



## Research Article

### ANTIBACTERIAL ACTIVITY OF *Marsilea quadrifolia*: A FLORAL SPECIES

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Article Received on: 19/07/18 Approved for publication: 12/08/18

DOI: 10.7897/2230-8407.098170

#### ABSTRACT

Investigation was undertaken to evaluate antibacterial activity of the aquatic fern named as *Marsilea quadrifolia* against various gram positive and gram negative multi drug resistant bacteria through disc diffusion assay. The ethyl acetate extract of this plant is more effective in terms of antibacterial activity than water extract which indicates that ethyl acetate solvent extracted the active components from the plant parts in a more efficient way compared to water extract.

**Key Words:** *Marsilea quadrifolia*, Antibacterial activity, Ethyl acetate, Water and Disc diffusion assay

#### INTRODUCTION

India is known as one of the mega biodiversity countries in the Global panorama. In India, the history of traditional medicine dates back to ancient human civilization and precious information on herbal medical practices have been documented<sup>1</sup>. Medicinal plants have important use in curing of multi drug resistant microorganisms. Crude extract or as standardized plant products are the natural products which gives several pathways for development of new drugs because of lack of availability of chemical diversity. Plant extract and drugs made from plants are used to treat several types of diseases<sup>2</sup>.

Traditional healers and their plant medicines provide the only health care to majority of people in a curative rather than a preventive approach in the developing countries for common ailments<sup>3</sup>. Natural herbal products were used to apply for treatment of diverse type of bacterial, fungal and viral diseases. WHO has reported that around 80% of the world's population in developing nations believe in plant-derived medicines for their healthcare needs<sup>4</sup>. Plant products and related drugs are used to treat 87% of all categorized diseases<sup>5</sup>.

51 medicinal plants are plants, which when administered to man or animal exert a sort of pharmacological action on them. Herbs make up most of the plant sources for the production of useful drugs that are being utilized by people worldwide<sup>6</sup>.

Pteridophytes (ferns and fern allies) are called as reptile group of plants and are one of the earliest groups of vascular plants. People mainly in rural areas are not aware of the application of pteridophytes since it is not easy to get them like flowering plants<sup>7</sup>. It is the high time to do research on new alternative

antimicrobial compounds with diverse chemical structure and novel mechanism of action for new and reemerging infectious diseases<sup>8</sup>.

The present research work was conducted after collection of the aquatic fern *Marsilea quadrifolia* from Singur region (22°81'50" N & 88°23'45" E) of the Hooghly district in West Bengal. The study was done to analyse antibacterial activity of this aquatic fern against various gram positive and gram negative multi drug resistant bacteria through disc diffusion assay.

#### MATERIALS AND METHODS

##### Sample Collection

Fresh plants of *M. quadrifolia* were taken 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).

##### Preparation of Plant Extracts

The plant samples were shed dried, grounded in powder form and preserved separately in airtight container for further use. 5 g of these powder samples were soaked separately into 50 ml of organic solvent like ethyl acetate and kept in rotary shaker at 30°C for overnight. After that they were filtered by using Whatman No. 1 filter paper and then the filtrate was concentrated by using a rotary evaporator. Each crude extract was transferred into air tight eppendorf tube and stored at -20°C till further use. The same process was carried out for water extract by using double distilled water.

### Bacterial Culture

The different microbial cultures used for this study are highlighted in Table 1. Standard microbial techniques were followed throughout the work. Mueller Hinton (MH) broth and Mueller Hinton (MH) agar were prepared following the manufacturer's instructions (Hi Media, Mumbai) and bacterial strains were inoculated in sterile media and then incubated at 30°C for 24 hours. Aseptic conditions were maintained during all microbial works.

### Disc Diffusion Assay

A disc diffusion assay was performed to determine antimicrobial activity of prepared extracts by following the standard protocols<sup>8</sup>. Briefly, each bacterial culture was spread on to the Mueller-Hinton agar media plates. Filter paper discs (6 mm in diameter) were placed on plate with the extracted samples (20 µl) individually and kept them in incubation at 30°C for 24 hours.

The dimension of the zone of inhibition was measured in millimeter unit. In this assay, we have used Antibiotic as positive control.

### Statistical Analysis

For calculation of the antibacterial activity, the data were expressed as mean ± standard deviation (SD) of the triplicate values.

### RESULTS

The antibacterial activity for *Marsilea quadrifolia* plant was screened against two Gram-positive (*B. subtilis*, and *Bacillus* sp.) and eight Gram-negative (*Escherichia coli*, *K. pneumoniae*, *Enterococcus faecalis*, *Agrobacterium tumefaciens*, *Stenotrophomonas* sp., *Pseudomonas* sp., *Delftia* sp. and *Brevundimonas* sp.) bacteria through disc diffusion assay is given in the following table and figure (Table 2 and Figure 1).

Table 1: The microbes used for this study with their accession numbers

Microorganism	Accession no.
<i>Delftia</i> sp. strain SR4	KY212526
<i>Brevundimonas</i> sp. strain SR3	KY229729
<i>Stenotrophomonas</i> sp. strain ASO-MDR1	KT363764
<i>Agrobacterium tumefaciens</i> strain SR5	KY229914
<i>Klebsiella pneumoniae</i>	ATCC 700603
<i>Enterococcus faecalis</i>	ATCC 29212
<i>Klebsiella pneumoniae</i> (C) <sup>a</sup>	ATCC BAA1705
<i>Escherichia coli</i>	ATCC 25922
<i>Bacillus subtilis</i>	ATCC 6051
<i>Staphylococcus aureus</i> subsp.	ATCC 25923
<i>Pseudomonas</i> sp.	Isolated clinically

<sup>a</sup>C, Carbapenemase producer

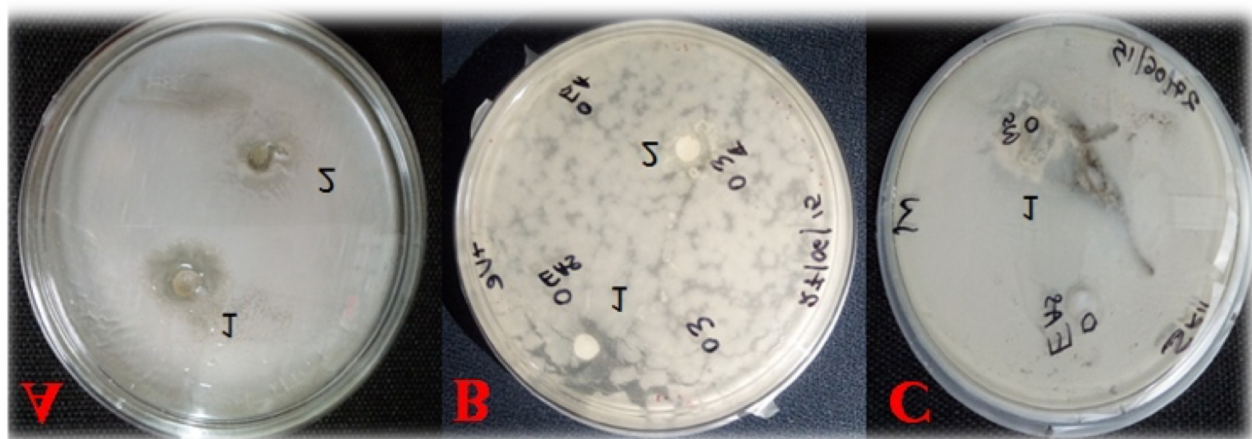
Table 2: Antimicrobial activity of the ethyl acetate and water extracts of *Marsilea quadrifolia*

Bacteria	Ethyl Acetate DD <sup>a</sup>	Water DD <sup>a</sup>
<i>Delftia</i> sp.	11±0.01	8±0.06
<i>Brevundimonas</i> sp.	13±0.11	na <sup>b</sup>
<i>Stenotrophomonas</i> sp.	16±0.02	na <sup>b</sup>
<i>Agrobacterium tumefaciens</i>	10±0.06	na <sup>b</sup>
<i>Klebsiella pneumoniae</i>	na <sup>b</sup>	na <sup>b</sup>
<i>Enterococcus faecalis</i>	12±0.15	na <sup>b</sup>
<i>Klebsiella pneumoniae</i> (C)	na <sup>b</sup>	na <sup>b</sup>
<i>Escherichia coli</i>	16±0.06	na <sup>b</sup>
<i>Bacillus subtilis</i>	12±0.11	11±0.03
<i>Staphylococcus aureus</i> subsp.	na <sup>b</sup>	na <sup>b</sup>
<i>Pseudomonas</i> sp.	11±-0.01	na <sup>b</sup>

<sup>a</sup> Disc Diffusion (diameter of zone of inhibition, including disc diameter of 6 mm; <sup>b</sup>na i.e. not active



Figure 1: *Marsilea quadrifolia* at Singur, West Bengal



**Figure 2:** Antibacterial activity of ethyl acetate and water extracts of *Marsilea quadrifolia* plant. A and B is for ethyl acetate extract of *Marsilea quadrifolia* plant extract. 3 is for water extract of *Marsilea quadrifolia* plant extract. 1: Control ethyl acetate (A and B) and water (C); A: *Pseudomonas sp* B: *Escherichia coli* C: *Bacillus subtilis*

## DISCUSSIONS & CONCLUSION

A huge number of complex and structurally diverse compounds mostly bioactive substances are synthesized by plant and other natural sources. Investigations were carried out by many researchers on plant and microbial extracts, essential oils, new synthesized molecules and pure secondary metabolites as potential antimicrobial agents<sup>8-12</sup>. However, review of the published articles on the anti-microbial activity of the medicinal plants, usually show mixed output which is often difficult to understand because of the traditional methods like inoculum preparation techniques, growth medium, inoculum size, end points determination and incubation conditions which are different non-standardized techniques. It is of utmost importance to determine antimicrobial activity of plant extracts through disc diffusion assay, but this preliminary part of data should be repeated thrice (as a part of quality assurance of the data) before coming to a conclusion. This will allow researchers to compare results through standard statistical tools and should avoid doing research which researchers use the antimicrobial activity investigation only as a complement to a phytochemical study. Several laboratory based methods are there to analyse or evaluate the *in vitro* antimicrobial activity of an extract or a pure compound. Disc diffusion assay and broth or agar dilution methods are the two most trusted methods used for the determination of antibacterial activity of plant extract. Other methods are applicable especially in antifungal assay, such as poisoned food technique. Time-kill test and flow cytometric methods are the two methods which are recommended for providing accurate and best results on the inhibitory effect (bactericidal or bacteriostatic) (time-dependent or concentration-dependent). Very few researchers are going on combating multidrug-resistant bacteria. So, it is the high time to develop a standard protocol for screening and/or quantifying the antibacterial activity of an extract or a pure compound for its applications in human health, agriculture and environment. The present results indicate that the antibacterial assay for *Marsilea quadrifolia* plant exhibited the development of zone of inhibition surrounding the discs. The ethyl acetate extract of this plant is more effective in terms of antibacterial activity than water extract which indicates that ethyl acetate solvent extracted the active components from the plant parts in a more efficient and effective way compared to water extract.

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**Cite this article as:**

Satarupa Roy et al. Antibacterial activity of *Marsilea quadrifolia*: A floral species. Int. Res. J. Pharm. 2018;9(8):86-89  
<http://dx.doi.org/10.7897/2230-8407.098170>

Source of support: Nil, Conflict of interest: None Declared

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