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PHYTOCHEMICALS STUDIES ON THREE EPIPHYTIC FERNS FROM MAHABALESHWAR AND PANCHGANI HILLS

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ABSTRACT: The current study has been proposed to find out the presence of different constituents in the methanol, ethanol, petroleum ether, chloroform and water extract of epiphytic ferns like *Asplenium indicum, Lepisorous nudus, Microsorum membranecium* by both qualitative and quantitative screening method. The phytochemical compounds such as alkaloids, flavonoids, steroids, tannins, phenolic, cardioglycosides, saponins, terpenoides, quinones, glycosides, were screened in ferns by using standard protocols. While In quantitative analysis the phytochemical compounds such as total tannins and total flavonoids were quantified. In qualitative analysis methanolic extract showed positive result for 10 phytochemical tests so in quantitative analysis tannins and flavonoids were tested in methanolic extracts. The result shows the highest value in extract of vegetative and reproductive phases of *Lepisorus nudus* than *Asplenium indicum* and *Microsorum membranecium.* All the studied fern shows variable bioactive compounds which can be used for medicinal purpose.

KEYWORDS: Pteridophyte, Phytochemical, Qualitative, Quantitative, bioactive compounds.

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1. INTRODUCTION

Pteridophytes are one of the oldest and primitive vascular plant groups on earth. The study of pteridophytes which occupy a unique position between non seed bearing and seed bearing plants. This group of plants has versatile in nature medicinal, ecological, and ornamental, ethanobotanical, environmental and evolutionary significance. There are about 45,000 plant species and more than

1300 species of pteridophytes are distributed in high altitudinal region of India [1]. Very few workers have been done on the phytochemical composition of ferns even their ethanomedicinal importance has been explored and studied by numerous authors [2]. The medicinal uses of some ferns and pteridophytes of India have been described [3], [4]. These plants groups had an important role in folklore medicine and are being used as valuable sources of food and medicine for the prevention of illness and maintenance of human and animal health. Later on different workers carried out modern biological and pharmaceutical studies on pteridophytes [5- 9]. In recent years, bioactive components prior with unknown pharmacological activities have been widely explored as source of medicinal agent [10]. This bioactive component of ferns mainly belongs to the phenolic; flavonoid, alkaloid, and terpenoid families [11] .Studies have shown that many of these compounds possess anti-inflammatory, antitumor, antimutagenic, antibacterial, anticarcinogenic and antiviral activities [12]. In view of these *Aspenium indicum, Lepisorus nudus, Microsorum membraneceum* ferns was studied extensively for its potential phytochemical constituents to prove its medicinal values.

2. MATERIALS AND METHODS

Collection of plant material

The fresh fern plant material was collected from Mahabaleshwar and Panchgani hills and authenticated from Shivaji University Kolhapur. The collected plant material was washed thoroughly with tap water and then rinsed with distilled water then shied dried. The dried leaf material was powdered using mortle and pestle 0.5 gm. of powder was extracted with 100 ml of ethanol, methanol, acetone, chloroform, petroleum ether and water using soxhlet apparatus for 6 hours at 55 -65 temperature. The extraction were collected in petriplates and the solvents were evaporated to dryness the residue left over were transferred to small vial and stored at 400^0 C in refrigerator for further analysis using the standard procedure. [13], [14], [15].

Pre phytochemical screening

1) Test for alkaloids

Mayer's reagent: 1 ml of extract, 2 ml of Mayer's reagent is added formation of yellow colored precipitate indicates the presence of alkaloids.

Dragendroff's reagent: 1 ml of extract, 2 ml of Dragendroff's reagent is added presence of alkaloids conformed by the formation of red colored precipitate.

2) Test for flavonoids

3 ml of leaf extract added with 4 ml of 1 N NaOH. Formation of intense yellow colour. On addition of dilute acid which become colorless indicates the presence of flavonoids.

3) Test for carbohydrates

Fehling's reagent: 1 ml of Fehling's A (copper sulphate in distilled water) and 1 ml of Fehling's B (Potassium tartarate and sodium hydroxide in distilled water) reagents were added and boiled for minute. Then above mixture add equal volume of test solution. The solution was heated in boiling water bath. Formation of brick red precipitate indicates the presence of carbohydrates.

Benedict's reagent: 1 ml extracts were treated with Benedict's reagent and heated gently .Orange red ppt form.

4) Test of glycosides

Borntragder's test: 1 ml extract, 1 ml benzene and 0.5 ml dilute ammonia solution were added. Formation of rose pink color in the ammonical layer indicates the presence of glycosides.

Legal's test: 1 ml extract were treated with sodium nitroprusside in pyridine and sodium hydroxide. Formation of pink to red color indicates presence of glycosides.

5) Test for phenols

Ferric chloride test: 1 ml extract, 1 ml of 5% ferric chloride solution was added. Formation of bluish black color indicates the presence of phenol.

6) Test for Quinones

1 ml of the leaf extract, mixed with 1 ml of concentrated sulphuric acid. Formation of red color showed the presence of quinones.

7) Test for Saponins

Foam test: 0.5 gm of extract was shaken with 2 ml of boiling water allowed to cool and shake well formation of the presence of saponins.

8) Test for Steroids

1 ml of the leaf extract, 2 ml of chloroform and 1 ml of sulphuric acid were added .Formation of reddish brown ring at interface indicates the presence of steroids.

9) Test for Tannins

1 ml of leaf extract added with 5% $FeCl_3$ added to the filtrate formation of brownish green color showed the presence of tannins.

10) Test for terpenoides

1 ml of extract was added with 2 ml of chloroform and concentrated sulphuric acid was mix carefully. Formation of reddish brown color at the interface showed the presence of terpenoid.

Quantitative phytochemical analysis

Determination of total tannins content

The tannins were determined by Folin-Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of FolinCiocalteu

phenol reagent, 1 mi of 35% sodium carbonate solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. a set of reference standard solutions of tannic acid (20, 40, 60, 80, 100 µg/ ml) were prepared in tannin content was expressed in terms of mg carried out in triplicate of tannic acid equivalents/ g of dried sample [16] [17]. The same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 700 nm with an UV/ Visible spectrophotometer.

Determination of total flavonoids content

Total flavonoids content was determined by aluminum chloride method using rutin as standard. 0.5 ml of leaf extracts of plants material at a concentration of 1 mg/ml was taken, and the volume was made up to 3 ml with methanol. Then 0.1 ml $AlCl_3$ (10%), 0.1 ml of potassium acetate and 2.8 ml distilled water were added sequentially. The test solution was vigorously shaken .Absorbance was recorded at 415 nm after 30 minutes of incubation. A standard calibration plot was generated at 415 nm using known concentration of rutin .The concentrations of flavonoids in the test sample were calculated from the calibration plot and expressed as mg rutin equivalent /g of sample. [18]

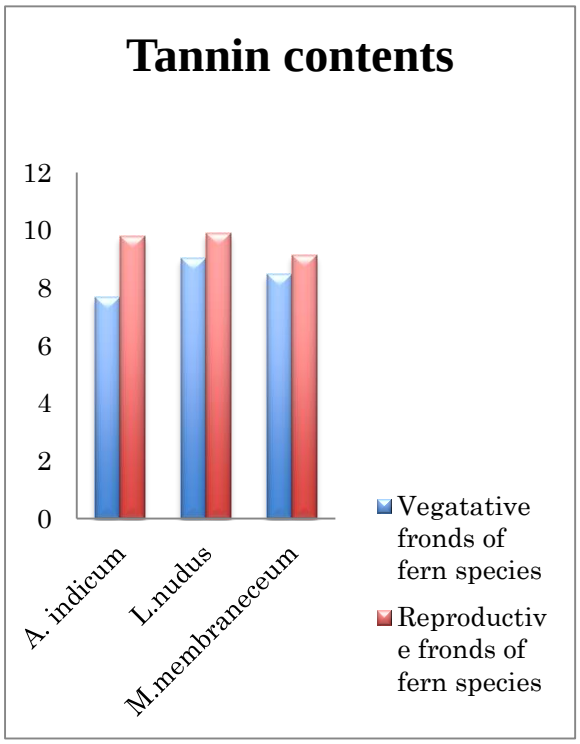
Table 1: Qualitative Phytochemical analysis of Extracts of Pteridophyte Species in Different Solvent System

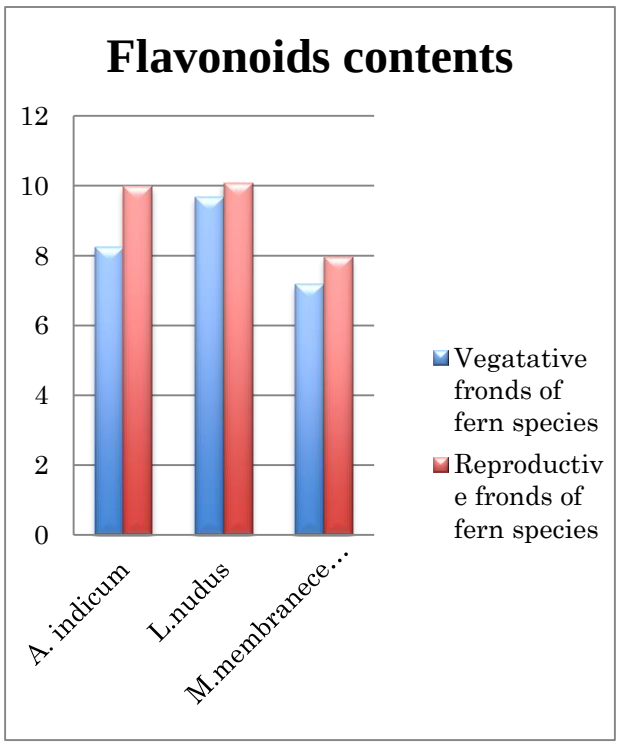
Phytochemicals	*A. indicum*					*L. nudus*					*M. membraneceum*				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Alkaloids	-	+	+	+	-	-	+	+	-	-	-	+	-	+	-
Flavonoids	+	+	+	-	-	+	+	+	+	-	+	+	+	+	-
Carbohydrates	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Glycosides	-	-	+	-	-	-	-	+	-	-	-	-	+	+	-
Phenols	+	+	+	-	-	+	+	+	-	-	+	+	+	+	-
Quinones	-	-	+	-	-	+	-	+	-	-	+	+	+	-	-
Saponins	+	-	-	+	-	+	-	+	-	-	+	-	+	+	-
Steroids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	-	-	-	+	+	+	-	-	+	+	-	-
Terpenoides	+	-	-	-	-	+	-	-	-	-	-	+	+	+	-

1.-Chloroform 2.-Ethanol 3.-Methanol 4.-Petroleum ether 5.-Water (+):-Positive (-):- Negative

Table 2: Quantitative analysis of phytochemicals (mg/g) in three fern species

Phytochemicals	*Asplenium indicum*		*Lepisorus nudus*		*Microsorum membraneceum*	
	Veg-Stage	**Rep-Stage**	**Veg-Stage**	**Rep-Stage**	**Veg-Stage**	**Rep-Stage**
Total Flavonoids	8.24 ± 0.3	9.97 ± 0.1	9.67 ± 0.2	10.08 ± 0.4	7.18 ± 0.1	7.96 ± 0.1
Total Tannins	7.67 ± 0.4	9.78 ± 0.2	9.01 ± 0.1	9.89± 0.1	8.47 ± 0.1	9.12 ± 0.3





3. RESULTS AND DICSUSSION

Qualitative phytochemical analysis

When the environmental condition is not favor for plants they produce secondary metabolites [19]. The secondary metabolites produced against the various adverse environmental conditions are alkaloids, flavonoids, tannin,saponins, phenols steroids, quinones etc. [20].Therefore in the present study 3 ferns were screened for the phytochemical constituents for five different solvents. The present investigations of phytochemical screening of methanolic extracts of *Asplenium indicum, Lepisorus nudus*, and *Microsorum membraneceum* showed strong positive result for ten phytochemicals. In ethanolic extracts of the ferns *Asplenium indicum* and *Lepisorus.nudus* showed 6 and 8 bioactive compounds in *microsorum mebranecium*. Alkaloids occur in ethanolic, methanolic and petroleum ether extracts of *Asplenium indicum, Lepisorus nudus* and M*icrosorum membraneceum*. Flavonoids are present in extracts of chloroform, ethanolic and methanoic of three ferns and in petroleum ether extracts of *Lepisorus nudus* and *Microsorum membraneceum*. Carbohydrates are present in ethanolic, methanolic, petroleum ether extracts of all ferns and water extract of *Asplenium indicum, Lepisorus nudus* and chloroform extract of *Lepisorus nudus* and *Microsorum membraneceum*. Glycosides, Quanines and terpenoides mostly present in methonolic extracts. Phenols are present in chloroform, ethanolic mathanolic extracts of all ferns and petroleum ether extract of *Microsorum membraneceum* plant. Chloroform extracts of all three ferns shows saponins. Petroleum ether extracts of *Asplenium indicum* and *Microsorum. Membraneceum* and methonolic extracts of *Lepisorus nudus* and *Microsorum membraneceum* .Steroids are present in all extracts of ferns. Tannins are present in methanolic and ethanolic extracts of all ferns and chloroform extracts of *Asplenium indicum* and petroleum extracts of *Lepisorus nudus*.

Quantitative phytochemicals analysis

Analysis shows total flavonoids in vegetative and reproductive stage of *Asplenium indicum* (8.24 ± 0.3 mg/,g 9.97 ± 0.1 mg/g) *Lepisorus nudus* (9.67 ±0.2 mg/g ,10.08 ± 0.4 mg/g) *Microsorum membraneceum* (7.18 ± 0.1 mg/g, 7.96 ± 0.1 mg/g) .Total tannins in vegetative and reproductive stages of *Asplenium indicum* (7.4 ±0.4 mg/g ,9.38±0.2 mg/g) *Lepisorus nudus* (9.01 ± 0.1 mg/g, 9.89 ± 0.1mg/g) *Microsorum membranecium* (8.47±0.1 mg/g, 9.12± 0.3 mg/g).

DISCUSSION

Phytochemical screening is important method to identify new source of therapeutically and industrially valuable compound having medicinal significance to make the best and judicious use of available natural wealth [21], [22]. The amount of phytochemicals which are found in the methanolic fern extracts was quantitatively determined by standard procedures. A John De Britto et al. 2012 [2] performed the petroleum ether, benzene, chloroform, methanol, water extract of the fern *P.biauriata, L. flexiuosam, H. arifolia, A. radiate, A. latifolium* for preliminary phytochemical screening in which methanolic extract showed maximum numbers of bioactive compounds.

Similarly in the present study chloroform, methanolic, ethanolic, petroleum ether and aqueous extracts of ferns are screened for phytochemical analysis and methanolic solvent extract showed strong positivity for 10 bioactive compounds. D Herin Sheeba Gracelin et al.(2012) [23] also reported preliminary phytochemical analysis and quantification of total alkaloids, flavonoids, phenolics, saponins, tannins of methanolic extracts of *P. confusa, P. vittata, P. argyreae, P. biaurita, P. multiaurita.* Muraleedharannair et al. (2012) [20] evaluated the phytoconstituents of *Adiantum caudataum*, *Adiantum latifolium, Adiantum lunulatum, Christella dentate* and *Christella parasitica* to provide chemical marker and inter-specific variation between the medicinally important geniuses. In present study total three plants and five solvent systems are carried out for phytochemical screening. Flavonoids are important secondary metabolites soluble in water free radical scavengers that protect the cells from oxidative cell damage.[24] Flavonoids are important secondary metabolites play important role in anti-allergic, anti-inflammatory, anti-microbial, and anti-cancer activity.[25]. Some earlier studies have also reported the presence of flavonoids *Equisetum arvense* [26], *Pteridium aquilinum* [27], and *Adiantum venustum* [28] *Asplenium septentrionale* [29]. In the present study Flavonoids are present in extracts of in plant protection from predation. They possess anti-parasitical anti-inflammatory, anti-microbial, cytotoxicity, and antioxidant activity. This was quantified in our study. Kalpana Devi Rajesh et al. [30] evaluated qualitative and quantitative phytochemical analysis of some important pteridophytes of western ghat with the solvent aqueous, ethanolic and petroleum ether extracts of *Actinopteris rediata, Drynaria quercifolia, Dryopteris cochleata, Pityrogramma calomelanos*. Analysis exhibit tannin present in most of tested plants Tannins may have the potential values as cytotoxic agents [31]. In our study tannin estimated qualitatively and quantitatively.

4. CONCLUSION

The present study reveals that among the five solvents employed methanolic extracts performed well to chloroform, ethanolic and methanoic of three ferns and in petroleum ether extracts of *Lepisorus nudus* and *Microsorum membraneceum*. Alkaloids are important bioactive components. They act on the nervous system as stimulators or sometimes as poisons. Literature survey has also exhibited the presence of alkaloids in ferns like *Adiantum venustum* [28] *Pteridium aquilidum* [27] and *Equisetum arvense* [26]. In our study alkaloids occurs in ethanolic, methanolic and petroleum ether extracts of three plants. Showed the phytochemicals rather than ethanolic, petroleum ether, chloroform and aqueous extracts. Quantitative analysis showed in *Lepisorus nudus* higher amount of flavonoids and tannins presents in both phases as compared to *Asplenium indicum* and *Microsorum membranecium.* The results clearly showed that three ferns own rich medicinal properties.

CONFLICT OF INTEREST

Authors have no conflict of interest.

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