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Phytochemical investigation of Dwarf Water Clover

Arnab Roy*[1]
[*1Department of Pharmacology & Toxicology, , Bengal School of Technology, Delhi Rd, Chinsurah, Sugandha, WB 712102, India.]

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ABSTRACT
The aim of this study was to investigate the presence of phytochemicals in Marsileaminuta Linn (belonging to the family **Marsileaceae**), a common aquatic medicinal fern. The extracts obtained from petroleum ether, benzene, chloroform, methanol, and water were subjected to both qualitative and quantitative screening methods. In the qualitative analysis, various phytochemical compounds including steroids, reducing sugars, triterpenoids, sugars, alkaloids, phenolic compounds, flavonoids, saponins, tannins, anthraquinones, and amino acids were examined in the five different solvent extracts. Among them, the methanol extract of the fern displayed positive results for 10 phytochemical tests, while the benzene extract exhibited positive results for 9 tests. The chloroform and petroleum ether extracts showed positive results for 7 tests, and the aqueous extract showed positive results for 5 phytochemical tests. In the quantitative analysis, the concentrations of important secondary metabolites such as alkaloids, phenolic compounds, flavonoids, saponins, and tannins were measured in all the fern extracts. The methanol extract showed the highest amount of phytochemicals compared to the other solvent extracts.
In this study, our objective was to identify and analyze the bioactive compounds within the plant, which could potentially contribute to its therapeutic properties. The results of our investigation offer valuable knowledge regarding the chemical composition of this plant, laying the groundwork for future exploration of its pharmacological effects.
Key Words: Marsileaminuta, flavonoids, phenolic compounds, alkaloids, saponins and tannins

I. INTRODUCTION

Plants have long been recognized as a valuable source of medicinal compounds due to their production of various bioactive molecules. These molecules, which likely evolved as a defence mechanism against predators and infections, offer potential therapeutic benefits[1]. Surprisingly, despite the vast number of flowering plants on Earth, only a mere one percent has been thoroughly examined to uncover their chemical composition and medicinal properties[2].

Herbs, commonly utilized in traditional medicine, have been extensively documented for their healing potentials. However, a comprehensive assessment of the local flora used in traditional medicine is crucial to identify their biological activities and isolate active compounds, ultimately leading to drug development [3].

One such plant genus of interest is Marsilea, a widespread water fern found globally. Among its species, Marsileaminuta Linn (Belonging from the family Marsileaceae) is a common Indian plant abundant in wet and flooded lowlands. In Ayurveda, the traditional Indian medicine system, the entire plant is utilized for its sweet, astringent, cooling, digestive, diuretic, hypnotic, and expectorantproperties. It has also been mentioned as a treatment fordiarrhea, psychopathy,skin diseases,cough, bronchitis and fever[4].

Plants possess an array of phytochemical compounds, including terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, known for their antioxidant activity. Numerous studies have revealed that many of these compounds exhibit anti-inflammatory, antitumor, antiatherosclerotic, antimutagenic, anticarcinogenic, antibacterial, and antiviral property [5].

Considering the promising attributes of Marsileaminuta, exhaustive research was conducted to identify its potential phytochemical constituents and validate its medicinal value.

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Fig.1: Dwarf water clover

II. MATERIALS AND METHODS

Plant extract preparation: Fresh and undamaged leaves of the M. minuta fern, known to be in good health and free from any diseases, were carefully collected from the fields located in Arambagh (near PC Sen railway station), specifically in the Hooghly district (WB, India). To ensure accurate Authentication, the collected leaves were compared with existing herbarium specimens housed in Shibpur Botanical Garden (WB, India).

Following collection, the leaves were thoroughly washed to remove any impurities and then dried in a shaded area. Once completely dried, the leaves were transformed into a fine powder using a blender. A quantity of 25 grams of this powder was subjected to successive extraction processes using different solvents in a Soxhlet extractor for a period of 48 hours. The solvents used in the extraction process included petroleum ether, benzene, chloroform, methanol, and distilled water. After the extraction process, all the obtained extracts were concentrated and carefully stored in airtight containers for future use[6].

Qualitative Analysis of Phytochemicals: A standard procedure was employed to conduct a qualitative phytochemical analysis of methanol, petroleum ether, chloroform, benzene and aqueous extracts obtained from M. minuta.

Quantitative Analysis of Phytochemicals: Standard procedures were employed to identify and measure the phytochemicals found in the methanol extract of M. minuta[7,8].

Determination of total phenolic compounds[9, 10]

An accurately weighed sample extract weighing 100 mg was dissolved in 100 ml of triple distilled water (TDW). Subsequently, 1 ml of this solution was transferred to a test tube. Then, 0.5 ml of 2N Folin-Ciocalteu reagent and 1.5 ml of 20% Na2CO3 solution were added. The volume was adjusted to 8 ml with TDW, followed by vigorous shaking. The mixture was allowed to stand for 2 hours, and the absorbance was measured at 765 nm. These measurements were used to determine the total phenolic content by referring to a standard calibration curve created using various diluted concentrations of gallic acid.

Determination of total Saponins[11]

The ground samples, weighing 20 g each, were placed into a conical flask. To this, 100 cm3 of a 20% aqueous ethanol solution were added. The flask was then heated on a hot water bath, with continuous stirring at approximately 55°C, for a duration of 4 hours. After heating, the mixture was filtered, and the residue was subjected to another extraction using 200 ml of 20% ethanol. The combined extracts were concentrated to 40 ml using a water bath at around 90°C.

Next, the concentrated solution was transferred to a 250 ml separatory funnel. To this, 20 ml of diethyl ether was added and vigorously shaken. The aqueous layer was separated andretained, while the ether layer was discarded. This purification process was repeated, and then 60 ml of n-butanol was introduced. The combined n-butanol extracts were washed twice with 10 ml of a 5% aqueous sodium chloride solution.

Subsequently, the remaining solution was heated in a water bath. After evaporation, the samples were dried in an oven until a constant weight was achieved. The saponin content was then calculated based on these dried samples.

Determination of total Flavonoids[12]

The method relies on the creation of a complex between flavonoids and aluminum, which exhibits its maximum absorption at 415nm. To perform the analysis, 100µl of plant extracts dissolved in methanol at a concentration of 10 mg/ml were combined with 100µl of 20% aluminumtrichloride in methanol and a drop of acetic acid. The resulting mixture was then diluted with methanol to a final volume of 5ml. After 40 minutes, the absorbance at 415nm was measured. Blank samples were prepared using 100µl of plant extracts, a drop of acetic acid, and methanol, which were also diluted to 5ml. additionally, the absorbance of a standard rutin solution (0.5 mg/ml) in methanol was measured using the same procedure. All measurements were performed in triplicate.

Determination of total tannins[13]

To start the experiment, a 500 mg portion of the sample was carefully weighed and placed

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inside a plastic bottle with a 50 ml capacity. Next, 50 ml of distilled water was added to the bottle, and the contents were vigorously shaken for a duration of 1 hour using a mechanical shaker. The mixture was then filtered into a 50 ml volumetric flask, and the flask was filled up to the mark to ensure accurate volume.

Subsequently, 5 ml of the filtered solution was transferred via pipette into a test tube. In the test tube, the solution was combined with 2 ml of 0.1 M FeCl3 solution in a mixture containing 0.1 N HCl and 0.008 M potassium ferrocyanide. To assess the absorbance, the resulting solution was measured at a wavelength of 120 nm within a time span of 10 minutes.

Determination of total alkaloids[14]

5 grams portion of the sample was carefully measured and placed into a 250 ml beaker. Subsequently, 200 ml of a 10% acetic acid solution in ethanol was added to the beaker, which was then covered and left undisturbed for a duration of 4 hours. Afterward, the mixture was filtered, and the resulting extract was concentrated on a water bath until it reached one-quarter of its original volume.

To achieve complete precipitation, concentrated ammonium hydroxide was gradually added drop by drop to the concentrated extract. The entire solution was then allowed to settle, enabling the precipitate to form. The formed precipitate was carefully collected and subjected to washing using dilute ammonium hydroxide. Once washed, the precipitate was filtered, leaving behind a residue containing the alkaloid. Finally, the alkaloid residue was dried and weighed for further analysis.

III. RESULTS

Qualitative Phytochemical Analysis

In a qualitative analysis of five solvents (aqueous, methanol, chloroform, petroleum ether and benzene), various extracts of M. minuta were tested for their phytochemical properties (**Table.1**). The results indicated that all five solvents yielded positive results in at least one of the ten phytochemical tests.

The methanol extract of the fern exhibited positive results for all ten phytochemical tests, indicating the presence of a wide range of chemical compounds. The benzene extract showed positive results in nine tests, suggesting a slightly lower diversity of phytochemicals compared to methanol.

The chloroform and petroleum ether extracts of the plant displayed positive results in seven and five tests, respectively. This implies a smaller number of phytochemicals present in these extracts compared to methanol and benzene.

The aqueous extract of the fern exhibited positive results in five tests, indicating a relatively lower abundance of phytochemicals compared to the other solvents. Overall, the methanol extract had the highest number of positive results, followed by benzene, chloroform, and petroleum ether extracts. The aqueous extract had the lowest number of positive results among all the solvents tested.

The study focused on screening phytochemical compounds in five solvent extracts. The compounds investigated included steroids, reducing sugars, triterpenoids, sugars, alkaloids, phenolic compounds, flavonoids, saponins, tannins, anthraquinones, and amino acids. Among these compounds, alkaloids, phenolic compounds, flavonoids, saponins, and tannins were identified as important secondary metabolites, known for their medicinal properties in the respective plant. These five compounds were found in all the extracts except for the aqueous extract of the tested fern, which lacked tannin. The researchers also conducted further analytical tests to quantify the phytochemical compounds in the extracts.

Table.1
Qualitative phytochemical analysis of M. Minuta

Compounds	Aqueous	Methanol	Chloroform	Petroleum ether	Benzene
Amino acids	-	+	-	-	+
Steroids	+	+	+	+	+
Anthroquinones	-	+	-	-	-
Triterpenoids	-	+	+	-	+
Tannins	-	+	+	+	+
Reducing sugars	-	-	-	+	+
Saponins	+	+	+	+	+

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Sugars	-	+	-	-	-
alkaloids	+	+	+	+	+
Flavonoids	+	+	+	+	+
Phenolic compounds	+	+	+	+	+
Catechins	-	-	-	-	-

Quantitative phytochemical analysis

The quantitative analysis of phytochemicals in the extract of ferns was conducted using established methods. Various extracts of M. minuta exhibited varying levels of phytochemicals. Among the different components, flavonoids were found to be the most abundant in the specific fern species, M. minuta. This was followed by alkaloids and phenolic compounds, as indicated in **Table 2**. On the other hand, the presence of tannins and saponins in the fern extract was found to be minimal.

Table 2
Quantitative phytochemical analysis of M. Minuta

Phytochemicals	Aqueous	Methanol	Chloroform	Petroleum ether	Benzene
Tannins	-	05.7 ± 0.83	03.25 ± 0.10	02.15 ± 0.22	04.70 ± 0.15
Saponins	04.65 ± 0.40	10.25 ± 0.50	06.45 ± 0.30	05.90 ± 0.05	07.20 ± 0.40
Flavonoid	10.30 ± 0.12	16.40 ± 0.25	12.30 ± 0.15	11.90 ± 0.50	13.90 ± 0.50
Alkaloid	08.92 ± 0.20	15.12± 0.16	10.20 ± 0.25	09.27 ± 0.20	11.25 ± 0.38
Phenolic compounds	06.10 ± 0.30	13.40 ± 0.23	07.60 ± 0.50	08.50± 0.15	09.20 ± 0.25

The Aqueous extract was found to contain 4.65 mg of saponins, 10.30 mg of flavonoids, 8.92 mg of alkaloids and 6.10 mg of phenolic compounds.

The Methanol extract exhibited 5.7 mg of tannins, 10.25 mg of saponins, 16.40 mg of flavonoids, 15.12 mg of alkaloids and 13.40 mg of phenolic compounds.

The chloroform extract showed3.25 mg of tannins, 6.35 mg of saponins, 10.20 mg of alkaloids, 12.30 mg of flavonoids and 7.60 mg of phenolic compounds.

Petroleum ether extract exhibits, 2.15 mg of tannins, 5.90 mg of saponins, 11.90 mg of flavonoids, 9.27 mg of alkaloids and 8.50 mg of tannins were present.

Finally, the Benzene extract contained 4.70 mg of tannins, 7.20 mg of saponins, 13.90 mg of flavonoids, 11.25 mg of alkaloids and 9.20 mg of Phenolic compound.

All the above data are combined and shown inChart number 1.

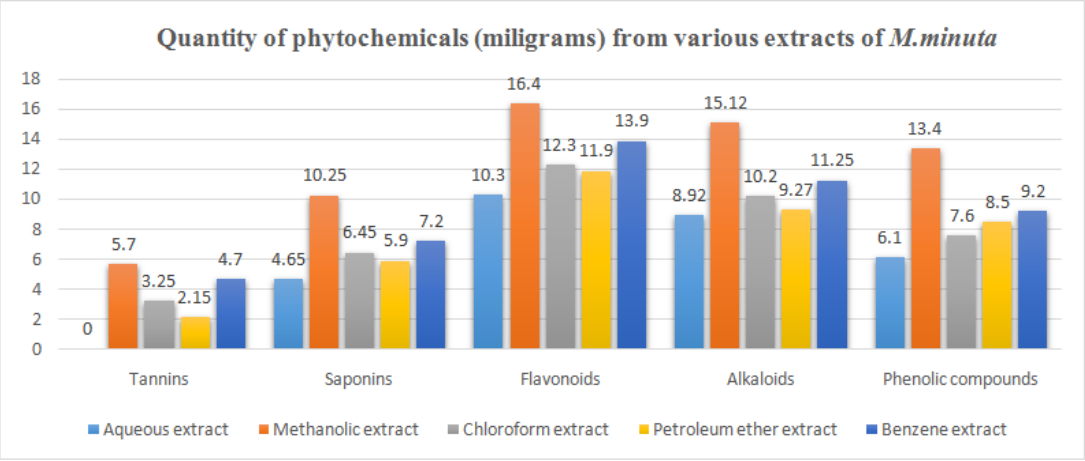

Chart.1: Quantitative phytochemical analysis of M. Minuta

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IV. DISCUSSION

The bioactive compounds found in Marsileaminuta have been recognized for their promising medicinal properties. Alkaloids demonstrate a range of biological effects, such as antimicrobial and antioxidant activities. Flavonoids, on the other hand, exhibit properties that combat inflammation and potentially hinder cancer growth. Phenolic compounds and tannins are associated with antioxidant and hepatoprotective properties[15, 16]. Additionally, the presence of saponins in Marsileaminuta indicates potential antitumor effects[17]. Thesefindings highlight the possibility of pharmacological activities within Marsileaminuta that deserve deeper exploration.

V. CONCLUSION

The analysis of the chemical components in Dwarf Water Clover (Marsileaminuta) has unveiled a diverse range of bioactive compounds that possess promising therapeutic properties. The plant contains alkaloids, flavonoids, phenolic compounds, tannins, and saponins[18], all of which contribute to its medicinal significance. These discoveries establish a starting point for future investigations into the pharmacological effects and potential uses of Marsileaminuta in the realm of natural medicine. Additional research is required to uncover the mechanisms by which these bioactive constituents act and to evaluate their safety and effectiveness.

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