Int J Pharm Bio Sci 2013 Jan; 4(1): (P) 800 - 805

Research Article **Natural Chemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

QUALITATIVE AND QUANTITATIVE ANALYSIS OF PHYTOCHEMICALS IN *MARSILEA MINUTA* LINN.

A. JOHN DE BRITTO,* D. HERIN SHEEBA GRACELIN AND P. BENJAMIN JEYA RATHNA KUMAR

Plant Molecular Biology Research Unit, Post Graduate and Research Department of Plant Biology and Biotechnology, St. Xavier's College (Autonomous), Palayamkottai - 627 002, Tamil Nadu, India.

ABSTRACT

The objective of the present study was to find out the presence of phytochemicals in the petroleum ether, benzene, chloroform, methanol and aqueous extracts of *Marsilea minuta* Linn (Marsileaceae), a common aquatic medicinal fern by both qualitative and quantitative screening 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 five solvent extracts. The methanol extract of the fern showed positive results for 10 phytochemical tests. The benzene extract exhibited positive results for 9 tests. In chloroform and petroleum ether extracts of the plant showed positive results for 7 tests and in aqueous extract of the fern 5 phytochemical tests were positive. In quantitative analysis the important secondary metabolites such as alkaloids, phenolic compounds, flavonoids, saponins and tannins were tested in all the extracts of the fern. The methanol extract showed highest amount of phytochemicals when compared with other solvent extracts.

KEY WARDS: ***Marsilea minuta,*** **alkaloids, phenolic compounds, flavonoids, saponins and tannins**

A. JOHN DE BRITTO

Plant Molecular Biology Research Unit, Post Graduate and Research Department of Plant Biology and Biotechnology, St. Xavier's College (Autonomous), Palayamkottai - 627 002, Tamil Nadu, India.

Corresponding author

INTRODUCTION

Plants have been a rich source of medicines because they produce a wide array of bioactive molecules, most of which probably evolved as chemical defense against predation or infection. It is estimated that only one percent of 2, 65,000 flowering plants on earth have been studied exhaustively for their chemical composition and potential against the important medicinal value [1]. Herbs are widely exploited in the traditional medicine and their curative potentials are well documented [2]. Large scale evaluation of the local flora exploited in traditional medicine for various biological activities is a necessary first step in the isolation and characterization of the active principle and further leading to drug development. The genus *Marsilea* is the common water fern, distributed worldwide. Water clover or *Marsilea minuta* Linn (Marsileaceae) is one of the common Indian species widely found in wet and flooded low lands [3]. The plant as a whole is used as sweet, astringent, cooling, digestive, diuretic, hypnotic, and expectorant. Treatment for psychopathy, diarrhoea, cough, bronchitis, skin diseases and fever have also been reported in Ayurveda [4]. Plants are endowed with the phytochemical compounds such as terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity [5, 6]. Studies have shown that many of these compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral activities [7]. In view of these *M. minuta* plant was studied exhaustively for its potential phytochemical constituents to prove its medicinal values.

MATERIALS AND METHODS

(i) Preparation of plant extracts

Healthy, disease free leaves of the fern *M. minuta* were collected from the fields at Karisal (Tirunelveli district) and their identification was confirmed with the help of herbarium specimens in XCH (Xavier's College Herbarium), St. Xavier's college, Palayamkottai. Thoroughly washed leaves were shade dried and then powdered with the help of a blender. 25 g of the powder was extracted successively with 250 ml of petroleum ether, benzene, chloroform, methanol and distilled water using a Soxhlet extractor for 48 h. All the extracts were concentrated and preserved in airtight bottles until further use.

(ii) Qualitative phytochemical analysis

Qualitative phytochemical analysis of petroleum ether, methanol, benzene, chloroform and aqueous extracts of *M. minuta* was conducted following the standard procedures [8].

(iii) Quantitative phytochemical analysis

The phytochemicals which are present in the methanol extract of *M. minuta* was determined and quantified by standard procedures.

(a) Determination of total phenolic compounds [9]

100 mg of the extract of the sample was weighed accurately and dissolved in 100 ml of triple distilled water (TDW). 1 ml of this solution was transferred to a test tube, then 0.5 ml 2N of the Folin-Ciocalteu reagent and 1.5 ml 20% of Na_2CO_3 solution was added and ultimately the volume was made up to 8 ml with TDW followed by vigorous shaking and finally allowed to stand for 2 hours after which the absorbance was taken at 765 nm. These data were used to estimate the total phenolic content using a standard calibration curve obtained from various diluted concentrations of gallic acid.

(b) Determination of total flavonoids [10]:

The method is based on the formation of the flavonoids - aluminium complex which has an absorptivity maximum at 415nm. 100µl of the plant extracts in methanol (10 mg/ml) was mixed with 100 µl of 20 % aluminum trichloride in methanol and a drop of acetic acid, and then diluted with methanol to 5ml. The absorption at

415 nm was read after 40 minutes. Blank samples were prepared from 100 ml of plant extracts and a drop of acetic acid, and then diluted to 5ml with methanol. The absorption of standard rutin solution (0.5 mg/ml) in methanol was measured under the same conditions. All determinations were carried out in triplicates.

(c) Determination of total alkaloids [11]

5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

(d) Determination of total tannins [12]

500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.I N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

(e) Determination of total saponins [13]

The samples were ground and 20 g of each were put into a conical flask and 100 cm^3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated.

RESULTS

1. Qualitative phytochemical analysis

In qualitative analysis of five solvents such as petroleum ether, benzene, chloroform, methanol and aqueous extracts of *M. minuta* exhibited positive results for ten phytochemical tests. 10 phytochemical tests were positive in methanol extract of the fern. In benzene extract 9 tests were positive. In chloroform and petroleum ether extracts of the plant showed 7 tests and 5 tests were positive in aqueous extract of the fern. Maximum tests were positive in methanol extract followed by benzene, chloroform and petroleum ether extracts of the fern. Minimum tests were positive in aqueous extract of the fern (Table 1).

Table 1
Phytochemical analysis of M. minuta

Compounds	P	B	C	M	A
Steroids	+	+	+	+	+
Triterpenoids	-	+	+	+	-
Reducing sugars	+	+	-	-	-
Sugars	-	-	-	+	-
Alkaloids	+	+	+	+	+
Phenolic compounds	+	+	+	+	+
Flavonoids	+	+	+	+	+
Catechins	-	-	-	-	-
Saponins	+	+	+	+	+
Tannins	+	+	+	+	-
Anthroquinones	-	-	-	+	-
Amino acids	-	+	-	+	-

P- Petroleum ether B- Benzene C- Chloroform M- Methanol A- Aqueous

Phytochemical compounds such as steroids, reducing sugars, triterpenoids, sugars, alkaloids, phenolic compounds, flavonoids, saponins, tannins, anthroquinones and amino acids were screened in five solvent extracts. Among these compounds alkaloids, phenolic compounds, flavonoids, saponins and tannins are important secondary metabolites and are responsible principles for medicinal values of the respective plant. These five compounds were present in all the extracts except aqueous extract of the tested fern. Tannin was absent in aqueous extract. All the extracts were subjected to further analytical tests for the quantification of phytochemical compounds.

2. Quantitative phytochemical analysis

The amount of phytochemicals which are found in the ferns extract was quantitatively determined by standard procedures. All the extracts of *M. minuta* showed different amount of phytochemicals. Among the five components flavonoids content was highest in the selected fern *M. minuta* followed by alkaloids and phenolic compounds (Table 2). The amount of tannins and saponins was very low in the fern extract.

Table 2
Quantitative analysis of phytochemicals (mg/g) in M. minuta

Phytochemicals	P	B	C	M	A
Alkaloids	09.25 ± 0.18	11.20 ± 0.35	10.33 ± 0.29	15.10 ± 0.15	08.90 ± 0.25
Flavonoids	11.85 ± 0.51	13.80 ± 0.55	12.35 ± 0.10	16.45 ± 0.20	10.32 ± 0.15
Phenolic compounds	08.53 ± 0.10	09.25 ± 0.30	07.55 ± 0.45	13.45 ± 0.20	06.13 ± 0.24
Saponins	05.92 ± 0.04	07.22 ± 0.44	06.50 ± 0.35	10.20 ± 0.55	04.70 ± 0.35
Tannins	02.16 ± 0.20	04.76 ± 0.10	03.30 ± 0.06	05.65 ± 0.80	-

P- Petroleum ether B- Benzene C- Chloroform M- Methanol A- Aqueous

The petroleum ether extract contained 9 mg of alkaloids, 11 mg of flavonoids, 8 mg of phenolic compounds, 5 mg of saponins and 2 mg of tannins. In benzene extract 11 mg of alkaloids, 13 mg of flavonoids, 9 mg of phenolic compounds, 7 mg of saponins and 4 mg of tannins were found. In chloroform extract 10 mg of alkaloids, 12 mg of flavonoids, 7 mg of phenolic compounds, 6 mg of saponins and 3 mg of tannins were observed. The methanol extract contained 15 mg of alkaloids, 16 mg of flavonoids, 13 mg of phenolic compounds, 10 mg of saponins and 5 mg of tannins. In aqueous extract 8 mg of alkaloids, 10 mg of flavonoids, 6 mg of phenolic compounds and 4 mg of saponins were found.

DISCUSSIONS

Rembold (1989) [14] reported that *A. indica* contains substances like nimbin, terpenoids, azadirone, azadiractin and these substances are useful in antimicrobial activities and treating various infectious diseases. Pranithanchai *et al.* (2009) [15] reported that the flowers, leaves-sap, and other parts of *C. pulcherrima* are used to treat swelling, earache, muscular and rheumatic pain and various cardiovascular diseases. Various constituents like diterpenoids, and flavonoids has been isolated from *C. pulcherrima* [16]. Alkaloids and flavonoids are the source of antimicrobial activities. Tannins may have the potential values as cytotoxic agents [17]. Saponins have been implicated as bioactive antibacterial agents [18]. Gopalakrishnan and Vadivel (2011) [19] identified the twenty phytochemical constituents from the ethanolic extracts of *Mussaenda frondosa* with the aid of GCMS technique. They also suggested that isolation of individual phytochemical constituents and subjecting it to biological activity will definitely give fruitful results. In the present study all the extracts of *M. minuta* showed the presence of alkaloids, flavonoids and saponins. Hence these compounds would be screen using chromatography techniques in future.

CONCLUSIONS

This study also leads to the further research in the way of isolation and identification of the active compound from the selected fern.

ACKNOWLEDGEMENT

The authors are grateful to the Council of Scientific and Industrial Research (CSIR), New Delhi for financial support (Ref. No: 38(1260)/10/EMR-II 17/05/2010).

REFERENCES

1. Cox P and Balick M, The ethnobotanical approach to drug discovery. Sci. Am. J, 270: 60 – 65, (1994).
2. Dubey N, Kumar R and Tripathi P, Global promotion of herbal medicines: India's opportunity. Current Science, 86: 37 - 41, (2004).
3. Sivarajan V and Balachandran I, Ayurvedic drugs and their plant sources, Oxford & IBH Publishing Co. Pvt. Ltd: 455, (1994).
4. Warrier P, Nambiar V and Ramankutty C, Indian Medicinal Plants, Orient Longman Ltd: 5, (1994).
5. Zheng W and Wang S, Antioxidant activity and phenolic compounds in selected herbs**.** J Agric Food Chem, 49 (11): 5165 – 5170, (2001).
6. Cai Y, Sun M and Corke H, Antioxidant activity of betalains from plants of the Amaranthaceae**.** J Agric Food Chem, 51 (8): 2288 – 2294, (2003).
7. Sala A, Recio M, Giner R, Manez S, Tournier H, Schinella G and Rios J, Antiinflammatory and antioxidant properties of *Helichrysum italicum***.** J Pharm Pharmacol, 54 (3): 365 – 371, (2002).
8. Brinda P, Sasikala B and Purushothaman K, Pharmacognostic studies on Merugan kilzhangu,. BMEBR, 3: 84-96, (1981).
9. Hagerman A, Muller I and Makkar H, Quantification of tannins in tree foliage. A laboratory manual, Vienna: FAO/IAEA, 2000, pp. 4 - 7.
10. Kumaran A and Karunakaran R, Anti oxidant and free radical scavenging activity of an aqueous extracts of *Coleus aromaticus*. Food chemistry, 97: 109-114, (2006).
11. Harborne J, Phytochemical methods. Chapman and Hall, Ltd London: 49-188, (1973).
12. Van-Burden T and Robinson W, Formation of complexes between protein and Tannin acid. J. Agric. Food Chem, 1: 77, (1981).

13. Obdoni B and Ochuko P, Phytochemical studies and comparative efficacy of the crude extracts of some homostatic plants in Edo and Delta States of Nigeria. Global J. Pure Appl. Sci, 8: 203 – 208, (2001).
14. Rembold H, Azadiractin: Their structure and mode of action, In: Insecticides of plant origin. American chemical society, Washington DC USA, 1989, pp. 150.
15. Pranithanchai W, Karalai C, Ponglimanont C, Subhadhirasakul S and Chantrapromma K. Cassane diterpenoids from the stem of *Caesalpinia pulcherrima.* Phytochemistry, 70 (2): 300 – 304, (2009).
16. Rao Y, Fang S and Tzeng Y, Anti-inflammatory activities of flavonoids isolated from *Caesalpinia pulcherrima*. J Ethnopharmacol, 100 (3): 249 – 253, (2005).
17. Aguinaldo A, Espeso E, Guevara B, Nonato M, Phytochemistry. In: Guevara BQ. (ed.) A guidebook to plant screening: phytochemical and biological. Manila: University of Santo Tomas, 2005, pp. 121 - 125.
18. Mandal P, Sinha Babu S, Mandal N, Antimicrobial activity of saponins from Acacia auriculiformis. Fitoterapia, 76 (5): 462 – 565, (2005).
19. Gopalakrishnan S, Vadivel E, GC-MS analysis of some bioactive constituents of *Mussaenda frondosa* Linn. International Journal of Pharma and Bio Sciences, 2 (1): 313 -320, (2011).