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Preliminary Phytochemical Investigations of *Blepharis maderaspatensis* (L.) Heyne ex Roth. and *Blepharis molluginifolia* Pers. whole Plant

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ABSTRACT

The plants for the present investigation were collected from Mysore (Karnataka). *Blepharis maderaspatensis* (L.) Heyne ex Roth. and *Blepharis molluginifolia* Pers. They belong to the family Acanthaceae. Extracts are taken by using chloroform, petroleum ether, ethyl acetate and ethanol. Solvents were screened for secondary metabolites. Among all the four extracts, maximum phytochemicals were found dissolved in ethanol. The preliminary phytochemical tests indicated presence of steroids, cardiac glycosides, flavonoids, saponins and phenolic compounds.

Keywords: *Blepharis maderaspatensis* (L.) Heyne ex Roth., *Blepharis molluginifolia* Pers., Acanthaceae, Secondary metabolites, phytochemistry.

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INTRODUCTION

Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [1].

Phytochemical studies have attracted the attention of plant scientist due to development of new and sophisticated techniques. These techniques played a significant role in giving the solution to systematic problems on the one hand and in the search for additional resources of raw material for pharmaceutical industry on the other hand. Plant synthesizes a wide variety of chemical compounds, which can be sorted by their chemical class, biosynthetic origin and functional groups into primary and secondary metabolites [2].

Secondary metabolites present in plants have been linked with the healing properties of plants. In addition to their active ingredients, plants contain minerals, vitamins, volatile oils, glycosides, alkaloids, bioflavonoids, and other substances that are important in supporting a particular herb's medicinal properties [3].

Blepharis (Acanthaceae) is an Afro-asiatic genus comprising 129 species which occur in arid and semi-arid habitats. This is the only genus in the family which is reported to have some C4 species [4].

According to our knowledge some of the species belonging to *Blepharis* genus have been previously investigated from different points of view [5].

Blepharis edulis Pers. is traditionally used for gastrointestinal, respiratory and inflammatory disorders. *Blepharis edulis* Pers. (family: Acanthaceae**)** is commonly known as “Utangan”. It is used in folk medicine to treat asthma, cough, fever, inflammation of throat. It is applied locally to heal fastering wounds and ulcers .It is appetizer, astringent to bowels. Seeds contain Allantoin, a bitter glycoside and Blepharin, a glucoside [6]. *B. edulis* seeds are used as food to increase sperm count and as aphrodisiac plant [7].

Blepharis boerhaaviafolia leaves commonly sold in Indian market, are reported to be useful in wounds, ulcers, nasal haemorrhage, asthma, throat inflammation, ascites, liver and spleen disorders. Seeds are considered to be expect de-obstruent and useful in strangury and conjunctivitis [8].

The genus has many medicinally important plants. The plants selected for the study are unexplored plants with many medicinal properties.

Blepharis maderaspatensis is used to treat headache. Seeds are used as dysuria, diseases of nervous system, diuretic, aphrodisiac [9], it is used to cure cuts and wounds [10], juice extracted from leaf is heated with gingelly oil and applied on affected places to heal wound [11]. Dry seeds of this plant contain steroids and the plant is used for brain disorders [12].

Leaves of *Blepharis molluginifolia* crushed and the paste is applied in head ache [13]. The plant is used traditionally to treat bone fractures, skin diseases, urinary discharges and allergies [14]. Flat branches of the plant is heated and tied in case of joint pains. Leaves are roasted and then extract is obtained, this extract is drunk as a remedy against flatulence. Roots are employed as antidote on snake- bite [15].

Thus for the present study two species of *Blepharis* were selected for primary screening of secondary metabolites present in them.

MATERIALS AND METHODS

Plant materials

For the present study the two plants *Blepharis maderaspatensis* (L.) Heyne ex Roth. and *Blepharis molluginifolia* Pers. belonging to the family Acanthaceae has been selected. These plants have been collected from the Mysore district, Karnataka, India in the month of September 2013.

Processing of plant material

The whole part of the plant have been collected, cleaned, chopped into small pieces and dried under shade at room temperature. The dried whole plant was pulverized using a stainless steel mixer grinder. After pulverization, the powder has been sieved using a commercial sieve (mesh size approx. 1mm) to make the particle size uniform. Powdered and stored in air tight containers for the phytochemical investigation. This powder has been subjected to solvent extraction in a Soxhlet apparatus using various solvents viz., petroleum ether, chloroform, ethyl acetate and ethanol.

Extraction of plant material

The plant materials were extracted with chloroform petroleum ether, ethyl acetate and ethanol using soxhlet extraction apparatus continuously for 16 hours. For extraction, the dried plant material was used. Initially 50 g of plant material was packed in filter paper and loaded into the thimble of soxhlet apparatus. 300 ml of solvent was poured into the flask (distilling pot) and the whole apparatus was set. The soxhlet extraction was performed for 12- 16 hours until the collected solvent in siphon tube appears to be clear. Later the extracted solvent was evaporated under reduced pressure using a rotary vacuum evaporator to get solid/ semi solid extract. The extract was weighed, physical characters were noted. The percentage yield was calculated and the extracts were suitably labelled and stored in clean and dry specimen bottles.

Preliminary phytochemical screening

Both the plant extracts were screened for the presence of various secondary metabolites such as alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, glycosides and steroids were screened according to the standard phytochemical methods described by Harborne[16].

RESULTS AND DISCUSSIONS

The petroleum ether, chloroform, ethyl acetate and ethanol extracts of *Blepharis maderaspatensis* showed the presence of alkaloids, flavonoids, phenols, tannins, saponins, diterpenoids, triterpenoids, cardiac glycosides and phytosterols. Alkaloids are present in ethyl acetate and ethanolic extracts but they are absent in petroleum ether and chloroform extract. Diterpenes are present in ethanolic extract. Flavonoids are present in petroleum ether, ethyl acetate and ethanolic extracts. Phytosterols are seen in petroleum ether, ethyl acetate and ethanolic extracts (Table 1).

The petroleum ether, chloroform, ethyl acetate and ethanol extracts of *Blepharis molluginifolia* showed the presence of flavonoids. Triterpenoids and phytosterols are found in chloroform, ethyl acetate and ethanolic extracts. Phenols and saponins are present in ethyl acetate and ethanol extracts. Alkaloids, tannins and cardiac glycosides are present in ethanolic extracts. Alkaloids are seen only in alcoholic extracts (Table 1).

Table: 1: Phytochemical analysis of different extracts of *Blepharis maderaspatensis* (L.) Heyne ex Roth. and *Blepharis molluginifolia* Pers. whole plant

Phytochemicals	Test	*Blepharis maderaspatensis*				*Blepharis molluginifolia*			
		PE	C	EA	E	PE	C	EA	E
ALKALOIDS	Dragendorff's	-	-	-	-	-	-	-	-
	Mayer's	-	-	-	-	-	-	-	-
	Hager's	-	-	+	+	-	-	-	+
FLOVONOIDS	Lead acetate	+	-	+	+	+	+	+	+
	Zn-Hcl reduction test	-	-	+	+	-	-	+	+
	Alkaline reagent test	-	-	+	+	-	-	+	+
PHENOLS	Ferric chloride	-	-	-	+	-	-	-	+
	Ferric sulphate test	+	+	+	+	-	-	+	+
TANNINS	Gelatin	+	+	+	+	-	-	-	+
SAPONINS	Foam Test	+	+	+	+	-	-	+	+
DITERPENES	Copper acetate	-	-	-	+	-	-	-	-
TRITERPENOIDS	Noller's test	-	+	+	+	-	+	+	+
	Tshugajen test	-	+	+	+	-	+	+	+
CARDIAC GLYCOSIDES	Brontrager's test	+	+	+	+	-	-	-	+
	Legal test	+	+	+	+	-	-	-	+
PHYTOSTEROLS	Salkowskis test	-	-	+	+	-	-	-	-
	Leibermann Burchard test	-	+	+	+	-	+	+	+

*+ sign indicates the presence of the metabolite.
*- sign indicates the absence of the metabolite.
*P = petroleum ether, C= Chloroform, EA = ethyl acetate, E = ethanol,

The biological function of flavonoids, apart from their antioxidant properties, include protection against allergies, inflammation, platelet aggregation, microbes, ulcers, heptatotoxins, viruses and tumors [17]. Flavonoids have anti-inflammatory effects. The anti-inflammatory effects of flavonoids are due to actions on blood vessels, inflammatory cells & inflammatory mediators [18].

Tannins may be employed medicinally in anti-diarrheal, haemostatic, and anti-haemorrhoidal compounds. The anti- inflammatory effects of tannins help control all indications of gastritis, esophagitis, enteritis, and irritating bowel disorders. Diarrhoea is also treated with an effective astringent medicine that does not stop the flow of the disturbing substance in the stomach; rather, it controls the irritation in the small intestine [19].

Many pharmacological activities have been reported about saponins such as antibiotic, antifungal, antiviral, hepatoprotective anti-inflammatory and anti-ulcer [20]. Saponins are widely used as adjuvants in oral and injected vaccines, saponins improve the effectiveness of orally administered vaccines by facilitating the absorption of large molecules, and oral administration of saponins increases the resistance of animals to a disease challenge, suggesting that saponins have immunostimulatory effects [21].

Terpenes have found to inhibit the growth of cancerous cells, decreases tumor size, decrease cholesterol level and also decrease micro-organism concentration [22].

Phenolics are the largest group of phytochemicals and have been touted as accounting for most of the antioxidant activity of plants or plant products [23].

Cardiac glycosides have a long history of therapeutic application. The early understanding of their positive inotropic effects facilitated their use as effective drugs for the treatment of heart-related pathologies. More recently, considerable *in vitro, in vivo* and epidemiological data support novel roles for such drugs for the treatment of several diseases. Most notably, it is now established that cardiac glycosides can induce apoptosis and inhibit the growth of cancer cell lines at concentrations close to those found in the plasma of patients with cardiac conditions [24].

The anticancer activity of plant sterols has been reviewed. It has reviewed the potential role of phytosterols both in the etiology as well as in the prevention of immunological diseases. The antiinflammatory activity of phytosterols most likely relies on the inhibition of secretion of inflammatory mediators, such as Interleukin -6 and Tumour necrosis factor α [25].

The medicinal properties of the plant could be attributed to the presence of one or more of the detected natural products.

The results of the present study support the folklore use of these plants as a medicinal source. Further studies are needed to clarify the *in vivo* potential of these plants in the management of human diseases.

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