



***Marsilea quadrifolia*: A floral species with unique medicinal properties**

Suresh Kumar Agarwal¹◇, Sitangshu Roy²#, Prosenjit Pramanick²†, Prosenjit Mitra²§,
Ricardo Gobato³♀ and Abhijit Mitra⁴✧

¹Pragyan International University, Ranchi, India; City Office: 2F Maurya Center, 48, Gariahat Road, Kolkata, 700019, West Bengal, India.

²Department of Oceanography, Techno India University, Salt Lake Campus, Sector V, Kolkata 700091, West Bengal, India.

³Laboratory of Biophysics and Molecular Modeling Genesis, State Secretariat of Education of Paraná, CEJC, St. R. Pombo, 953, Bela Vista do Paraíso, Paraná, 86130-000, Brazil.

⁴Department of Marine Science, University of Calcutta, 35 B. C Road, Kolkata, 700019, West Bengal, India.

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Abstract

Marsilea quadrifolia is a unique plant with high medicinal value. It is widely distributed in the state of West Bengal, which is a maritime state in the north-east part of Indian sub-continent. The present paper is a snapshot of the quantitative analysis of total carbohydrate, protein, fat, flavonoids, amino acids, saponins in the plant extract prepared by dissolving the powdered form of the plant in distilled water in the ratio of 1:10. Our analysis showed the highest value for total carbohydrate, followed by protein, amino acid, flavonoids, saponins and fat.

Keywords: *Marsilea quadrifolia*, Medicinal property, Biochemical composition, West Bengal, Saponins, Flavonoids.

◇Email: drskagarwal@yahoo.com

#Email: isitangshuroy@gmail.com

†Email: ppramanick660@gmail.com

§Email: mitra.prosenjit2016@gmail.com

♀Email: ricardogobato@seed.pr.gov.br

✧Email: abhijit_mitra@hotmail.com



1. Introduction

India is considered as one of the mega biodiversity countries in the Global panorama. In India, the history of herbal medicine dates back to ancient human civilization and valuable information on traditional medical practices are available [1]. Medicinal plants are used for curing diverse types of bacterial, fungal and viral diseases. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for discovery of new drugs because of the unmatched availability of chemical diversity. Plant products and related drugs are used to treat 87% of all categorized diseases [2].

Phytochemicals and chemical compounds that are produced during the plants normal metabolic processes are known as secondary metabolites. Secondary metabolites include several classes like alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids [3]. Plants have the potential to synthesize allometric substances, mainly secondary metabolites of which 12,000 have been isolated, a number estimated to be less than 10% of the total [4]. These active components serve as molecules of the plant defense against attack by microorganisms as well as exhibit the medicinal properties for treating several diseases.

In the present research programme, the aquatic fern *Marsilea quadrifolia* collected from Singur region (22°81'50" N & 88°23'45" E) of the Hooghly district in West Bengal was analysed to

evaluate the levels of carbohydrate, protein, lipid, total flavonoids, total amino acids and saponins. The collection and analysis was carried out during 2nd June to 28th June 2018.

2. Materials and Methods

2.1. Sample preparation

Fresh plants of *M. quadrifolia* were collected from natural habitat of Singur region in the Hooghly district, West Bengal (India). The collected plant was identified by Botanical Survey of India, Shibpur, Howrah (India). Collected plants were washed with deionized water to remove the adherence impurities, shed dried for about 10 days, powdered and stored in refrigerator. Plant extracts were prepared by dissolving the powder in the distilled water in the ratio of 1:10. The containers were intermittently agitated for 48 hr & filtered through Whatman No. 1 filter paper. The filtrate was condensed using rotary evaporator & stored in the refrigerator in air-tight condition.

2.2. Qualitative analysis of biochemical constituents

The qualitative analysis to determine the presence / absence of carbohydrate, protein, lipid, total flavonoids, total amino acids and saponins was conducted through a series of experiment (Table 1).

Table 1: Qualitative analysis of biochemical composition

Sl. No.	Experiment	Expected outcome	Inference
1	Test solution + 2 ml of Fehling's reagent and 3 ml of water and boil	Red or Orange colour	Presence of reducing sugars
2	Test solution + few drops of 4% NaOH + few drops of 1% CuSO ₄	Violet or Pink colour	Presence of Proteins
3	Test solution in alcohol + a bit of magnesium and one or two drops of con. HCl	Red or Orange colour	Presence of Flavonoids
4	Test Solution + 1% Ninhydrin in alcohol	Blue or violet colour	Presence of Amino acids
5	Test solution + H ₂ O + and shaken well	Foamy lather	Presence of Saponins



2.3. Quantitative analysis of biochemical constituents

2.3.1. Estimation of total carbohydrate

The carbohydrates concentration was measured by Anthrone method [5]. Hundred milligrams (100 mg) of plant sample was weighed and taken into a boiling tube, hydrolysed by keeping it in a boiling water bath for three hours with 5 ml of 2.5 N HCl and the mixture was cooled at room temperature. Using sodium carbonate it was neutralized and the volume was made up to 100 ml. Then it was centrifuged and supernatant was collected. 0.5 ml of aliquots was used for estimation. The working standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard glucose solutions and '0' served as blank. 0.1 and 0.2 ml of the test sample solution was taken in two separate test tubes. In all the test tubes, the volume was marked up into 1 ml with water and blank was set with 1 ml of water. 4 ml of anthrone reagent was added to each tube, mixed well and kept it in water bath for 10 minutes. The contents were cooled rapidly and the absorbances were read at 630 nm and the amount of total carbohydrate present in the sample was calculated.



Figure 1: Morphology of *Marsilea quadrifolia*.
Source: (Photograph taken from Singur by Sitangshu Roy).

2.3.2. Estimation of total protein

The plant sample was homogenized in 10% cold Tri Chloro Acetic acid TCA (10 mg: 5 ml) and was centrifuged at 5000 rpm for 10 minutes. Supernatant was discarded and pellets were saved. Pellets were again suspended in 5 ml of 10% cold TCA and re-centrifuged for 10 minutes. Supernatant was again discarded and the

precipitate was dissolved in 10 ml of 0.1 N NaOH. 0.1 ml of this solution was used for protein estimation. In 1 ml of plant sample, total protein content was estimated using the protocol of Lowry *et al* [6]. A stock solution (1 mg/ml) of bovine serum albumin was prepared in 1 N NaOH; five concentrations (0.2, 0.4, 0.6, 0.8 and 1 ml) from the working standard solution were taken in series of test tubes. In another set of test tubes 0.1 ml and 0.2 ml of the sample extracts were taken and the volume was raised up to 1 ml in all the test tubes. To each test sample, 5 ml of freshly prepared alkaline solution was added at room temperature and left undisturbed for a period of 10 min. Subsequently, to each of these mixture tubes 0.5 ml of Folin-Ciocalteu reagent was rapidly added and incubated at room temperature for 30 minutes until the blue colour develops. The absorbances were read at 750 nm and the amount of total protein present in the sample was calculated.

2.3.3. Estimation of total fat

The total fat in the samples was estimated by Soxhelt method using petroleum ether (80 °C) as per the procedure [7].

2.3.4. Estimation of total flavonoids

Aluminium chloride colorimetric method was used with some modifications to determine flavonoid content. 1 ml of sample plant extract was mixed with 3 ml of methanol, 0.2 ml of 10% aluminium chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water and left at room temperature for 30 minutes. The absorbance was measured at 420 nm. All the tests were performed in triplicates. Flavonoid contents were determined from the standard curve and were expressed as quercetin equivalent (mg/g of extracted compound) [8].

2.3.5. Estimation of total amino acids

Five hundred milligrams (500 mg) sample were weighed and ground in a mortar and pestle with a small quantity of acid-washed sand. To this homogenate, 5 to 10 ml of 80% ethanol was added. The supernatant was collected by centrifugation. The extraction was repeated twice with the residue and pooled all the supernatants. The volume was reduced if needed by evaporation



and used the extract for the quantitative estimation of total free amino acids.

For the preparation of Standard, 50 mg leucine was dissolved in 50 mL of distilled water in a volumetric flask. From that 10 mL was diluted to 100 mL in another flask for working standard solution. A series of volume from 0.1 to 1 mL of this standard solution gives a concentration range 10 µg to 100 µg. Proceeded as that of the sample and read the color. 0.1 mL of plant extract was taken, and to that 1 mL of ninhydrin solution was added and made up the volume into 2 mL with distilled water. The tube was heated in boiling water bath for 20 min. Five milliliters (5 mL) of the diluents was added and mixed. After 15 min the intensity of the purple color against a reagent blank in a colorimeter at 570 nm was read. The color was stable for 1 hour. The reagent blank was prepared as above by taking 0.1 mL of 780% ethanol instead of the extract. A standard curve was drawn using absorbance versus concentration. The concentration of the total free amino acids in the sample was estimated and expressed as percentage equivalent of leucine [9].

2.3.6. Estimation of saponin

The method used was that of Obadoni and Ochuko [10]. Twenty grams (20 g) of sample powder were put into a conical flask and 100 ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 hours 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 separating 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 and the saponin content was calculated as percentage.

3. Results

The quantitative analysis of biochemical constituents of *M. quadrifolia* reveals the highest concentration of total carbohydrate followed by

total protein, total amino acid, total flavonoids, saponins and total fat (Table 2).

Table 2: Quantitative analysis of biochemical constituents of *M. quadrifolia* extract.

Contents (Total)	Concentration (mg/g)
Carbohydrate	195 ± 9.7
Protein	49 ± 3.2
Amino acid	30 ± 3.5
Flavonoids	3.5 ± 0.8
Saponins	2.6 ± 0.6
Fat	2.1 ± 0.2

Results are expressed as mean ± standard deviation.

4. Discussion

M. quadrifolia Lin. is an aquatic fern under the family Marsileaceae, which is commonly called Sushni Saak in Bengali. It is an aquatic fern with 4 parted leaf resembling 4-leaf clover, Figure (1). The leaves float in deep water or sometimes even seen in erect position in shallow aquatic habitat. Presence of a long stalked petiole is a marked feature of *M. quadrifolia*. The plant is also abundantly available in moist or wet soil. The presence of carbohydrate, protein and amino acid in considerable amount reveals the nutritional value of *M. quadrifolia*. Along with this, flavonoids and saponins indicate the role of the floral species in improving the health of the human beings. Saponins are antidiarrhoeal as they inhibit histamine release in vitro, anticancer as they possess membrane permeabilizing properties and antihelminthic as they lead to vacuolization and disintegration of teguments. The flavonoids are antimicrobial as they complex with the cell wall and bind with adhesions, and antidiarrhoeal as they inhibit the release of autocoids and prostaglandins, inhibit contractions caused by spasmogens [11]. Juice made from the leaves of *M. quadrifolia* is diuretic and febrifuge. It is also used to treat snakebite and applied to abscesses etc.

The medicinal value of the floral species may be transferred to human system through production of food items preferably desserts, which can be easily prepared with the extracts of the leaves of the species. Such desserts will not only have



considerable health benefits, but will also open up an alternative livelihood for unemployed population of the country. Earlier works on preparation of herbal desserts have been revealed by the present group of researchers [12, 13], which have been widely accepted by the people of all ranks of the society in the state of West Bengal (India) and exhibited a boom in the marketing domain through Roy's Satyanarayan Mistanna Bhandar of Singur, India (Figures 2-4).

Conclusions

Indian sub-continent is blessed with a wide variety of medicinal plants. The state of West Bengal, a maritime state in the north east part of the country has rich medicinal plants distributed in the wetlands, marshy area and hilly terrains. The nutritional values of *Marsilea quadrifolia* has given the plant a special natural medicinal flavor, which can be linked with the dessert making cottage industry to provide nutrition to population as well as generate alternative livelihood.

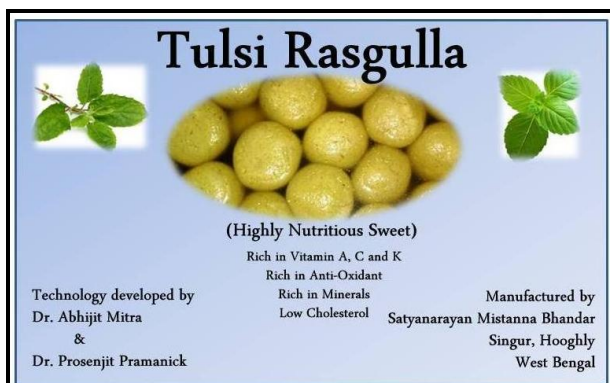


Figure 2: Label of herbal (Tulsi) Rasgulla developed by researchers. Source: (Mitra, 2018) [13].



Figure 3: Marketing of herbal dessert in festival. Source: (Mitra, 2018) [13].



Figure 4: Media coverage of Tulsi Rasgulla. Source: (Mitra, 2018) [13].

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