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QUALITATIVE AND QUANTITATIVE ESTIMATION OF PHYTOCHEMICAL COMPOUNDS AND ITS ANTIBACTERIAL SCREENING OF THE PLANT *MARSILEA QUADRIFOLIA*

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ABSTRACT

This paper highlights the qualitative and quantitative estimation of phytochemical compounds present in aqueous, ethanol, methanol and ethyl acetate extracts of the aquatic fern *Marsilea quadrifolia*. The result illustrated that carbohydrates (11.33 ±0.08mg/g), flavonoids (17.68±0.04mg/g), tannins (24.38±0.01mg/g) and phenols (12.42±0.02mg/g) were present more in methanol extract and proteins (12.09±0.08mg/g) were rich in ethanolic extract. The extracts were subjected to test for antibacterial activity against organisms like *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*. The antibacterial activity was carried using pour plate method and its activity was found to be moderate.

INTRODUCTION:

In developing countries, it is estimated that about 80% of the world population currently uses herbal medicine for some aspects of primary health care (Fransworth, 1993; Houghton, 1995). The importance of medicinal and aromatic plants has been emphasized from time to time due to their more safety and less side effects (Manish Devgun et al., 2009; J.Srivastava et al., 1996). Many conventional drugs or their precursors are derived from plants. However, there is a difference between administering a pure isolated chemical and the same chemical in a plant matrix. Many higher plants accumulate extractable organic substances in quantities sufficient to be economically management of disease. Plants have been a rich source of medicines because they produce wide array of bioactive molecules, most of which probably evolved as chemical defense against predation or infection (Cox, P.A. and M.J. Balick, 1994). The medicinal values of plants lie in their phytochemicals, which makes specific physiological actions on the human body. Phytochemicals are compounds found in plants that are utilized as food and medicine top reserve against illness and to ensure human health. Phytochemicals have antioxidant which helps in fighting against many diseases including cancer, heart disease, diabetes and high blood pressure (Prasad *et al.,* 2012) In recent years, multiple drug resistance has developed due to indiscriminate use of existing antimicrobial drugs in the treatment of infectious diseases. Antimicrobial resistance is a threat to mankind because most of the infection causing bacteria has become multidrug resistant. Antibiotic resistant bacteria may keep people sick longer, and sometimes people are unable to recover at all.

Because of the concern about the side effects of conventional medicine, the use of natural products as an alternate to conventional treatment in healing and treatment of various diseases has been on the rise in the last few decades (Kumari *et al.,* 2011). *Marsilea quadrifolia* Linn. is a pteridophyte belonging to Family Marsileaceae commonly known as European water clover. In eastern parts of India it is known as Sushni .The plant is widely distributed throughout India. Juice made from the leaves is diuretic and febrifuge and also used to treat snake bite and applied to abscesses etc (Duke J.A and Ayensu. E.S 1985). The plant is anti-inflammatory, diuretic, depurative, febrifuge and refrigerant (Schofield. J.J 1989). Plant spacifies vitiated pitta, cough, bronchitis, diabetes, psychiatric diseases, eye diseases, diarrhea and skin disease.

MATERIALS AND METHOD:

Collection of plant sample: The selected plant *M. quadrifolia* was collected from Kanya Kumari district, Tamil Nadu, India. The plant was then identified by the book "The flora of Presidency of Madras" (Gamble, 1958). The whole plant were cleaned and wet dried for three weeks and grounded into a fine powder, which was used for further extraction.

Preparation of plant extract: Crude plant extract was prepared by Soxhlet extraction method. The powdered plant materials (25gram) was extracted with aqueous, ethanol, methanol, and ethyl acetate at 40 - 80˚C depending upon the evaporation point of the solvent by Soxhlet extraction. The extraction was carried out using solvent of increasing polarity from ethyl acetate, ethanol, methanol and water respectively. The process of extraction continues for 24 hours or till the solvent in siphon tube of an extractor become colourless. After that, the extract was taken in a beaker, kept on hot plate and heated at 30 - 40ºC till all the solvent got evaporated. Dried extract was kept in refrigerator at 4ºC for further use.

QUALITATIVE ANALYSIS:

Tests for carbohydrates (Kokate, 1994)

Fehling's Test: 1 ml of Fehling's A and 1 ml of Fehling's B solution's were mixed and boiled for one minute, equal volume of test solution was added to the above mixture. The solution was heated in boiling water bath for 5-10 minutes. First yellow, then brick red precipitate was observed.

Tests for proteins (Ansari, 2006):

Biuret Test: Small quantity of aqueous, ethanol, methanol and ethyl acetate extracts was dissolved in few ml of water. To this test solution of 4% NaOH and a few drops of 1% CuSO4 was added. Appearance of violet colour showed the presence of proteins.

Tests for flavonoids (Kokate, 1994):

With Lead Acetate: To the small quantity of extract lead acetate solution was added. Formation of yellow precipitate showed the presence of flavonoids.

Tests for Phenols:

$FeCl_3$ Solution Test: On addition of 5% $FeCl_3$ solution to the extract, deep blue black colour appeared.

Test for Tannins (Mukherjee, 2002)

Lead Acetate Test: On addition of lead acetate solution to the extract white precipitate appeared.

Test for Glycosides (Ansari, 2006)

Keller-Killiani Test: To 2 ml of the extract, glacial acetic acid, one drop 5% $FeCl_3$ and conc. H_2SO_4 was added. Reddish brown colour appeared at junction of two liquid layers and upper layer turned bluish green indicating the presence of glycosides.

Test for Steroids (IP, 1996)

Salkowski Test: To 2 ml of extract, 2 ml of chloroform and 2 ml of conc. H_2SO_4 was added. The solution was shaken well. As a result chloroform layer turned red and acid layer showed greenish yellow fluorescence.

Test for Alkaloids (Ansari, 2006)

The extracts were evaporated in test tubes. To the residue dilute HCl was added, shaken well and filtered.

Mayer's Test: To the 2-3 ml of filtrate Mayer's reagent was added. Formation of yellow precipitate showed the presence of alkaloids.

Test for Saponin (Ansari, 2006)

Foam Test: Extracts were shaken vigorously with water. No persistent foam was formed.

Detection of triterpenes

To the extracts chloroform and conc. H_2SO_4 were added. Appearance of red colour indicated the presence of triterpenes.

QUANTITATIVE ESTIMATION OF PHYTOCHEMICAL COMPOUNDS

Estimation of carbohydrate (Miller, 1972): 100 mg of sample was weighed and sugars were extracted with hot 80% alcohol twice (5 ml each time). The supernatant was collected and evaporated on water bath and makeup the volume with 3 ml of water. 3 ml of Dinitrosalicylic acid (DNS) reagent was mixed with sample and heated for 5 minutes in a boiling water bath. After the colour development 1ml of 40% Rochelle salt (sodium-potassium tartarate) was added. The tubes were cooled under running tap water and measure the absorbance at 510 nm. The standard graph was plotted for working standard glucose solution (0 to 100µg/µl).

Estimation of total protein (Lowry *et al.,* 1951): 0.2 ml of supernatant was taken and made up to 1 ml of distilled water. To this add 2 ml of alkaline copper sulphate reagent. Mixed the solutions well and incubated at room temperature for 10 minutes. After that add 0.2 ml of Folin Ciocalteau reagent to each tube and incubate for 30 minutes. Read the optical density at 660 nm. Using the standard curve, the concentration of protein in the samples was determined.

Estimation of flavonoids (Kariyon *et al.,* 1953): Total flavonoids content was determined by aluminium chloride method using catechin as a standard. 1ml of test sample and 4 ml of water were added to a volumetric flask (10 ml volume).

After 5 minutes 0.3 ml of 5% sodium nitrite, 0.3 ml of 10% aluminium chloride was added. After 6 minutes incubation at room temperature, 2 ml of 1 M sodium hydroxide was added to the reaction mixture. Immediately the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm spectrophotometrically. Results were expressed as catechin equivalents (mg catechin/ g dried extract).

Estimation of tannins (Robert, 1971):

1 ml extract was mixed with 5 ml of vanillin hydrochloride reagent (mix equal volumes of 8% Hcl in methanol and 4% vanillin in methanol). The mixture was allowed to stand for 20 minutes and measure the absorbance at 500nm. The standard graph was plotted for working standard catechin solution (0 to 250 µg/µl).

Estimation of phenols:

0.5 ml of freshly prepared sample was taken and diluted with 8 ml of distilled water. 0.5 ml of Folin Ciocalteu Reagent (1 N) was added and kept at 40˚C for 10 min. 1 ml of Sodium Carbonate (20%) was added and kept in dark for one hour. The absorbance was read at 650 nm using UV Spectrophotmeter (Malick et al., 1980). The same procedure was repeated for all standard gallic acid solutions and standard curve obtained. The sample concentration was calculated as Gallic acid equivalent (GE).

ANTIBACTERIAL ACTIVITY

Disc diffusion method:

Preliminary antibacterial screening of the extracts was carried out by the disc diffusion method (Bauer *et al.,* 1966). The freshly grown liquid culture of the pathogenic bacteria was seeded over the Muller Hinton Agar with a sterile swab. Sterile disc of six mm diameter were soaked with 40µl of 250 mg/ml of the sample extract and air dried to evaporate the solvent and the disc were kept over the pathogenic bacteria seeded Muller Hinton Agar plate.

The plates were incubated at 37ºC for 18-24 hours. After the incubation period the plates were observed for the clearance of the zone around the disc which indicates that the particular plant extract have antibacterial activity each experiments were carried out in triplicates. The mean ± SD of the inhibition zone was taken for evaluating the antibacterial activity of the extracts.

Table 1: Qualitative estimation of the phytocompounds present in the plant *Marsilea quadrifolia*

S.no	Tests	Aqueous	Ethanol	Methanol	Ethyl Acetate
1	Carbohydrates	+	+	+	+
2	Proteins	+	+	+	+
3	Flavonoids	+	+	+	+
4	Alkaloids	_	_	_	_
5	Saponnins	_	_	_	_
6	Glycosides	_	_	_	_
7	Phenols	+	+	+	+
8	Tannins	+	+	+	+
9	Steroids	+	+	_	_
10	Triterpenes	_	_	_	_

Table 2: Quantitative Estimation of Phytochemicals for the Plant *Marsilea Quadrifolia*

S.no	Tests	Aqueous	Ethanol	Methanol	Ethyl Acetate
1	Carbohydrates(mg/g)	5.47±0.04	3.26±0.03	11.33±0.08	5.37 ±0.03
2	Proteins (mg/g)	9.60±0.06	12.09±0.08	8.97±0.03	4.51±0.03
3	Flavonoids(mg/g)	15.34±0.05	13.53±0.02	17.68±0.04	15.14±0.08
4	Tannins(mg/g)	17.30±0.01	11.41±0.01	24.38±0.01	17.68±0.13
5	Phenols(mg/g)	9.22±0.02	6.3±0.03	12.42±0.02	6.50±0.07

Table 3: Antibacterial Activity of the Plant *Marsilea Quadrifolia*

S.no	Pathogen	Aqueous	Ethanol	Methanol	Ethyl Acetate	Control
1	*Klebsilla pneumonae*	NZ	NZ	NZ	NZ	20
2	*Enterobacter*	NZ	NZ	NZ	NZ	22
3	*Escherichia coli*	NZ	10mm	11mm	NZ	21
4	*Pseudomonas aerogenes*	NZ	11mm	12mm	NZ	20
5	*Proteus vulgaris*	NZ	7mm	9mm	7mm	18

NZ- No Zone

RESULT AND DISCUSSION

Qualitative estimation of the phytocompounds presents in the plant *Marsilea quadrifolia.*

Preliminary phytochemical analysis for *M. quadrifolia* exhibit the presence of carbohydrates, proteins, flavonoids, tannins and phenols in aqueous, ethanol, methanol and ethyl acetate extracts. Uma and Pravin. (2013) reported the presence of carbohydrates, proteins, flavonoids and tannins in methanol, ethyl acetate and aqueous extracts. Meenatchi and Jenitha. (2015) also reported the presence of carbohydrates, proteins, flavonoids, tannins and phenols in the aqueous, methanol and petroleum ether extract of *M. quadrifolia* As well as carbohydrates was also present in the ethanol extract of *M. quadrifolia* reported by Ashwini et al. (2012) alkaloids, saponins, glycosides and triperpenes were completely absent in all the extracts of *M. quadrifolia.* Similarly Ashwini et al. (2012) reveled the absence of alkaloid,saponins and triterpenes and the presence of glycosides in the ethanolic extract of *M. quadrifolia.* Uma and pravin. (2013) reported the absence of saponins and the presence alkaloids and terpenes in the aqueous, methanol and ethyl acetate extract of *M. quadrifolia*, Meenatchi and Jenitha. (2015) also reported the absence of saponins and glycosides and the presence of alkaloids in the aqueous and methanolic extracts of *M. quadrifolia*.

Quantitative Estimation of Phytochemicals for the Plant *Marsilea Quadrifolia.*

Quantitative estimation was performed in aqueous, ethanol, methanol and ethyl acetate extract of the aquatic plant *M. quadrifolia*. It shows that maximum amount of carbohydrates (11.33 ±0.08mg/g) was present in the methanol extract, proteins (12.09±0.08mg/g) in ethanol extract, flavonoids (17.68±0.04mg/g), tannins (24.38±0.01mg/g) and phenols (12.42±0.02mg/g) in the methanol extract of *M. quadrifolia*. Tannins was found to be present rich in aqueous, ethanol, methanol and ethyl acetate extract, following to this flavonoid, phenols, proteins and carbohydrates was present.

Antibacterial Activity of the Plant *Marsilea Quadrifolia*

The study shows that the methanol and ethanol extracts of the plant *Marsilea quadrifolia* having antimicrobial activity against the bacteria's like *Escherichia coli*, *pseudomonas aeruginosa* and *proteus vulgaris*, methanol extract is having more activity when compared with ethanol. Similar results were shown by Sethi. (2014) for *P.aeruginosa* when treated with ethanolic extract. The ethyl acetate extract is having activity only against *proteus vulgaris*. In this study aqueous extract is doesn't show antibacterial activity for these organisms. Overall, seeing this plant shows only a moderate activity in 100µl concentration.

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