Research Article

PRELIMINARY PHYTOCHEMICAL ANALYSIS OF *PYRROSIA LANCEOLATA* (L.) FARWELLGnana Deepa Ruby R^{1*} and Catharin Sara S²¹Research Scholar, Department of Botany, Holy Cross College (Autonomous), Tiruchirappalli, Tamil Nadu, India²Assistant Professor, Department of Botany, Holy Cross College (Autonomous), Tiruchirappalli, Tamil Nadu, India

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DOI: 10.7897/2230-8407.0512178**ABSTRACT**

Pyrrosia lanceolata (L.) Farwell. was well-known as medicinal fern for skin disease and sore throat from ancient times in various literatures. There is no any report regarding the study of this plant earlier and hence it was designed to explore the secondary metabolites present in this lower plant *Pyrrosia lanceolata* (L.) Farwell. The plant parts such as frond and rhizome were treated with various solvents like water, ethanol, benzene, chloroform, petroleum ether and DMSO (Dimethyl Sulfoxide). 80 % of the solvent extracts were used for separating the secondary metabolites by using standard phytochemical techniques. All extracts from frond and rhizome of *P. lanceolata* (L.) Farwell, showed the presence of the following secondary bio constituents such as flavonoids, terpenoids, phenols, anthroquinones, catechins, glycosides and fixed oils and fats. The presence of specific bioactive compounds in this plant is under study now. Thus this work definitely opens a new avenue for the bio active compounds which has higher medicinal values and could be used for the treatment of many diseases in future.

Keywords: *Pyrrosia lanceolata* (L.) Farwell, qualitative analysis, phytochemicals, bioactive compounds, solvents, plant extracts.

INTRODUCTION

Phytochemistry is one of the rapidly expanding areas of Plant Taxonomy which utilizes chemical Information to improve the classification of plants. Plants are endowed with various phytochemical molecules such as vitamins, flavanoids, phenolic acids, lignins, tannins, flavanoids, terpenoids, quinones, coumarins, alkaloids, amines, betalains and other metabolites which are rich in antioxidant activity^{1,2}. Natural compounds may be considered alternative means for medicine, because of low or little toxicity due to their dietary properties or their long history as herbal medicines. In recent years identification and validation of the potential benefits of phytocompounds has become as important area of Pharmaceutical Science. These secondary plant metabolites (phytomedicine) previously with unknown pharmacological activities have been extensively investigated as a source of medicinal agents³. The antioxidant compounds possess anti-inflammatory, anti atherosclerotic, anti tumour, anti mutagenic, anti carcinogenic, anti microbial and antiviral activities^{4,5}. The ingestion of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes and other diseases associated with aging^{6,7}. In the plant World, Pteridophytes are said to be primitive vascular plants and provide important contribution to Earth's Plant Diversity. Pteridophytes are not infected by microbial pathogens, which may be one of the important factors for the evolutionary success of pteridophytes and the fact that they survived for more than 350 million years⁸. They are found scattered all over the globe and quite many of them occur in India. Many ferns among many other plants were used for medicinal purposes by the early Greeks and Romans and through the middle ages. Phytochemical analysis has been done in large scale in flowering plant as compared to non flowering plants. Most of the phytochemical works on Indian fern pertain to the primary metabolites. Medicinal ferns are gaining importance in recent days by the fact that several medicinal ferns from India have been subjected to

phytochemical analysis. Modern Biological and pharmaceutical studies were carried out on Pteridophytes by different workers⁹⁻¹³. Recently many pharmaceutical innovations are developed to find and separate the secondary metabolites from the ferns for designing the new medicines. In the present scenario, the use of the herbs and herbal medicine is at its peak and majority of researches are screening higher plants for the same but, very few researches are considering the fern plants for their medicinal potential. The fern *Pyrrosia lanceolata* (L.) Farwell. is being used ethno medicinally by the tribal people of Western Ghats¹⁴ for cuts and wounds. Plant decoction is used for curing colds and sore throats. Tea prepared from the frond is used as itch guard. But no work has been done about secondary metabolites. Thus the main objective of this work was to screen and evaluate the different parts of this unexplored fern *Pyrrosia lanceolata* plant for their phytomedicinal constituents.

MATERIALS AND METHODS**Collection and treatment of plants**

Pyrrosia lanceolata (L.) Farwell., plant was collected from Bonocord forest, Palode, Kerala, India. The plant was identified as Pteridophyte (Figure 2 - Identified Scanned Picture of the Experimental Plant) from Rapinat Herbarium, Tiruchirappalli and the voucher specimen is preserved in the Department of Botany, Holy Cross College (Autonomous), Tiruchirappalli, Tamil Nadu, India.

Botanical Description of a plant

Pyrrosia lanceolata (L.) Farwell. belongs to the family Polypodiaceae. Rhizome long creeping up to 2 mm thick, slender densely covered by scales lanceolate up to 5 × 1 mm, uniformly pale brown spot at the sub axial region, frond simple, lanceolate, elliptical or linear- lanceolate up to 14 × 1 cm, apex acute, base decurrent up to the winged type stipe. Sori irregularly distributed mainly in the distal part of the

pinna orbicular up to 2 mm in diameter. Dark brown, naked, spore reniform or planoconvex, $60 \times 50 \mu\text{m}$, pale brown, exine with few prominent tubercles.

Extraction of plant material

The plant was shade dried for two to three weeks until the moisture content of the plant was get drained. The plant parts frond and rhizome were get separated and ground into a fine powder. Then the powdered mass obtained was stored in a clean sterile bottle at room temperature, and used for the various extractions. Dried powder (3 g each) was macerated with 80 % of 50 ml each solvent for 48 hours at room temperature. Filtration and collection of the extract was done carefully and the extracts were preserved for further experimental analysis. Through this cold percolation method plant extracts were prepared. The plant materials were soaked in different solvents like aqueous, ethanol, benzene, petroleum ether, chloroform and Dimethyl Sulfoxide (DMSO) with occasional shaking at room temperature. After 15 days, the soluble materials were filtered off. The filtrate was concentrated under vacuum at 40°C temperatures using rotary evaporator to give thick syrup that constituted the plant crude extract. To extract the plant materials 80 % of the solvents were used.

Phytochemical screening

Phytochemical tests were carried out for frond and rhizome of *Pyrrosia lanceolata* (L.) Farwell, treated with water, ethanol, benzene, petroleum ether, chloroform, and DMSO using standard procedures to identify the constituents.

Qualitative Analysis

Qualitative phytochemical analysis was carried out with different organic extracts of frond and rhizome of the selected plant *P. lanceolata* for identification of their bioactive components. The extracts were tested for Alkaloids¹⁵, Carbohydrates¹⁶, Cardiac Glycoside, Saponins, Fixed oils and Fats¹⁷, Phenol¹⁸, Terpenoids, Tannins¹⁹, Flavonoids²⁰, phytosteroids²¹, Catechins, Anthroquinones and Phlobatanins. Their color reactions were observed to identify the presence of above said phytochemical compounds.

RESULTS

Preliminary qualitative phytochemical analysis of *P. lanceolata*

In the present preliminary phytochemical analysis, frond and rhizome solvent extracts of *Pyrrosia lanceolata* (L.) Farwell, showed the presence of phyto constituents. Six solvent extracts like Aqueous, Ethanol, Benzene, Chloroform, Petroleum ether and DMSO (Dimethylsulfoide) were used to analyze the frond and rhizome of *Pyrrosia lanceolata*. The phyto constituents tested were alkaloids, carbohydrates, glycosides, saponins, phenol, phytosterols, terpenoids, tannins, flavanoids, catechins, fixed oils and fats. Among the thirteen phytochemical tests analyzed, eight tests showed the positive results.

Table 1 shows the results of phytochemical constituents of frond of *Pyrrosia lanceolata* (L.) Farwell. Among the six solvent extracts DMSO and ethanol showed maximum positive results. Phytochemical constituents were high in DMSO and ethanolic extracts than the other solvent extracts. Terpenoids, flavanoids, anthroquinones and phenols gave good results. Alkaloids, carbohydrates, glycosides, fixed oils and fats showed medium results whereas saponins, tannins, phytosterols, catechins and phlobatanins showed nil results.

Phytochemical constituents of rhizome of six solvent extracts were tabulated in Table 2. In this analysis, the intensity of phytochemical constituents was highly noticed in ethanol and DMSO solvent extracts. Like frond, rhizome of *P. lanceolata* also showed the absence of saponins, tannins, phytosterols, catechins and phlobatanins. Terpenoids, flavanoids, phenols, anthroquinones were showed high intensity in all the extracts analyzed. Next to DMSO and ethanolic extract Petroleum ether, chloroform, benzene and aqueous extracts showed higher intensity of phyto constituents in both frond and rhizome analysis. When compared to frond the rhizome extract showed higher phytochemical constituents. Thus the preliminary screening of *Pyrrosia lanceolata* (L.) Farwell shows the presence of phytochemical constituents that would provide knowledge which will play important role in drug preparation.

DISCUSSION

The alkaloids, phenols, flavanoids and terpenoids, are present in all the extracts with maximum intensity. Alkaloids are significant for the protection and survival of plants because they ensure their survival against microorganisms (anti-bacterial and antifungal activities) insects and herbivores and also against other plants by means of allopathic active chemicals and they are the source of antimicrobial activities²². They function as signal compounds which attract pollinating or seed dispersing animals and represents adaptive characters that have been subject to natural selection during the evolution^{23,24}. To illustrate the natural occurrence of alkaloids, some of the more common alkaloids are present in the angiosperm families which are particularly rich in these bases, but it should be borne in mind that alkaloid distribution is very uneven and many families lack them together. Alkaloids are generally absent or infrequent in the gymnosperms, ferns, mosses and lower plants²⁰. An alkaloid called angiopteroside was isolated from the rhizome of *Angiopteris evecta*. Hoffm and it was confirmed using x-ray crystallography. Angiopteroside showed significant activity for inhibition of HIV and additionally cytotoxicity against lung cancer²⁵. To correlate with the above said result, our experimental plant *Pyrrosia lanceolata* (L.) Farwell also gave positive result for alkaloid in frond and rhizome samples (Table 1 and 2). Phenolic compounds are the important source for antimicrobial and insecticidal activities²⁶. All the six extracts of frond and rhizome of experimental plants showed the presence of phenolic compounds (Table 1 and 2 and Figure 1e). Usage of herbal compounds may be considered alternative means for melanoma prevention because of low or little toxicity due to their dietary properties or their long history as herbal medicines. Several studies support the positive effects of natural phenolic compounds in their inhibition of melanoma *in vitro* and *in vivo* results arresting the metastatic growth of tumor cells²⁷. In another study several ferns were examined for hydroxylated cinnamic acids and benzoic acids. Coumarin was shown to be present in *Dennstaedtia punctilobula*²⁸. Studies reveal that many ferns possess phenolic compounds, and the above mentioned plant and its phytocompounds coincides with the results obtained from our present investigation and hope our future work will supplement the same phytochemicals in the present plant. Flavanoids in the phytonutrient family are known for high antioxidant activity. Antioxidants are renewed for removing cell-damaging free radicals from the body thereby possibly reducing the symptoms of aging and the risks of chronic diseases such as cancer.

Table 1: Preliminary phytochemical constituents of six extracts of *Pyrrosia lanceolata* (L.) Farwell (Frond)

Name of the extract	Alkaloids	Carbohydrates	Glycosides	Saponins	Phenol	Phytosterols	Terpenoids	Tannins	Flavanoids	Catechins	Fixed oils and fatty acids	Anthroquinones	Phlobatannins
Aqueous	+	++	+	-	+	-	+++	-	+++	-	+	+	-
Ethanol	++	++	+	-	++	-	+++	-	+++	-	+++	++	-
Benzene	+	+	++	-	++	-	+	-	++	-	+	+	-
Chloroform	+	+	+	-	+	-	+	-	++	-	++	+	-
Petroleum ether	++	+	+	-	++	-	++	-	+	-	+	+	-
DMSO	+++	+++	++	-	+++	-	+++	-	+++	-	+++	+++	-

Maximum intensity- +++; Medium intensity- ++; Low intensity- +; Negative- (-)

Table 2: Preliminary phytochemical constituents of six extracts of *Pyrrosia lanceolata* (L.) Farwell (Rhizome)

Name of the extract	Alkaloids	Carbohydrates	Glycosides	Saponins	Phenol	Phytosterols	Terpenoids	Tannins	Flavanoids	Catechins	Fixed oils and fatty acids	Anthroquinones	Phlobatannins
Aqueous	+	+++	+	-	++	-	+++	-	+++	-	+	+	-
Ethanol	++	+++	++	-	+++	-	+++	-	+++	-	+++	++	-
Benzene	++	++	+	-	++	-	+++	-	++	-	+++	+	-
Chloroform	+++	++	+	-	++	-	++	-	+++	-	+	+	-
Petroleum ether	++	++	+	-	++	-	++	-	+++	-	++	++	-
DMSO	+++	+++	+++	-	+++	-	+++	-	+++	-	+++	++	-

Maximum intensity- +++; Medium intensity- ++; Low intensity- +; Negative- (-)

Numerous studies have shown that flavanoids such as apigenin, quercetin and kaempferol effectively suppress melanoma growth and metastasis^{29,30}. These flavanoid phytochemical compound results are in line with our experimental results. *Serpocaulon meniscifolium*, *Phlebodium aureum*, *Microgramma vacciniifolia* and *Pleopeltis pleopeltifolia* plant extracts evidenced with triterpenes compounds such as Hop-22-ene, Hop-21-ene, 22-hydroxy hopane, 22-acetoxy hopane, 22,29,30-tris-nor-hopan-21-one, Neo-hop-13-ene³¹. Terpenoids (Figure 1d) exert wide spectrum of activities such as anti-malarial, anti-plasmodial, anti-tuberculosis, anti-fungal and anti-cancer. Catechins are reported in the present study (Table 1 and 2) and they possess anti-tumorigenic activity against various cancers³². Yet another investigation was made to prove the presence of catechins in ferns. This study was made to find the caffeic acid derivatives in 40 species of ferns. Identifiable or partially identifiable compounds were observed in 14 species, unidentified catechol derivatives in 21 species. The presence of rosmarinic acid in *Blechnum brasiliense* was confirmed. Chlorogenic acid and 1-caffeoylglucose were found to occur, although not together, in several species. Some data are presented concerning the structure of blechnic acid, a new caffeic acid ester isolated from *B. spicant* and detected chromatographically in *Lastrea globuliferum*³³. But in Polypodiaceae family catechins are not available because of the absence of tannins. Catechins are widely distributed in woody plants and vegetable especially in ligneous fern plants

which contain tannin. These compounds may be polymerised and have similar properties as those of tannin³⁴. Thus it explains that the catechins and tannins have some relationship in their occurrence. This study also proves this relationship. Both tannins and catechins were absent in this plant. Anthroquinones are found in bacteria, fungi, lichens, gymnosperms and angiosperms. And have also been isolated from ferns belonging to the polypodiaceae³⁵. According to this literature reference the experimental plant of this study also showed the presence of anthroquinones and it is one of the polypodiaceae members. So phytochemicals have concern in the treatment of many diseases, and they are the rich compounds in ferns which show the similarity with our study. The phytochemical constituents of *Pyrrisia lanceolata* (L.) Farwell and their pharmacological activities are yet to be studied. Since as it is clear from our observations the plant has significant medicinal potential it would be of interest to isolate the responsible phytochemicals and study the mechanism of action responsible behind the observed effects. Our next course of studies will be precisely to isolate and identify the phytochemicals responsible for many effects from this plant and determine the extract mechanisms behind the observed effects. Further research on this particular *Pyrrisia* species may help in the isolation of therapeutically potent compound which can be finally be subjected to pharmacological activities and clinical trials, thus leading to opening up new avenue in the use of natural products for therapeutic purpose.

Figure 1: Various results of phytochemical analysis of *Pyrrisia lanceolata* (L.) Farwell

1a shows dried specimen of *Pyrrisia lanceolata*, 1b shows positive carbohydrate result, 1c shows positive alkaloid result, 1d shows positive terpenoid result, 1e shows positive phenol result, 1f shows positive fats and fixed oils result

Figure 2: Herbarium voucher Specimen of *Pyrrisia lanceolata* (L.) Farwell

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