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A Review on *Marsilea Quadrifolia* L. – A Medicinally Important Plant

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ABSTRACT

Marsilea quadrifolia Linn is an aquatic fern belongs to the family (Marsileaceae). All parts of the plant possess a multitude of phytochemical secondary metabolite which imparts an exceptional assortment of medicinal uses to the plant. Its use has been exposed as Antibacterial, Antioxidant, Neurodegenerative disorders, Anticonvulsive, Anticholinesterase etc. Several extracts like aqueous, Chloroform, Ethanol, Methanol and Petroleum ether has been used for different Pharmaceutical activities.

Key words: Marsileaceae, Petroleum ether, Phytochemical

INTRODUCTION

The ethnic and urban societies of the world have conserved immense traditional knowledge of medicinal plants since primitive time. It is well known fact that most of the expensive drugs used in allopathic medicinal are derived from plant resources. The ethnopharmacobotanical field studies in the urban and tribal areas play crucial role in sighting key information which is unknown or less known about many of the plant species of our rich flora [1-3].

In developing country like India, nearly 70% of the population still rely on herbs, which are explored by the cultural societies, exploit them for the treatment of various diseases. Nation accounts for 2500 plants species of 1000 genera utilized by traditional physiotherapies [4-9]. Millions of Indians are herbal drugs regularly, as species, home remedies, and health foods as well as over the counter (OTC) as self medication or also as drugs prescribed in the non – allopathic systems.

M. quadrifolia Linn is an aquatic fern belongs to the family (Marsileaceae) commonly named has Niraaikkirai, Aaraikeerai in Tamil, European water clover and Four leaf clover in English, Neerara in Malayalam and Caupiya, Sunsuniya in Hindi. It is an aquatic fern bearing 4 parted leaf resembling ‘4-leaf clover’ (trifolium). Leaflets obtained to ¾ long, glaucous, petioles to ‘8’ long; sporocarp (ferns) ellipsoid, to 3/16 long, dark brown, on stalks to ¾ long, attached to base of petioles. The plant roots both at the nodes and internodes of the rhizome [10]. Leaves floating in deep water or erect in shallow water or on land. It possess long stalked petiole with 4 clovers like lobes and are either held above the water or submerged, heavy clay to sandy substrates of permanent lakes and seasonal ponds and made habitats (ditches and rice fields). It is widely distributed in tropical and temperate regions of world and found throughout India, in marshy places and along the banks of canals and rivers [11]. It is also rooted in the bottom of clayey soil in submerged water [12]. *M quadrifolia* is enlisted in the Red Data Book of the International

Union for Conservation of Nature (IUCN) [13]. *M. quadrifolia* is also eaten by various tribal communities such as Kadar's, Pulaiyars, Malasars, Malaimalasars, Mudhuvars of Anamalais hills, Western Ghats, Coimbatore district Tamil Nadu, India as per seasonal availability [14], this review deals only with the medicinal importance and other related attributes of the plant.

Taxonomy of the Plant [Fig. No: 1]

Domain	: Eukaryote
Kingdom	: Planate
Phylum	: Sterptophyta (Polypodiophyta)
Class	: Pteridopsida
Order	: Salviniiales
Family	: Marsileaceae
Genus	: Marsilea L.
Species	: Marsilea quadrifolia – European water clover
Division	: Pteridophyta – ferns

Traditional Uses

Petiole and leaves (Matigora and Jaduguda village) for Cooked in oil with salt and masalas: Regular consumption relieves of hypertension, sleep disorders and headache (present investigation). Petiole and leaves (Jaduguda village) for 50 ml. warm mustard oil with garlic (2-5 gram) poured over Washed and chopped 250 Gms. Petiole and leaves cooked covered with bamboo basket followed by seasoning with 4-5 gram desi masala (mixture of red chilies, turmeric, coriander and zeera) and salt: Consume only in the evening for sound tension free night sleep (minimum 12- 14 hours)and relaxes them both physically and mentally, Hypertension and other nervous disorders all type of body aches, insomnia (present investigation). Entire fresh plant (Matigora) Juice with Garlic: Cure cough as well as convulsive condition of leg and muscles (present investigation), Whole plant (matura village) Juice or paste: Applied externally on the head relieves from sleep disorder and hypertension (present investigation). Young leaves (Rakha mines) Two drops juice of crushed leaves in the nostrils twice a day Migraine (present investigation). Whole plant Crushed plant with sugar candy or honey to cure infantile diarrhoea [15]. Whole plant Whole plant including roots is made into a paste with whole plant of *Centella asiatica* applied twice daily for 7 days around the nipple: Improving lactation after childbirth [16]. Leaves with one breath several leaves are to be plucked and the squeezed juice from the leaves massaged to all ten tendons of the body twice daily for 2-3 days: Tribe of Bangladesh lesions on tongue or in the mouth and rheumatism. Young stems and leaves cooked leaves and petioles a famine food, only used in times of scarcity [17].

Spores Spores ground and mixed with flour Used in making bread etc.

Medicinal Importance

Juice made from the leaves is diuretic and febrifuge and also used to treat snake bite and applied to abscesses etc [19]. The plant is anti-inflammatory, diuretic, depurative, and febrifuge and refrigerant [20].Plants pacifies vitiated pitta, cough, bronchitis, diabetes, psychiatric diseases, eye diseases, and diarrhoea and skin disease.

Phytochemical Constituents

Marsilea quadrifolia contain like marsilin (1-triacontanol-cerotate), 3-hydroxy-tri-acontan-11-one, hentriacotan-6-ol, methylamine, Beta-sitosterol, marsileagenin A, flavonol-O-mono & diglycoside, C-Glucoflavones and C-glucosyl xanthenes [21].

Pharmacological Review

Anticonvulsant activity [22]

Anticonvulsive potential of MQ extracts by using behaviour and electroencephalographic (EEG) analysis on pentylenetetrazole (PTZ) induced seizure model in rats. For anticonvulsant effect, 60minutes after administration of MQ, behaviour and EEG were analyzed during PTZ (60mg/kg) induced seizures. Changes of EEG power, latency of onset of seizure, seizure severity score, and duration of epileptic seizure were determined. Both the water and ethanol extract of MQ increased the latency of seizure but also decreased duration of epileptic seizure and seizure severity score. This reduction of seizure severity was also observed in EEG recording and EEG power analysis. The effectiveness of MQ ethanol extract is better than MQ water extract.

Antimicrobial activity [23]

The antimicrobial activity of different solvent extracts of leaf and stem of *M. quadrifolia* at different concentrations was analyzed. Among the concentrations, 5mg of both leaf and stem extracts showed best antimicrobial activity than other concentrations 2.5 and 3.75mg. The leaf and stem extracts showed antimicrobial activity and produced the zone of inhibition ranges from 8 to 23mm. The aqueous leaf extract showed maximum zone of inhibition 23mm against *Streptococcus pyogenes* followed by ethanolic stem extract showed 21mm against *Bacillus subtilis*. The minimum antibacterial activity 8mm was observed by diethyl ether stem extract against *Klebsiella pneumonia*. The antifungal activity of diethyl ether leaf extract showed positive results in all tested fungal strains when compared to other solvent extracts.

a) *Marselia quadrifolia* leaves

b) Aerial parts of *Marselia quadrifolia*

Figure no: 1. Aerial parts of *Marselia quadrifolia* Linn

The maximum zone of inhibition 13mm was observed against *Aspergillus terreus* at 5mg of diethyl ether leaf extract. Aqueous and methanolic leaf extracts had no antifungal activity in all tested fungal strains except 5mg of methanolic leaf extract. The aqueous and diethyl ether stem extracts showed potent antifungal activity and the maximum zone of inhibition 15mm was observed against *Aspergillus Niger*. Diethyl ether stem extract also showed maximum zone of inhibition 15mm against *Trichoderma viride*.

Invitro Cytotoxic activity [24]

The invitro cytotoxic activity of methanol, aqueous and ethyl acetate extracts of leaves of *Marsilea quadrifolia* on MCF-7 cells from human breast cancer. Qualitative phytochemical screening tests were performed to detect phytochemicals in the extracts. Antioxidant activity of the plant extract was then characterised using Diphenyl-picryl hydrazyl (DPPH) radical scavenging method. Antioxidant activity using DPPH was found to increase, in concentration dependent manner. All the three extracts ethyl acetate, aqueous and methanol exhibited potential antioxidant activity with an IC₅₀ value of 10, 125 and 25µg/ml respectively, when compared to the standard BHT with an IC₅₀ value of the 7.5 µg/ml. The cytotoxic activity of the extracts of *Marsilea quadrifolia* on MCF-7 cells from human breast cancer was investigated invitro 3-(4) 5-Dimethyl-thiazol-yl) - 2, 5 biphenyl tetrazolium bromide (MTT). The results showed decreased cell viability and cell growth inhibition in dose dependent manner. The IC₅₀ value of standard 5 fluorouracil, methanol, ethyl acetate and aqueous extracts were 9.3, 39.06, 47.82 and 187.5µg/ml respectively.

Methanol and ethyl acetate extracts of *M. quadrifolia* demonstrated strongest antioxidant and anti-proliferative activities. The findings from this study indicated that methanol and ethyl acetate extracts of *M. quadrifolia* leaf possessed vast potential as medicinal drug especially in breast cancer treatment. .

Antibacterial, Cytotoxic and Antioxidant Activity [25]

The antibacterial, antioxidant and cytotoxic activity of petroleum ether, chloroform and ethyl acetate extracts of *Marsilea quadrifolia* (Family: Marsileaceae). For antibacterial test, Disc diffusion technique was used against 5 Gram positive and 11 Gram negative human pathogenic bacteria. The range of zone of inhibition of chloroform and ethyl acetate extracts was 9 to 20 mm. The petroleum ether extract did not show any zone of inhibition against any tested pathogenic bacteria. The Brine shrimp lethality bioassay method was used to determine the cytotoxicity activities and Vincristin sulphate was used as a positive control. The LC₅₀ values of standard Vincristin sulphate, petroleum ether, chloroform and ethyl acetate extracts were 6.628µg/ml, 9.543µg/ml 7.820 µg/ml, and 8.589µg/ml respectively. All the fractions showed potent antioxidant activity, of which the ethyl acetate fraction demonstrated the strongest antioxidant activity with the IC₅₀ value of 50.1053 µg/ml.

Anti – Stress activity [26]

Anti-stress activity was evaluated using physical stress models viz. swimming endurance and post swimming motor function test, anoxic tolerance test and restraint stress test. Swiss albino mice (18-25 g) divided into

four groups of six animals each were used for the study. Control group received CMC as vehicle and standard group received *Withania somnifera* (100 mg/kg) while *Marsilea quadrifolia* ethanolic extract (200 and 400 mg/kg) were administered orally for seven days. Change in immobility time in swim endurance and first clonic convulsion in anoxic tolerance test and stress-induced behavioural and biochemical alterations in immobilization stress was recorded as parameters. *Marsilea quadrifolia* ethanolic extracts significantly reduces the immobility timing along with increases the swimming endurance time, post motor function and clonic convulsion timing in anoxic tolerance test as compared to control group and significantly reversed the behavioural and biochemical alterations in restraint stress.

Hypoglycaemic and in Vitro Antioxidant activity [27]

Hypoglycaemic effect was evaluated in alloxan induced diabetic rat. The oral administration of plant extract at a dose of 300 mg/kg body weight was given to fasting glucose loaded rat with regard to normal control during 1 hr. study period and in alloxan induced (110 mg/kg body weight i.p.) diabetic rat in comparison with reference drug Metformin Hydrochloride (100 mg/kg) during 3 days study period. The antioxidant potential of MEMQ was checked by qualitative method and quantitatively through DPPH (1, 1- diphenyl-2-picryl-hydrazyl) scavenging assay at 517 nm. Total phenolic content, total antioxidant capacity and reducing

Power activity was also assayed. Considerable drop in elevated blood glucose level was observed in the alloxan induced diabetic ($p < 0.05$ & $p < 0.001$) rat. At a dose of 300 mg/kg the extract showed glucose level reduction of 47.57% in alloxan induced rat while 44.38% was found for Metformin after 3 days. Antioxidant activity using DPPH was found to increase in a concentration dependent manner with an IC₅₀ value of $96.37 \pm 3.62 \mu\text{g/ml}$ higher than the standard one, IC₅₀ $16.59 \pm 0.59 \mu\text{g/ml}$. Total phenolic content was found $165.75 \pm 0.961 \text{ mg/g}$ in GAE and the total antioxidant capacity was equivalents of ascorbic acid ($224.90 \pm 1.42 \text{ mg/g}$).

DPPH Free Radical Scavenging Activity [28]

Antioxidants are the substances which inhibit oxidation, which have the ability to remove the potentially damaging oxidizing agents in a living organism. Many phytochemicals present in the plants are able to reduce or prevent the oxidative damage to the human cells which can cause even cancer in humans. It is highly vital to know about the antioxidant activities of each plant and the phytochemicals responsible for that. In this study, the DPPH free

radical scavenging activity of the extracts of *Marsilea quadrifolia* is analysed.

Antibacterial activity [29]

Four Leaf Clover or aalaik keera in Tamil is in use for more than 3000 years as part of food. It is a pteridophytic plant that is marketed and used by tribals of Eastern India as a nerve relaxant and curative agent due to its nutritional value. In the present study *M. quadrifolia* leaf extracts have showed effective inhibition against gram negative bacteria such as *Salmonella typhi*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa* and *Escherichia coli*. These results are in accordance with the previous results were *M. quadrifolia* revealed profound antibacterial, cytotoxic and antioxidant effects. Despite these uses, no published works are available for the antimicrobial property of this plant. The study was therefore aimed to investigate the antibacterial effects of ethanolic extract of the experimental plant, the pathogens were tested by disc diffusion assay method and minimum inhibitory concentration was evaluated. An attempt has been made to compare the activity of extract with standard ciprofloxacin.

Antianxiety Activity [30]

Marsilea quadrifolia a commonly available aquatic plant found to be used in different traditional system and by local people for anxiety and related disorders. The study was intended to evaluate the antianxiety potential of *Marsilea quadrifolia* and give a scientific basis for this. The CNS effect was screened by taking Swiss albino mice and Wistar rats. The extract was found to cause reduction in spontaneous activity, decrease in exploratory behavioural pattern by swimming and pole climbing test at a dose of 500mg/kg. In general behavioural screening the extracts found to show tranquilizing property. These Preliminary tests indicate that the Ethanolic extract of *Marsilea quadrifolia* in doses of 500 mg/kg has significant Antianxiety activity. The current study thus supports the traditional utilization of this plant against the inflammatory disorders.

Ethylene Induce Heterophyll in Marsilea Quadrifolia [31]

Individuals of *Marsilea quadrifolia*, an amphibious fern, experiencing extreme variations in environment develop heterophyll with different morphological characteristics. The objective of this study is to investigate if ethylene can induce floating type of leaves in this fern. To achieve this goal, ratio of stomatal density on abaxial and adaxial leaf surfaces (stomatal ratio) and the mass per unit length of petiole (PML), on leaves of terrestrial shoots sprayed with an ethylene gas releaser, Ethephon, were compared with those of leaves produced by submergence of terrestrial

shoots. Leaves with different stomatal ratio and PML, corresponding to that of terrestrial type and floating type of leaves, were produced when terrestrial shoots of *M. quadrifolia* were submerged. The result reveals that the plasticity of leaves to respond to submergence depends on leaf's age. Application of Ethephon significantly altered the stomatal ratio of young leaves on terrestrial shoot but not their PML. Leaves response to Ethephon treatment was also age dependent. These results indicate that ethylene might be involved in the formation of floating leaves in *M. quadrifolia*.

Remediation of nitrite contamination [32]

The study was carried out to determine the seasonal variation of nitrite levels in drinking and surface waters of urban, peri-urban and rural areas of Lucknow, during 2007-2008, and to evaluate the nitrite removal and accumulation potential of certain native aquatic macrophytes. Most of the drinking and surface water samples were collected from urbanized region of the city. All drinking water samples detected, showed higher nitrite level in winter, when compared with that in summer and rainy seasons. However, in drinking water samples nitrite level was below the permissible limit i.e. 3.29 mg l⁻¹ NO₂. The surface water showed more than 3 fold higher levels of nitrite over the permissible level i.e. 0.06 mg l⁻¹, and the level was higher during rainy season than in summer and winter seasons. Eight macrophytes viz. *Peltandra virginica*, *Utricularia vulgaris*, *Eichhornia crassipes*, *Trapa natans*, *Mimulus glabratus*, *Marsilea quadrifolia*, *Pistia stratiotes* and *Polygonum persicaria* were studied for phytoremediation potential of nitrite from the water under simulated laboratory conditions. The gradual diminution in the level of nitrite in the water and simultaneously its increase in the plant tissues was recorded at 5th, 10th and 15th d after plant culture. All the plants selected, removed nitrite from water but *Polygonum persicaria*, *Mimulus glabratus*, *Trapa natans* and *Pistia stratiotes* were found more efficient and removed nitrite upto 60.91, 58.09, 60.97 and 72.28%, respectively. Observations revealed that *Pistia stratiotes* can be used for the effective removal of nitrite from the contaminated water.

Valuable Culinary and Remedial Fern in Jaduguda, Jharkhand, India [33]

An investigation has been made on the relevance of one such lesser known species *Marsilea quadrifolia* Linn. (Vernacular name - sushni saag) commonly used by 'Ho' tribes in the mining belt of Jaduguda, Jharkhand (India) for its culinary and medicinal properties. Besides exploring indigenous knowledge of local tribal's, the study also incorporates the survey of local markets in and around Jaduguda. Information was collected from the local tribal people (Ho) as well as local drug sellers in the Haat (local market) pertaining

to use of *Marsilea quadrifolia* Linn. It is a pteridophytic plant that is marketed and used by tribals of that area for its nerve relaxant nature and curative properties for various other ailments of nervous system and its nutritional value. The present paper compiles botanical description of *M. quadrifolia* Linn. its traditional folk medicinal uses and marketing and monetary benefit to the villagers.

Psychopharmacological activity [34]

Hydro alcoholic extract of the entire plant *Marsilea quadrifolia* (HEMQ) was evaluated for different psychopharmacological actions such as behaviour, exploratory behaviour, muscle relaxant activity and phenobarbitone induced sleeping time. The extract was found to cause reduction in spontaneous activity, decrease in exploratory behavioural pattern by swimming and pole climbing test., reduction in the muscle relaxant by traction test. In addition, the extract significantly potentiated the phenobarbitone-induced sleeping time. Preliminary tests indicate that the hydro alcoholic extract of *Marsilea quadrifolia* in doses of 200-400 mg/kg has significant psychopharmacological activity.

In Vitro Morphogenetic Potential [35]

The present project is part of the actual concerns in biodiversity conservation. *Marsilea quadrifolia* species is protected both at national and European level (by Habitats Directive of EU and Bern Convention). The aspects of *in vitro* morphogenesis passing through all the stages from inoculation, multiplication to rooting and acclimation have been studied. The results are positive proving that the species responds well to *in vitro* conditions.

Herbal Drugs As Anti Diarrheal: A Review [36]

In past years herbal medicine has gained an exponential growth in the field of medicine in all over world. In comparison to other countries India is the largest producer of medicines. The current review focuses on herbal preparations and plants recently evaluated in the treatment of diarrhea disease in the world. This paper will focus on different beneficial aspects of herbal medicine as anti diarrheal.

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