Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI - 10.26479/2015.0102.04**

PHYTOCHEMICAL ANALYSIS OF FOUR *CHEILANTHES* SPECIES FROM NORTHERN WESTERN GHATS OF INDIA

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ABSTRACT: Background: The objective of the current study was to find out the presence of phytochemicals in the methanol extracts of four fern *Cheilanthes* sp.: *C.farinosa, C.anceps, C.tenu ifolia,* and *C. albomarginata* which is collected from Northern Western Ghats of India. Used for qualitative and quantitative screening methods. Methods: In qualitative analysis, the phytochemical compounds such as steroids, reducing sugars, triterpenoids, sugars, alkaloids, phenolic compounds, flavonoids, saponins, tannins, anthroquinones and amino acids were screened in four *Cheilanthes* sp extracts by using standard methods. *Results:* The methanol extract of the fern *C.farinosa,* presented positive results for 10 phytochemical tests. *C.anceps,* extract showed positive results for 9 tests. In methanol extracts of the *C.tenuifolia,* and *C. albomarginata* presented tests were positive. In quantitative analysis the significant secondary metabolites such as alkaloids, phenolic compounds, flavonoids, saponins and tannins were tested in four ferns *Cheilanthes* sp of extracts. The methanol extract of *C.farinosa* presented maximum amount of phytochemicals as soon as compared with further solvent extracts. *Conclusions:* More bioactive compounds will be isolated from four ferns in Pteridaceae family such as *Cheilanthes* sp.: *C.farinosa, C.anceps, C.tenuifolia,* and *C. albomarginata* and they may be used for medicinal determinations in future so enrich today's openings in nutraceuticals and food applications for human health. To the best of our knowledge, this is the first paper presenting complete data on phytochemical analysis of four cheilanthes species from Northern Western Ghats of India

KEYWORDS: Phytochemicals, Pteridaceae, Ferns, Qualitative and Quantitative screening

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1.INTRODUCTION

Traditional medicinal information is important not only for its potential contribution to the drug development as well as people's healthcare [1]. According to the World Health Organization 80% of the world's population mostly individuals of developing countries depend on plant-derived medicines for their healthcare needs [2]. Pteridophytes (ferns and fern allies) are called as reptile group of plants and are one of the primary groups of vascular plants. Most of the aboriginal people are not well identified about the uses of Pteridophytes Ever since it's not simply available like flowering plants. Pteridophytes have a vital role in the earth's biodiversity. Commercial and medicinal values of higher plants have been investigated carefully, unfortunately Pteridophytes have been unnoticed. That's why there is lack information available on the literature regarding medicinally important except a few studies [3-7]. For that reason, this study was undertaken in order to discover the detailed information on Pteridophytes used by ethnic and non-tribal people in Northern Western Ghats India. Plants are gifted with numerous phytochemical molecules such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and additional metabolites, which are rich source available in antioxidant activity, [8, 9]. Studies have revealed that lots of these antioxidant compounds have anti-inflammatory, antitumor, antimutagenic, anticarcinogenic, antibacterial, antiatherosclerotic, as well as antiviral activities [10,11].The eating of natural antioxidants has been related with reduced hazards of cancer, diabetes, cardiovascular disease and other diseases associated with ageing [12, 13] In modern years, there has been a universal trend on the way to the use of the natural phytochemicals existing in berry crops, herbs, oil seeds, fruits and vegetables beans, teas etc. [14-16].In modern centuries, secondary plant metabolites (phytochemicals), up to that time unknown pharmacological activities have been widely studied as a source of therapeutic agents [17]. Therefore, it is predicted that phytochemicals with adequate antibacterial value will be used for the handling of bacterial infections [18]. Pteridophytes are not infected by microbial pathogens, which may be one of the significant aspects for the evolutionary achievement of pteridophytes and the information that they stay alive for more than 350 million years. In view of the rich diversity of Indian medicinal plants as well as Pteridophytes, it is predictable that, the screening of plant extract for antibacterial activity might be helpful for humans and plants diseases [19]. The purpose of this study was to evaluate the phytochemicals from methanol extracts of four Cheilanthes species

2. MATERIALS AND METHODS

2.1 Collection of plant materials

Plant materials of four Cheilanthes species were obtained from different localities of Northern Western Ghats of India (Cheilanthes farinosa (Forssk.) Kaulf. — Molem locality, GPS: 150 22l09ll N′,740 12l 44ll E; Cheilanthes anceps Sw, — Mahabaleshwar locality, GPS: 170 55l 31ll N, 730 39l 45ll E; Cheilanthes tenuifolia (Burm.f.) Sw, — Gaganbawada locality, GPS: 160 31l 58ll N′,730 49l 5ll E; Cheilanthes albomarginata Clarke — Amboli locality, GPS: 150 57l 42ll N, 730 59l 48ll E;). Specimens were authentically identified with help of Dr.Manisha kale (Associate Professor Department of Botany, Jaysingpur College Jaysingpur, Maharashtra, India. The Whole Plant along with rhizome of Cheilanthes sp. was collected from Northern Western Ghats, India. The Cheilanthes species were cleaned and separated into dry powder form. The CSWPR was stored in a freezer (−20°C) until further analysis**.**

2.2 Methanol extraction

10 g of each plant powder was added to 100 ml of methanol in a conical flask as well as plugged with cotton wool. The supernatant was collected after 24 hours and the solvent was evaporated to make the crude extract and stored at 4℃.

2.3 Qualitative phytochemical analysis

The qualitative phytochemical analysis of methanol extracts of Cheilanthes farinosa (Forssk.)Kaulf. Cheilanthes anceps Sw, Cheilanthes tenuifolia (Burm.f.) and Cheilanthes albomarginata Clarke was conducted following the standard procedures [20].

2.4 Quantitative phytochemical analysis

The quantitative phytochemicals which are present in the methanol extracts of Cheilanthes farinosa (Forssk.)Kaulf.Cheilanthes anceps Sw, Cheilanthes tenuifolia (Burm.f.); Cheilanthes albomarginata Clarke were determined and quantified by standard procedures.

2.5 Determination of Total Phenolic Content (TPC)

The total phenolic content in the Whole Plant with rhizome of Cheilanthes species (CSWPR) extracts was determined spectrophotometrically utilizing the Folin-Ciocalteu method. A portion of a total amount of a solution of 125µl extract mixed with 1.8 ml of Folin Ciocalteu reagent and which is distilled water diluted 10-fold with previously. Before adding 1.2 ml of 15% sodium Carbonate solution in distilled water the solution was allowed to stand at 25°C for 5 min and absorbance was measured at 765 nm using a spectrophotometer. After 90 min at room temperature, this was compared as expressed mg of Gallic acid equivalents per g (mg GAE g−1) of dry powder to standard curve of gallic acid concentrations. [21].

2.6 Determination of Total Flavonoids Content (TFC)

The colorimetric method was measured by Total flavonoid contents of all four extracts [13].

Cheilanthes species extracts (0.5 ml) were mixed with methanol (1.5 ml), to which 10% aluminum chloride (0.1 ml), 1 M potassium acetate (0.1 ml) and distilled water (2.8 ml) were added. The solution was vortexed, allowed to stand for 30 min at room temperature. The UV–vis spectroscopic analysis using an absorbance of reaction mixture was measured at 415 nm (Hitachi U-2800; Hitachi, Tokyo, Japan). According to the standard curve prepared for rutin and the concentration of flavonoids was reported as mg of rutin equivalents per g (mg RE g−1) of sample was quantified the total flavonoids content.

2.7 Determination of Total Alkaloids

Five grams of the plant sample was taken in a 250ml beaker and 200ml of 10% acetic acid in ethyl alcohol was added. The mixture was covered and allowed to stand for 4 hours. It was before filtered and the filtrate was concentrated on a water bath till it spreads a quarter of its original volume. Concentrated Ammonium hydroxide was added till precipitation was complete. The mixture was allowable to settle and the precipitate collected on a weighed filter paper and washed with dilute Ammonium hydroxide. The precipitate, alkaloid, was dried and weighed. The percentage alkaloid was calculated by variance.[22]

2.8 Determination of Total Tannins

500 mg of the plant sample was taken into a 50 ml plastic bottle and added 50 ml of distilled water as well as shaken for 1 hour in a mechanical shaker. it was filtered into a 50 ml volumetric flask and prepared to the mark. At that time 5 ml of the filtered was pipetted out into a test tube and simultaneously mixed with 2 ml of 0.1 M FeCl3 in 0.I N HCl and 0.008 M potassium Ferro cyanide. The absorbance was measured at 120 nm within 10 min [23].

2.9 Determination of Total Saponins

The plant samples were powdered and 20 gms of weighed into a 250ml conical flask.100 ml of 20% aqueous ethanol was added. The mixture was heated over a hot water bath for 4 hours with incessant stirring at about 55°C. The mixture was filtered with a Whatman No.42 paper and the residue re-extracted with one more 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was moved into a 250 ml centrifuge funnel and 20 ml of diethyl ether was added and shaken dynamically. The aqueous layer was recovered whereas the ether layer was discarded. The purification procedure was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed two times with 10 ml of 5% aqueous NaCl. The residual solution was heated in a water bath. After evaporation the residue was dried in the oven to a constant weight. The % saponin was calculated by difference.[24].

3. RESULTS AND DISCUSSION

3.1 Qualitative phytochemical analysis

In qualitative analysis of methanol extracts of *Cheilanthes sp.: C.farinosa, C.anceps, C. tenuifolia*

and C. albomarginata revealed positive results for 10 phytochemical tests. 10 phytochemical tests be present positive in methanol extract of the fern *C.farinosa.* In *C.anceps*, extract 9 tests were positive. In methanol extracts of the ferns C.tenuifolia and C. albomarginata 7 tests are present positive. Maximum tests be present positive in methanol extracts of C.farinosa, followed by C.anceps, C.tenuifolia, and C. albomarginata extract (Table 1)

Sr.No	Plant Parts	Compound	*C.farinosa*	*C.anceps*	*C.tenuifolia*	*C.albomarginata*
1	Whole Plant along with Rhizome	Steroids	+	+	+	+
2		Triterpenoids	-	+	+	+
3		Reducing sugars	+	+	-	-
4		Sugars	-	-	-	+
5		Alkaloids	+	+	+	+
6		Phenolic Compounds	+	+	+	+
7		Flavonoids	+	+	+	+
8		Catechins	-	-	-	-
9		Saponins	+	+	+	+
10		Tannins	+	+	+	+
11		Anthroquinones	-	-	-	+
12		Amino acids	-	+	-	+

*Note: + sign indicates compounds are present and - Sign indicates compounds are absent

Table 1: Qualitative analysis of phytochemicals of four *Cheilanthes* sp

Phytochemical compounds such as alkaloids, phenolic compounds, flavonoids, saponins, steroids, reducing sugars, triterpenoids, sugars, tannins, anthroquinones also amino acids was screened in four cheilanthes ferns extracts. Among these secondary metabolites compounds such as alkaloids, phenolic compounds, flavonoids, saponins and tannins are significant as well as responsible for medicinal values of the relevant plant. Thesefive compounds are present in all the four Cheilanthes sp.: C.farinosa, C.anceps, C.tenuifolia, and C. albomarginata. All the four Cheilanthes sp extracts were exposed to further analytical tests for the quantification of phytochemical compounds

3.2 Quantitative phytochemical analysis

The amount of phytochemicals which are instigating in the four Cheilanthes sp extract was quantitatively determined using standard procedures. All the four Cheilanthes sp extract indicated diverse amount of phytochemicals. Between the five constituents flavonoids content are maximum in all the four Cheilanthes sp followed by alkaloids and phenolic compounds (Table 2).

Sr.No	Plant Parts	Compounds	*C. farinosa*	*C. anceps*	*C. tenuifolia*	C. *albomarginata*
5	Whole Plant along with Rhizome	Alkaloids	11.05 ± 0.10	10.10 ± 0.15	10.02 ± 0.30	14.05± 0.35
6		Flavonoids	0.23±0.017	0.21±0.012	0.21±0.013	0.17±0.019
7		Phenolics	0.40±0.012	0.31±0.018	0.43±0.014	0.36±0.016
9		Saponins	05.32 ± 0.14	09.00 ± 0.30	06.10 ± 0.25	09.10 ± 0.65
10		Tannins	02.18 ± 0.10	06.40± 0.15	03.40 ± 0.65	08.25 ± 0.30

Table 2: Quantitative analysis of phytochemicals (mg/g) of four *Cheilanthes* sp.:

DISCUSSION:

The amount of tannins and saponins was very low in the four Cheilanthes sp ferns extract. C.farinosa extract contained 11 mg of alkaloids, 0.23 mg of flavonoids, 0.40 mg of phenolic compounds, 5 mg of saponins and 2 mg of tannins. In C.anceps, extract 10 mg of alkaloids, 0.21 mg of flavonoids, 0.31 mg of phenolic compounds, 9 mg of saponins and 6 mg of tannins are occurred. In C.tenuifolia extract contains 10 mg of alkaloids, 0.21 mg of flavonoids, 0.43 mg of phenolic compounds, 6 mg of saponins and 3 mg of tannins were identified. C. albomarginata extract contained 14 mg of alkaloids, 0.17 mg of flavonoids, 0.36 mg of phenolic compounds, 9 mg of saponins and 8 mg of tannins.[25] Assessed the petroleum ether, chloroform, acetone, ethanol and aqueous extracts of fern Hemionitis arifolia for earliest phytochemical screening. The ethanolic and aqueous extracts indicated the presence of flavonoids, carbohydrates, phenolic compounds and sterols remained the major Phyto constituents. In the current study methanol extracts of ferns are screened for phytochemical analysis. [26] Examined the Phyto-constituents of Adiantum caudataum, Adiantum latifolium, Adiantum lunulatum, Christella dentate and Christella parasitica, to deliver chemical marker and inter-specific variation among the medicinally important genus. A total of four cheilanthes plants and 30 extracts were studied for the phytochemical screening. The crude extracts of A. caudataum, A. latifolium, A. lunulatum, C. dentata and C. parasitica presented diverse degree of Phyto-constituents using reference to solvents of the plant extracts. Pragada et al. (2011)[27]The Preliminary phytochemical analysis and quantification carried out of total phenols, antibacterial activities and in-vitro antioxidant of the hydro alcoholic (70% ethanol).The extract of Acalypha indica.[28] screened the methanol extract of roots of Gentiana kurroo Royle (Gentianaceae) a significant and widespread medicinal plant of Kashmir Himalaya for the occurrence of several bioactive plant metabolites and analgesic activity. The screened Adiantum capillus - veneris for phytochemical analysis discovered that the presence of tannins, alkaloids, saponins, cardiac glycosides, terpenes, flavonoids, phenolics, and carbohydrates [29,30]. Hence in the current study

of all the four Cheilanthes sp.: C.farinosa, C.anceps, C.tenuifolia and C. albomarginata extracts revealed that the presence of alkaloids, flavonoids and saponins. This study gives the information to further research in the technique of isolation and identification of the bioactive compounds from the selected Cheilanthes fern species using chromatographic and spectroscopic techniques. The Cheilanthes species whole plant along with rhizome an abundant source of phytochemicals as well as phenolic compounds and compacts to the expansion of value-added products from Cheilanthes species whole plants along with rhizome therefore enrich today's opportunities in pharmaceuticals, nutraceuticals and food applications for Human Strength.

5.ACKNOWLEDGEMENT

P.N Ghorpade and S.B Thakar two of the authors are Thankful to Department of Biotechnology Shivaji University, Kolhapur Maharashtra India for Laboratory Facilities.

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