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Original Article

Screening and Preliminary Phytochemicals Study of *Microlepia speluncae* (L.) Moore collected from Ponmudi, Kerala, India

Amster Regin Lawrence R [1], John Peter Paul J [2,*]

[1] Research Scholar, Centre for Advanced Research in Plant Sciences (CARPS), Department of Botany, St. Xavier's College (Autonomous), Palayamkottai - 627 002, Tamil Nadu, India.

[2] Assistant Professor of Botany and Director, Centre for Advanced Research in Plant Sciences (CARPS), St. Xavier's College (Autonomous), Palayamkottai - 627 002, Tamil Nadu, India

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ABSTRACT

The present study was intended to discover the preliminary phytochemicals of *Microlepia speluncae* (L.) Moore from Ponmudi, India. The preliminary phytochemical analysis was conducted in five extracts namely ethanol, acetone, ethyl acetate, chloroform and hexane by Harborne method. The preliminary phytochemical analysis showed the presence of alkaloids, anthocyanin, anthroquinone, catechin, cardiac glycosides, coumarins, flavonoids, glycosides, phenolic groups, phlobatannins, phytosteroids, quinones, saponins, tannins, terpenoids, emodins and diterpenes. Among the various phytochemicals studied, flavonoids, terpenoids and diterpenes showed the maximum presence, being found in five different extracts and catechin was observed in only one extract. From the results, it was noted that the extracts of *Microlepia speluncae* (L.) Moore was found to be the presence of a number of active secondary metabolites. This report will lead to the isolation and characterization of these active secondary metabolites for bio-efficacy and bioactivity.

Keywords: Phytochemical, Bioactive compounds, *Microlepia speluncae*, Pteridophytes, Dennstaedtiaceae

Corresponding author *
Dr J. John Peter Paul
Assistant Professor of Botany and Director, Centre for Advanced Research in Plant Sciences (CARPS), St. Xavier's College (Autonomous), Palayamkottai - 627 002, Tamil Nadu, India.
E Mail: johnarock2008@yahoo.com

1. INTRODUCTION

Pteridophytes are the primitive land plants group on earth and established large group of vascular cryptograms. The position of the pteridophytes is in between the lower cryptograms and higher vascular plants. Pteridophytes have a long ecological history on our planet. The Pteridophytes are mostly distributed in the high altitude mountainous

regions such as Himalayas, Western Ghats and Eastern Ghats. More than 300 species of ferns and fern allies were reported from the Western Ghats, South India [1]. Use of plants as a source of medicine has been inherited and is an important component of the health care system. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties which are naturally occurring chemicals found in plant sources.

Plants are a rich source of secondary metabolites with interesting biological activities. In general these secondary metabolites are an important source with a variety of structural arrangements and properties [2]. Ferns are expected to have many useful secondary metabolites than other plants. Ferns were reported to have many useful phytochemicals (secondary metabolites) such as flavonoids, steroids, alkaloids, phenols, triterpenoid compounds, varieties of amino acids and fatty acids [3]. They can be used directly or in extracted forms for the management of various ailments due to the presence of various secondary metabolites [4]. Many species of this plant division are highly ignored and are determined to have potential secondary metabolites that act against various diseases [5]. The phytochemical potential of ferns is relatively unexplored, although they possess great economic potential due to some interesting medicinal properties [6]. In the present study, an effort has been made to screen the preliminary phytochemicals present in *Microlepia speluncae* (L.) Moore using the standard method for the search in the field of pharmaceutical and health care and can be properly recognized as medicine in future.

2. MATERIALS AND METHODS

2.1 Collection of Plant Sample

The plant materials used in the present study was *Microlepia speluncae* (L.) Moore belonging to the family Dennstaedtiaceae. The plant materials were collected from Ponmudi, Kerala, India and identified and confirmed by Pteridophyte flora of the Western Ghats - South India [7].

2.2 Preparation of extracts

For the preparation of different extracts, the plant specimens were washed thoroughly and placed on blotting paper and spread out at room temperature in the shade condition for drying. The shade dried samples were grounded to fine powder using a tissue blender. The powdered samples were then stored in the refrigerator for further use. 30g powdered samples were packed in Soxhlet apparatus and extracted with ethanol, acetone, ethyl acetate, chloroform and hexane for 12 h separately [8].

2.3 Preliminary phytochemical analysis

The different extracts (ethanol, acetone, ethyl acetate, chloroform and hexane) of *Microlepia speluncae* (L.) Moore were tested for alkaloids, anthocyanin, anthroquinone, catechin, cardiac glycosides, coumarins, flavonoids, glycosides, phenolic groups, phlobatannins, phytosteroids, quinones, saponins, tannins, terpenoids, emodins and diterpenes. Phytochemical screening of the extracts was carried out according to the standard methods [8].

2.3.1 Test for alkaloids

1ml of 1% HCl was added with 2ml of extract and was treated with few drops of Mayer's reagent. A creamy white precipitate indicates the presence of alkaloids.

2.3.2 Test for anthocyanin

1ml of 2N HCl was added to the 1ml of extract and was treated with NH_3. Pink red colour turns blue violet.

2.3.3 Test for anthraquinone

2ml of extract was mixed with 1ml of benzene and 1ml of 10% ammonia solution was added. The presence of red or violet color indicates the anthraquinones.

2.3.4 Test for cardiac glycosides

0.4ml of glacial acetic acid was added with 1ml extract and trace amount of $FeCl_3$. Blue colour indicates the presence of cardiac glycosides.

2.3.5 Test for catechin

1ml of plant extract was mixed with few drops of Ehrlich's reagent was treated with few drops of conc. HCl. pink color indicates catechin.

2.3.6 Test for Coumarins

1ml of seaweed extract was added with 1ml of 10% NaOH. Formation of yellow colour indicates the presence of coumarins.

2.3.7 Test for diterpenes

1ml extract was added with 1ml dis. H_2O and 10 drops of copper acetate solution. Emerald green colour indicates the presence of diterpenes.

2.3.8 Test for emodins

1ml of plant extract was mixed with 2ml of NH_4OH and treated with 3ml of benzene. Red color indicates emodins.

2.3.9 Test for flavonoids

A few drops of 1% NH_3 solution was added to 2 ml of extract in a test tube. Yellow coloration indicates the presence of flavonoids.

2.3.10 Test for glycosides

2ml of 50% H_2SO_4 was added to 2ml of extract in a boiling tube. The mixture was heated in boiling water bath for 5 min. 10ml of Fehling's solution was added and boiled. A brick red precipitate indicates the presence of glycosides. .

2.3.11 Test for phenols

1ml extract, add 2ml distilled water followed by few drops of 10% ferric chloride. The formation of blue or black colour indicates the presence of phenolic groups.

2.3.12 Test for phlobatannins

1ml extract was added with 1% aqueous HCl and then boiled. Red precipitate indicates the presence of phlobatannins.

2.3.13 Test for quinones

1ml seaweed extract added with 1ml of alcoholic KOH. Red to blue colour indicates the presence of quinones.

2.3.14 Test for saponins

2ml of extract was shaken vigorously with 5ml distilled water to obtain stable persistent foam. The formation of emulsion indicates the presence of saponins.

2.3.15 Test for steroids

1ml of extract added to 1ml $CHCl_3$ and few drops of Conc. H_2SO_4. Golden red colour or Brown colour indicates the presence of phytosteroids.

2.3.16 Test for tannins

To 2ml extract, 1ml of distilled water and 1-2 drops of ferric chloride solution was added and observed for brownish green or a blue black coloration indicates the presence of tannins.

2.3.17 Test for terpenoids

2ml extract was mixed with 2ml of $CHCl_3$ in a test tube. 3ml Conc. H_2SO_4 was added carefully along the wall of the test tube to form a layer. An interface with a reddish brown coloration confirms the presence of terpenoids.

3. RESULTS AND DISCUSSION

Microlepia speluncae (L.) Moore, was analyzed for the presence or absence of seventeen various secondary metabolites such as alkaloids, anthocyanin, anthroquinone, catechin, cardiac glycosides, coumarins, flavonoids, glycosides, phenolic groups, phlobatannins, phytosteroids, quinones, saponins, tannins, terpenoids, emodins and diterpenes were tested in five different extracts of *Microlepia speluncae* (L.) Moore (ethanol, acetone, ethyl acetate, chloroform and hexane). Thus, out of (1x5x17) 85 tests for the presence or absence of the above compounds, 53 tests gave positive results and the remaining gave negative results.

The 53 positive results showed the presence of flavonoids, terpenoids and diterpenes in all the five different extracts, alkaloids, coumarins, and anthocyanin in the four extracts, anthroquinones, cardiac glycosides, phenolic groups, phytosteroids and quinones in the three extracts, emodins, glycosides, phlobatannins, saponins and tannins in two extracts, followed by catechin present in only one extract. Among the five different extracts, the ethanol extract showed the presence of the maximum number (12) of compounds. Next to ethanol extract, chloroform and acetone extract showed the presence of eleven compounds, ethyl acetate showed presence of ten compounds, followed by hexane extract with nine compounds (Table-1).

Table 1: Preliminary phytochemical analysis of *Microlepia speluncae* (L.) Moore

Compounds	Solvents				
	Ethanol	Acetone	Ethyl Acetate	Chloroform	Hexane
Alkaloids	-	+	+	+	+
Anthraquinone	-	-	+	+	+
Anthocyanin	+	+	+	+	-
Cardiac glycosides	+	-	+	+	-
Catechin	-	+	-	-	-
Coumarins	-	+	+	+	+
Diterpenes	+	+	+	+	+
Emodins	+	-	-	+	-
Flavonoids	+	+	+	+	+
Glycosides	+	-	-	+	-
Phenolic groups	+	+	-	-	+
Phlobatannin	+	+	-	-	-
Phytosteroids	+	+	+	-	-
Quinones	-	-	+	+	+
Saponins	+	-	-	-	+
Tannins	+	+	-	-	-
Terpenoids	+	+	+	+	+

4. CONCLUSION

From the present study, it was concluded that *Microlepia speluncae* (L.) Moore showed the presence of a number of active secondary metabolites such as alkaloids, anthocyanin, anthroquinone, catechin, cardiac glycosides, coumarins, flavonoids, glycosides, phenolic groups, phlobatannins, phytosteroids, quinones, saponins, tannins, terpenoids, emodins and diterpenes. From the results, it can be observed that the different extracts of *Microlepia speluncae* (L.) Moore was found to be the presence of a number of active secondary metabolites. These phytochemicals can be reported to be beneficial in the management of more than one disease condition. This claim has been attributed to the numerous constituents found in the plant, each having its respective pharmacological effects. Some of the constituents act synergistically, while others have separate and distinct pharmacological activities. The increasing demand for medicinal plant products has renewed interest in the pharmaceutical industry in the production of herbal health care formulations, herbal-based cosmetic products, and herbal nutritional supplements. Thus, in addition to serving medical and cultural functions, medicinal plants in India have economic importance.

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