichens are an underutilised source of biological activities with industrial implications, and their full potential has yet to be identified. Bioactive compounds derived from lichens show great promise as antimicrobial, antioxidant, anticancer, and cytotoxic agents in biopharmaceutical applications, as well as in the development of new formulations or developments for human benefit.[5]

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