



Phytochemical Analysis of Four Traditionally Important Aquatic Species

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Abstract

The present study deals with four aquatic plants such as *Marsilea quadrifolia*, *Centella asiatica*, *Trapa natans* and *Ipomea aquatica* which were collected from different localities of Marthandam, Kanyakumari District, Tamilnadu which are being consumed raw or as vegetable. These species were extracted with five different solvents viz. petroleum ether, chloroform, acetone, ethanol, distilled water and evaluated for their biomolecules and phytochemicals. The findings of the study provided evidences that various solvent extracts of these tested plants contain medicinally important bioactive compounds and it justifies their use in the traditional medicines for the treatment of various diseases as well as for nutritive purposes.

Keywords: Biomolecules, *Centella asiatica*, *Ipomea aquatica*, *Marsilea quadrifolia*, phytochemicals, *Trapa natans*.

Introduction

Nutraceutical a term combining the words ‘nutrition’ and ‘pharmaceutical’ is a food or food product that provides health and medical benefits including the prevention and treatment of disease health. The contribution of local plant foods reducing health risks have always been recognized as part of the local knowledge. Edible plants have traditionally occupied an important position in the socio-cultural, spiritual and health arena of rural and tribal lives of India. India has one of the oldest richest and most diverse cultural traditions associated with the use of traditional systems of medicine. The use of nutraceutical as an attempt to accomplish desirable therapeutic outcomes with reduced side effects as compared with other therapeutic agents has met with great monetary success.

In India most rural inhabitants depend upon wild edible plants to meet their additional food requirement. Sometimes the nutritional value of traditional wild plants is higher than several known common plants. The search of new molecules, now a days has taken a slightly different route where the science of ethnobotany and ethnopharmacognosy are being used as guide to lead the chemist towards different sources and classes of compounds. The conventional food plants provide most nutrients needed for energy, body building, maintenance and regulation of body processes. Due to increasing population, economic crisis in most developing nations, food insecurity have posed a serious threat to growth development and survival. Most people are now incorporating the nonconvention food plants in their diets to provide not only nutrients but also traditional treatment for various ailments¹.

Aquatic plants have economic and environmental uses depending on their natural characteristics. Some are consumed in human diet, while other species have medicinal values and still others species are good resources of minerals and vitamins. Aquatic plants possess rich content of carbohydrates and

proteins, hence they are used as food and feed². The leaf protein extracted from unwanted aquatic weed could be used for food and feed purposes³. Biologically active compounds present in the medicinal plants have always been of great interest to scientist working in this field. It is reported that because of rich content of carbohydrates and proteins in aquatic plants they can be utilized as food and feed². In the present work qualitative and quantitative analysis were carried out in four wetland plants such as *Marsilea quadrifolia*, *Centella asiatica*, *Trapa natans* and *Ipomea aquatica* using five different solvents viz. petroleum ether, chloroform, acetone, ethanol and distilled water.

Marsilea quadrifolia is an aquatic fern of the family Marsileaceae. It is commonly known as water clover. It possess long stalked petiole with four clover like lobes and are either held above the water or submerged. Juice made from the leaves of *Marsilea quadrifolia* is diuretic and febrifuge and also to treat snakebite and applied to abscesses. The plant is anti-inflammatory, diuretic, depurative, febrifuge and refrigerat^{4,5}. The plant contains an enzyme named thiaminase⁵.

Centella asiatica of Apiaceae family is widely used for its medicinal properties like sedative, analgesic, antidepressive, antimicrobial, antiviral and immunomodulatory⁶. In classical Indian ayurvedic literature it is considered to be one of ‘Rasayana’ (rejuvenator) drugs⁷. The stem is slender, creeping stolon, green to reddish green in colour, connecting plants to each other. It has long stalked green reniform leaves. The rootstock consists of rhizomes growing vertically down. The plant contains several valuable compounds viz., centellsaponin, asiaticosida, madecassoside and scelefoleoside^{8,9}. Pectin¹⁰, castilliferol I and castillicetin II¹¹. A bitter principle vallarin, pectic acid and resin present in the leaf and root; asiaticoside and oxyasiaticoside shown to be active in the treatment of leprosy and tuberculosis¹². The plant is highly effective in ulcer preventive¹³, antidepressive sedative and ability to improve the

venomous insufficiently¹⁴. It is also found to improve the power concentration, general ability and behaviour of mentally retarded children¹⁵ and to treat rheumatic disorders¹⁶.

Trapa natans, belongs to the family Trapaceae, commonly known as water chestnut is an annual aquatic floating herb occurring in ponds and lakes throughout the Indian subcontinent¹⁷. It is commercially cultivated across different parts of India for its consumable seasonal fruit. The water caltrop's submerged stem reaches 12 to 15 ft in length, anchored into the mud by very fine root. It has two types of leaves feather like submerged leaf and a rosette like floating leaves. Four petalled white flower form in early summer and is insect pollinated. The fruit is a nut with four barbed spine. Traditionally the plants have been used in India for several important medicinal purposes. It has been used as nutritive, appetizer, astringent, diuretic, aphrodisiac, cooling, tonic and also useful in lumbago, sore throat, bilious affections, bronchitis, fatigues and inflammation. Fruits are used in making liniments for the cure of rheumatism sores and sunburn. Its stem is used in the form of juice in eyes disorders¹⁷⁻¹⁹. The dried kernels of its fruits are recommended abortion, dysuria, poluria and oedema²⁰.

Ipomea aquatica, commonly known as water spinach belongs to the family convolvulaceae. This plant is semiaquatic, tropical plant grown as a leafy vegetable²¹. It grows in water or on moist soil. Stem of this plant is 2-3m or more long, rooting at the nodes and they are hollow and can float. The leaves vary from typically sagittate to lanceolate. The flowers are trumpet-shaped usually white in colour. The plant possesses a good medicinal property which includes inhibition of liver disease, constipation, gastric troubles and intestinal disorders. The purpose of the study is to supplement existing knowledge on these selected aquatic plants by evaluating their nutritional and medicinal composition.

Material and Methods

Collection of plant material: Fresh plant parts of *Marsilea quadrifolia* L., *Centella asiatica* L., *Ipomea aquatica* and seeds of *Trapa natans* were collected from different water bodies in and around Marthandam, Kanyakumari District, Tamilnadu. The collected plants were identified and voucher specimen was deposited to the Herbarium, Department of Botany, Nesamony Memorial Christian College, Marthandam (NMCCH – 5044, 5045, 5046, and 5047). The plant parts were washed thoroughly with normal tap water, followed by sterile distilled water. These plant samples were then shade dried separately in room condition, powdered and stored for further use.

Sample extraction: 50g of each powdered plant samples were weighed and taken separately. These samples were extracted with petroleum ether, chloroform, ethanol, acetone and distilled water individually using Soxhlet's apparatus. The organic extracts obtained were evaporated to dryness by kept open in room temperature. However in case of aqueous extraction, the extract was evaporated to dryness by heating in a water bath to

obtain a semisolid mass. Dried extract was stored in refrigerator for their future use in phytochemical analysis.

Qualitative screening of phytochemicals: Proteins were estimated by Lowry method²² and aminoacids by Ninhydrin test. Carbohydrates are estimated by Anthrone method²³. Presence of fat or lipids was determined by solubility test. Flavanoids was estimated as method suggested by²⁴. Steroids by Salkowshi test. Presence of saponin was determined by the method suggested by^{25,26} and phenol by Folin Ciocalteu Reagent Method.

Quantitative screening of phytochemicals: Chemical tests were carried out on petroleum ether and chloroform extracts of four plants selected, to quantify total phenol and saponin constituents by utilizing standard methods of analysis^{27,28}.

Results and Discussion

The phytochemical characteristics of selected aquatic medicinal plants were summarized in the table-1. The results revealed that phenol, fat, saponin and carboxylic acid were present in all the plants. These results were supported by the reports in *I.aquatica*²⁹, in *C.asiatica*³⁰. Whereas, quinones and alkaloids were absent in these plants and this result was authenticated by the report in *C.asiatica*³¹. Carbohydrates and proteins were absent only in *I.aquatica*, whereas its presence was reported in the same species^{29,32}. Similarly, steroids were absent in *C.asiatica*, coumarins in *T.natans* and xanthoproteins in *M.quadrifolia*. Flavanoids were absent in *T.natans* and also in *I.aquatica*.

The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites³³. They possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection, improvement of endothelial function, inhibition of angiogenesis and cell proliferation activities^{34,35}. There was a report on the presence of an antioxidant compound from aquatic plant *I.aquatica*³⁶. Further presence of polyphenols in some Indian vegetables was also reported³⁷. From this study, the total phenolic contents obtained were 60.2 ± 5.4 mg/g, 136.4 ± 2.7 mg/g, 57.1 ± 0.1 mg/g, 115.8 ± 85.2 mg/g in petroleum ether extract and 165.0 ± 50.9 mg/g, 55.5 ± 2.7 mg/g, 57.1 ± 4.8 mg/g, 111.0 ± 89.6 mg/g in chloroform extract for the plants *M.quadrifolia*, *C.asiatica*, *T.natans* and *I.aquatica* as reported in table 2. These results obtained were corroborative with the reports in *I.aquatica*²⁹. Similarly the presence of 18.8mg/g of phenolic content was quantified from crude extracts of *I.aquatica*³⁵.

Total saponin content quantified in petroleum ether extract were 0.06 ± 0.03 mg/g, 0.17 ± 0.18 mg/g, 0.07 ± 0.05 mg/g, 0.08 ± 0.05 mg/g and in the case of chloroform extract, the values were 0.04 ± 0.02 mg/g, 0.16 ± 0.02 mg/g, 0.10 ± 0.07 mg/g, 0.10 ± 0.07 mg/g of the extract for the plants *M.quadrifolia*, *C.asiatica*, *T.natans* and *I.aquatica* as reported in table 2. The absence of saponin content in *C.asiatica* was also reported³⁰, whereas its presence was positive in our study. Similar results were reported in *R.dumatorium*³⁸.

Conclusion

Phytochemicals are naturally occurring substances found in fruits, vegetables and grains. Unlike vitamins and minerals, they have no nutritional value. They can however influence various body processes. They work together with nutrients and dietary fiber to protect the body against diseases, slow the aging process and reduce the risk of many diseases such as cancer, heart disease, stroke, high blood pressure etc²⁹. The results revealed the presence of many medicinally important constituents in the plants studied apart from its nutritive values. Many evidences gathered were also in confirmatory with this. These plants were used as food by various tribals and other peoples across the world. *T.natans* is cultivated across different parts of India for its consumable seasonal fruit, *I.aquatica* is grown as a leafy vegetable. Now-a-days due to the destruction and reduction of waterbodies, the availability and utility of these valuable aquatic plants for nutritional as well as medicinal purpose also gets reduced. Hence these species are needed to be domesticated for further utilization in nutraceutical preparations.

References

1. Hassan L.G., Umar K.J. and Tijjani A.A., Preliminary investigation on the feed quality of *Monechma ciltion* seeds, *Chem.class Journ.*, **4**, 81-83 (2007)
2. Rahman A.H.M.M., Rafiul Islam A.K.M., Naderuzzaman A.K.M., Hossain M.D. and Rowshatul A., Studies on the aquatic angiosperms of the Rajshahi University campus, *Res. J. Agri. and Biol. Sci.*, **3**, 474-480 (2007)
3. Anjana Dewanji, Aminoacid composition of leaf proteins extracted from some aquatic weeds, *J. Agric. Food. Chem.*, **41**, 1232-1236 (1993)
4. Duke J.A. and Ayensu E.S., Medicinal plants of China, *Reference publications Inc.*, ISBN 0-917256-20-4 (1985)
5. Schofield J.J., Discovering wild plants, Alaska, Western Canada, the Northwest, Alaska Northwest Books, G.TE *Discovery Publications, Inc.*, 22023 20th Ave. S.E.Bothell, WA.98021 (1989)
6. Brinkhaus B., Lindner M., Schuppan D. and Hahn E.G., Chemical, pharmacological and clinical profile of the East Asian medical plant *Centella asiatica*, *Phytomedicine*, **7(5)**, 427-48 (2000)
7. Jayashree G., Kurup M., Sudarslal S. and Jacob V.B., Anti-oxidant activity of *Centella asiatica* on lymphoma-bearing mice, *Fitoterapia.*, **74**, 431-434 (2003)
8. James J.T. and Dubery I.A., Pentacyclic triterpenoids from the medicinal herb, *C.asiatica* (L.), *Urban.Molecules*, **14**, 3922-41 (2009)
9. Matsuda H., Morikawa T., Ueda H. and Yoshikawa M., Medicinal food stuffs, XXVII. Saponin constituents Gotu Kola(2): Structures of new ursane and oleanane type triterpene oligoglycosides, centellsaponin B,C and D from *C.asiatica* cultivated in Srilanka, *Chem. Pharm.Bull.*, **49**, 1368-71 (2001)
10. Wang X.S., Liu L. and Fang J.N., Immunological activities and structure of pectin from *C.asiatica*, *Carbohydr Polym.*, **60**, 95-101 (2005)
11. Subban R., Veerakumar A., Manimaran R., Hashim K.M. and Balachandran I., Two new flavanoids from *C.asiatica* (L.), *J. Nat. Med.*, **62**, 369-73 (2008)
12. Chopra R.N., Nayar S.L. and Chopra I.C., Glossary of Indian medicinal plants. New Delhi, Council of Scientific and Industrial Research, (1980)
13. Cho K.H., Clinical experiences of madecassol (*C.asiatica*) in the treatment of peptic ulcer, *Korean J. Gastroenterol.*, **13**, 49-56 (1981)
14. Zheng C.J. and Qin L.P., Chemical components of *C.asiatica* and their bioactivities, *J. Chin Integ Med.*, **5**, 348-51 (2007)
15. Appa Rao M.V.R., Srinivas K. and Koteshwar Rao T., The effect of Mandookaparni (*C.asiatica*) on the general mental ability of mentally retarded children, *J.Res. Indian Med.*, **8**, 9-16 (1973)
16. Howes M.R. and Houghton P.J., Plants used in Chinese and Indian traditional medicine for improvement of memory and cognitive function, *Pharm. Biochem. Behav.*, **75**, 513-527 (2003)
17. Anonymous, The ayurvedic Pharmacopoeia of India 1st ed. Part 1, Vol.IV. New Delhi, India: Government of India, Ministry of Health and Family Welfare, **452** (2003)
18. Anonymous, The Wealth of India. A Dictionary of Indian Raw Material and Industrial Products. Vol.X. New Delhi, India: Council of Scientific and Industrial Research, **197** (1976)
19. Anjaria J., Parabia M. and Dwivedi S., Ethnoveterinary heritage Indian ethnoveterinary medicine-an overview, Ahmedabad, India:Pathik enterprise, **223** (2002)
20. Khare C.P., Indian medicinal plants: an Illustrated dictionary, Berlin, Heidelberg:Springer, **667** (2007)
21. Oomen H.A.P.C. and Grubben G.J.H., Tropical leaf vegetables in human nutrition. Communication 69, Dept. of Agr. Research, Royal Tropical Institute, Amsterdam, Netherlands, Orphan Publishing Co.,Willemstad, Curacao, (1978)
22. Lowry O.H., Rosebrough N.J., Farr A.C. and Randall R.J., Protein measurement with folin-phenol reagent, *J.Biol.Chem.*, **193**, 265-275 (1951)
23. Jermyn M.A., Increasing the sensitivity of anthrone method for carbohydrate, *Anal.Biochem.*, **68**, 332-335 (1975)
24. Zhisen J., Meng Cheng T. and Jianming W., *Food Chemistry*, **64**, 555-559 (1999)
25. Obdoni B.O. and Ochuko P.O., Phytochemical Studies and Comparative Efficacy of the Crude Extract of some Homostatic Plants in Edo and Delta States of Nigeria, *Glob. J. Pure Appl. Sci.*, **8b**, 203-208 (2001)

26. Edeoga H.O., Okwu D.E. and Mbaebie B.O., Phytochemical constituents of some Nigerian medicinal plants, *African J. Biotech.*, **4**, 685-688 (2005)
27. Gibbs R.D., Chemotaxonomy of flowering plants McGill-Queen's university press, Montreal and London, **1**, 523-619 (1974)
28. Jayaraman J., Laboratory manual in Biochemistry, Wiley Eastern Ltd (1981)
29. Igwenyi I.O., Offor C.E., Ajah D.A., Nwankwo O.C., Ukaomah J.I. and Aja P.M., Chemical compositions of *Ipomea aquatica* (Green Kangkong), *Int. J. Pharma and Bio.*, **2(4)**, B 594-598 (2011)
30. Thangavel Arumugam., Muniappan Ayyanar., Yesudason Justin Koil Pillai and Thangavel Sekar, Phytochemical screening and antibacterial activity of leaf and callus extracts of *Centella asiatica*, *Bangladesh J. Pharmacol.*, **6**, 55-60 (2011)
31. Harshal A.D. and Sanjivani R.B., Phytochemical analysis of *Cassia obtusifolia*, *Cassia auriculata*, *Tephrosia purpurea*, *Helictres isora* and *Centella asiatica*, *Int. J. Pharma & Bio.*, **2(3)**, B 363-367 (2011)
32. Kandukuri Vasu, Jakku Vinayasagar Goud, Aruri Suryam and Singara Charya M.A., Biomolecular and phytochemical analyses of three aquatic angiosperms, *Afr. J. Microbiol. Res.*, **3(8)**, 418-421 (2009)
33. Singh R., Singh S.K. and Arora S., Evaluation of antioxidant potential of ethyl acetate extract/fractions of *Acacia auriculiformis*, *A.Cunn. Fod Chem. Toxicol.*, **45**, 1216-1223 (2007)
34. Han X., Shen T. and Lou H., Dietary polyphenols and their biological significance, *Int. J. Mol. Sci.*, 950-988 (2007)
35. Yadav R.N.S. and Munin Agarwala, Phytochemical analysis of some medicinal plants, *J. Phytol.*, **3(12)**, 10-14 (2011)
36. Nagendra Prasad K., Shivamurthy G.R. and Aradhya S.M., *Ipomea aquatica*, an under utilized green leafy vegetables: A review, *Int. J. Bot.*, **1**, 123-129 (2008)
37. Daniel M., Polyphenols of some Indian vegetables, *Curr.Sci.*, **58**, 1332-1333 (1989)
38. Neelu Singh, Wild edible plants: a potential source of nutraceuticals, *Int. J. Pharma Sci. Res.*, **2(12)**, 216-225 (2011)

Table-1
Qualitative screening of phytochemicals in selected aquatic species

Sl. No.	Plant	Extracts	Phytochemicals											
			Carbohydrates	Proteins	Fat	Alkaloid	Saponin	Flavanoid	Phenol	Steroid	Coumarins	Xantho protein	Quinones	Carboxylic acid
1	<i>Marsilea quadrifolia</i>	Pet. Ether	-	-	+	-	+	-	+	-	+	-	-	-
		Chloroform	+	-	+	-	+	-	+	-	-	-	-	-
		Ethanol	+	+	-	-	-	-	+	+	-	-	-	+
		Acetone	+	-	-	-	-	-	+	+	-	-	-	+
		Dis.water	-	-	-	-	+	+	+	-	+	-	-	-
		Pet. Ether	-	-	-	-	+	-	+	-	+	-	-	-
		Chloroform	+	-	+	-	+	-	+	-	-	+	-	-
		Ethanol	-	+	+	-	-	+	+	-	-	+	-	+
		Acetone	-	-	+	-	-	-	+	-	-	-	-	+
		Dis.water	-	-	-	-	+	+	+	-	+	-	-	-
		Pet. Ether	+	-	-	-	+	-	+	+	-	-	-	-
		Chloroform	+	-	+	-	+	-	+	+	-	+	-	-
3	<i>Trapa Natans</i>	Ethanol	+	+	+	-	-	-	+	-	-	-	-	+
		Acetone	+	-	+	-	-	-	+	+	-	+	-	+
		Dis.water	-	-	-	-	+	-	+	-	-	+	-	-
		Pet. Ether	-	-	+	-	+	-	+	+	-	-	-	-
4	<i>Ipomea aquatica</i>	Chloroform	-	-	+	-	+	-	+	+	-	-	-	-
		Ethanol	-	-	-	-	-	-	+	-	-	+	-	+
		Acetone	-	-	+	-	-	-	+	-	-	-	-	-
		Dis.water	-	-	+	-	-	-	+	-	+	-	-	-

Table-2
Quantitative screening of phytochemicals in selected aquatic species

Sl.No.	Plant	Phytochemicals			
		PHENOL (mg/g)		SAPONIN (mg/g)	
		Pet. ether extract	Chloroform extract	Pet. ether extract	Chloroform extract
1	<i>Marsilea quadrifolia</i>	60.2 ± 5.4	165.0 ± 50.9	0.06 ± 0.03	0.04 ± 0.02
2	<i>Centella asiatica</i>	136.4 ± 2.7	55.5 ± 2.7	0.17 ± 0.18	0.16 ± 0.02
3	<i>Trapa natans</i>	57.1 ± 0.1	57.1 ± 4.8	0.07 ± 0.05	0.10 ± 0.07
4	<i>Ipomea aquatica</i>	115.8 ± 85.2	111.0 ± 89.6	0.08 ± 0.05	0.10 ± 0.07