Research Article

GC–MS Characterization and Evaluation of Antimicrobial and Anti-Inflammatory Activities of Ethanolic Leaf Extract of *Marsilea quadrifolia*

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ABSTRACT

The therapeutic potential of the medicinal water fern *Marsilea quadrifolia* is widely recognized. Using gas chromatography–mass spectrometry (GC–MS), the current study examines the phytochemical composition of the ethanolic leaf extract and assesses its antibacterial and anti-inflammatory properties. Numerous bioactive substances with established pharmacological characteristics, including fatty acids, phenolics, hydrocarbons, and terpenoids, were detected by GC-MS analysis. The zone of inhibition increased in a concentration-dependent manner when the antibacterial activity was evaluated against specific Gram-positive and Gram-negative bacterial strains using the agar well diffusion method. In vitro techniques were used to assess the anti-inflammatory potential, and the results showed significant inhibitory activity. The synergistic activity of identified phytoconstituents may be responsible for the biological effects that have been reported. Overall, the results support *M. quadrifolia's* potential for additional therapeutic investigation by indicating that it may be a promising natural source of antibacterial and anti-inflammatory compounds.

Keywords: GC-MS Analysis, *Marsilea quadrifolia*, Antimicrobial Activity, Anti-inflammatory.

INTRODUCTION

Water clover, or *Marsilea quadrifolia* L., is an aquatic fern belonging to the Marsileaceae family that is found in many tropical and subtropical areas. Folk medicine has long used it to treat fever, inflammation, sleep difficulties, and microbial infections[1]. The need to identify new bioactive compounds from medicinal plants of ethnopharmacological significance has been highlighted by the growing problem of antimicrobial resistance[2]. Secondary metabolites such as alkaloids, flavonoids, terpenoids, and phenolic compounds are abundant in these plants and exhibit a variety of biological activities, including anti-inflammatory and antibacterial properties [3]. A potent analytical method for identifying phytoconstituents, gas chromatography–mass spectrometry (GC–MS), allows for the accurate detection of volatile and semi-volatile chemicals and their correlation with biological activity [4]. According to earlier research, M. quadrifolia has antioxidant, anti-inflammatory, and antibacterial qualities [5]. Nevertheless, there is still a lack of thorough GC-MS profiling of its ethanolic leaf extract and its association with anti-inflammatory and antibacterial properties. In order to validate its traditional therapeutic use, the current study will use GC-MS to examine the phytochemical content of the ethanolic leaf extract and assess its antibacterial and anti-inflammatory properties.

MATERIALS AND METHODS

Plant Collection and Extraction:

Fresh *Marsilea quadrifolia* leaves were collected from their natural source. The leaves were washed, shade-dried, and powdered by grinding to obtain a uniform coarse material. The powdered form of the plant was collected and macerated with ethanol at room temperature for 73 hours with occasional stirring. The extracts were separated from residues by filtering through several layers of muslin cloth for coarse filtration and then through Whatman No. 1 filter paper. The residue was further extracted using the same procedure. The filtrates obtained were combined and then evaporated to dryness at a temperature not exceeding 35 °C. The extracts were kept in a cool place till use.

GC–MS Analysis:

GC-MS is now widely acknowledged as a critical technology platform for secondary metabolite profiling in materials that are of plant origin. Here, using the characteristics of both mass spectrometry and gas chromatography helps to separate various chemicals within a test sample according to how long they retain. In this investigation, the active principles are displayed in Figures 1 and Table 1, demonstrating the presence of several bioactive phytochemical compounds in the ethanolic extract of *Marsilea quadrifolia,* including the parameters such as retention time (RT), molecular formula, molecular weight (MW), concentration (peak area%), chemical structures, and biological activities.[6]

One of the best, quickest, and most reliable techniques for identifying different phytochemicals, such as polyphenolic compounds, alkaloids, long-chain hydrocarbons, and unsaturated fatty acids, is GC-MS, which only needs a tiny sample of plant extract.[7] As a result, the phytochemical components of the chosen medicinal plant were found and identified using the GC-MS technique in the current experiment.

GC-MS analysis of the Ethanolic extract of the *Marsilea quadrifolia* was performed using the Shimadzu GC-MS-QP2010 Ultra Version of the apparatus. There are further uses for this instrument in ultra-high separation and

accurate two-dimensional GC. Helium is the mobile phase carrier gas in this system, and it is employed at a Total flow rate of 14.1 ml/min, Column Flow of 1ml/Min, and Purge flow of 3.0 ml/min via a capillary standard non-polar fused silica column. The column oven was set at 70 °C, while the injector was operating at 280 °C. At a pressure of 61.3 kPa (psi), the split-less method of injection technique was employed with the AOC-20i auto injector. After inserting the 1 µl sample ethanolic extract into the device, it was completely operational to identify molecules having a mass per charge (m/z) ratio between 40 and 850 atomic mass units (amu).

In addition, the GC-MS settings include an interface temperature of 260 °C, an ion source temperature of 200 °C, a solvent cut time of 4.5 min, and an elution length of 63 min. The data were compared using the NIST 20 (2020) mass spectral library search tool. For the research of 25 compounds, retention periods ranging from 5 to 63 minutes were recorded, together with the molecular formula, molecular mass, and percentage area of each chemical.

The GC-MS chromatogram shown in Figure 1 and other critical factors for analysis for individual molecules retention time (RT), molecular formula, molecular weight (MW), concentration (%), and previously reported biological activity by other research work, are presented in Table 1. These tools aid in predicting the formulas and structures of several biomolecules in the current study.

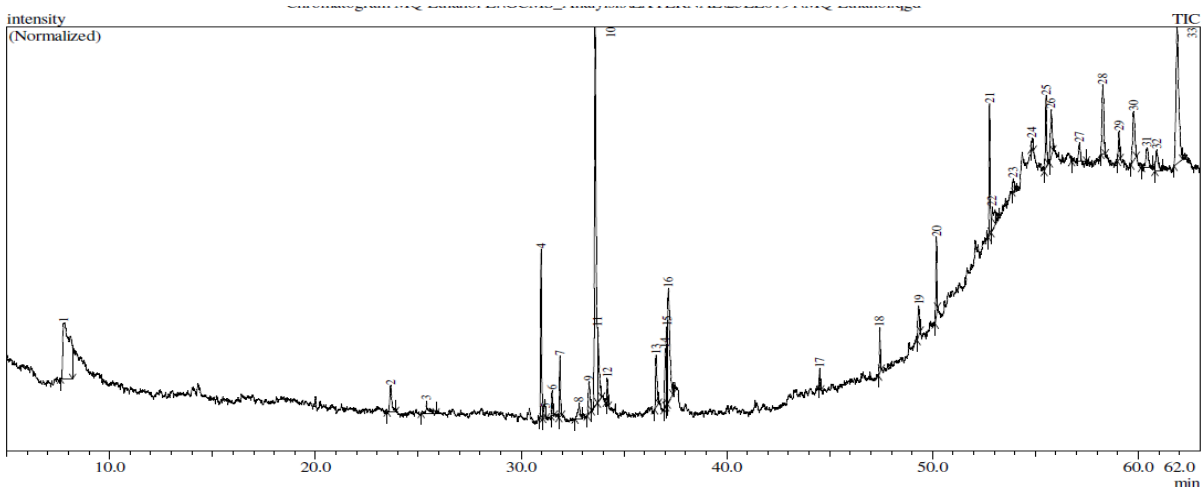
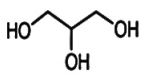
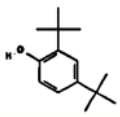
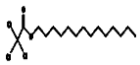
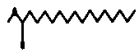
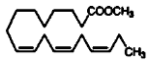
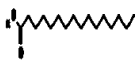
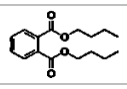
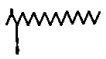
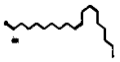
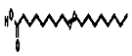
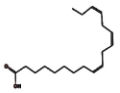
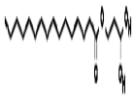
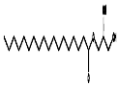
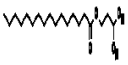
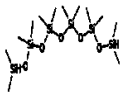
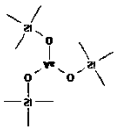
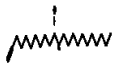


Figure 1: GC-MS chromatogram for ethanolic extract of *Paederia foetida*

Table 1: GC-MS spectral analysis of ethanolic extract of *Paederia foetida*

Peak No	RT	Compound Name	Area %	Mol. Formula	M.W (g/mol)	Reported bioactivity	Structure	Ref
1	7.767	Glycerin	11.27	C3H8O3	92	Anti-microbial, Antioxidant, Antifungal, Anti-inflammatory Cosmetics.		*8*
2	23.666	2,4-Di-tert-butylphenol	1.59	C14H22O	206	Anti-microbial, Antioxidant, Antifungal, Anticancer		*9*
3	25.407	Trichloroacetic acid, tetradecyl ester	0.87	C16H29Cl3O2	358	Anti-microbial, Antioxidant		*10*
5	31.183	2-Pentadecanone, 6,10,14-trimethyl	0.81	C18H36O	268	Anti-inflammatory Anti-microbial Anticancer		*11*
8	32.829	Hexadecanoic acid, methyl ester	1.05	C17H34O2	270	Antioxidant		*12*
9	33.314	Hexyl linolenate	1.70	C24H42O2	362	Antioxidant		*13*
10	33.601	n-Hexadecanoic acid	17.52	C16H32O2	256	Antitumor, Antioxidant, Anti-inflammatory		*14*

11	33.742	Dibutyl phthalate	3.63	C16H22O4	278	Antimicrobial		***15***
12	34.193	Hexadecanoic acid, ethyl ester	0.61	C17H34O2	270	Antioxidant		***16***
13	36.569	Phytol	2.09	C20H40O	296	Antioxidant, Anti-inflammatory		***17***
14	37.017	10E,12Z-Octadecadienoic acid	1.66	C18H32O2	280	Anticancer Antiviral		***18***
15	37.081	9-Octadecenoic acid, (E)-	2.86	C18H34O2	282	Anti-inflammatory Anti-microbial		***19***
16	37.172	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	7.32	C18H30O2	278	Antioxidant Antibacterial		***20***
17	44.516	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	0.47	C19H38O4	330	Antimicrobial Anti-inflammatory Antioxidant		***21***
18	47.442	Octadecanoic acid, 2,3-dihydroxypropyl ester	1.29	C21H42O4	358	Antimicrobial Anticancer Anti-inflammatory		***22***
19	49.327	n-Tetracosanol-1	1.19	C24H50O	354	Anti-inflammatory Antimicrobial Anticancer		***23***
21	52.772	Pentadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	4.10	C18H36O4	316	Antioxidant Anti-inflammatory Antimicrobial		***24***
22	52.905	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	1.65	C12H38O5Si6	430	Antimicrobial Antibacterial		***25***
23	53.919	Arsenous acid, tris(trimethylsilyl) ester	0.52	C9H27AsO3Si3	342	Antioxidant Anti-inflammatory		***26***
24	54.836	Undec-10-ynoic acid, undecyl ester	0.40	C22H40O2	336	Antioxidant		***27***

Assessment of *in vitro* antimicrobial activity:

Using the agar well diffusion method and normal microbiological methods, the antibacterial activity of the ethanolic leaf extract of Marsilea quadrifolia was assessed. The broad-spectrum antibacterial properties of the extract were evaluated in this investigation using soil-derived microorganisms as the test organisms. Microbial cultures were isolated and kept on nutrient agar after aseptic collection of soil samples. Freshly generated soil microorganisms were suspended in sterile saline solution to provide the inoculum. To maintain equal microbial density, the turbidity was adjusted to match the 0.5 McFarland standard, or roughly 1×10^2 CFU/mL [28].

Aseptic conditions were used to prepare sterile nutrition agar plates and let them solidify. To create a confluent lawn of growth, the standardized microbial suspension was evenly applied to the agar plates using a sterile cotton swab. Using a sterile cork borer, wells with a diameter of approximately 6 mm were aseptically drilled into the agar, ensuring sufficient spacing between wells to prevent overlap of inhibitory zones [29].

Using a micropipette, various ethanolic extract concentrations were carefully added to the wells. The antibacterial efficacy of the plant extract was compared using ciprofloxacin as the standard antibiotic (positive control), and the absence of solvent-induced activity was confirmed using ethanol as the negative control. After that,

the plates were incubated for 24 hours at 37°C to promote microbial growth and extract diffusion into the surrounding medium. The plates were inspected for distinct zones of inhibition surrounding the wells following incubation. A calibrated scale was used to measure the zones' diameters in millimeters. Every experiment was carried out in triplicate, and the mean ± standard deviation was used to express the results. Significant antibacterial activity of the extract against soil-derived microorganisms was demonstrated by a concentration-dependent increase in the zone of inhibition.[30]

Zone of inhibition with standard deviation:

Concentration	Standard	MQME	MQEE	MQCE	MQEAE
(µg/ml)	(ZOI in mm)	**(**ZOI in mm)	(ZOI in mm)	(ZOI in mm)	(ZOI in mm)
100	**15**	**12**	**10**	**13**	**11**
150	**17**	**13**	**15**	**14**	**12**
200	**22**	**19**	**17**	**15**	**13**
250	**25**	**22**	**20**	**17**	**16**

Graphical Representation:

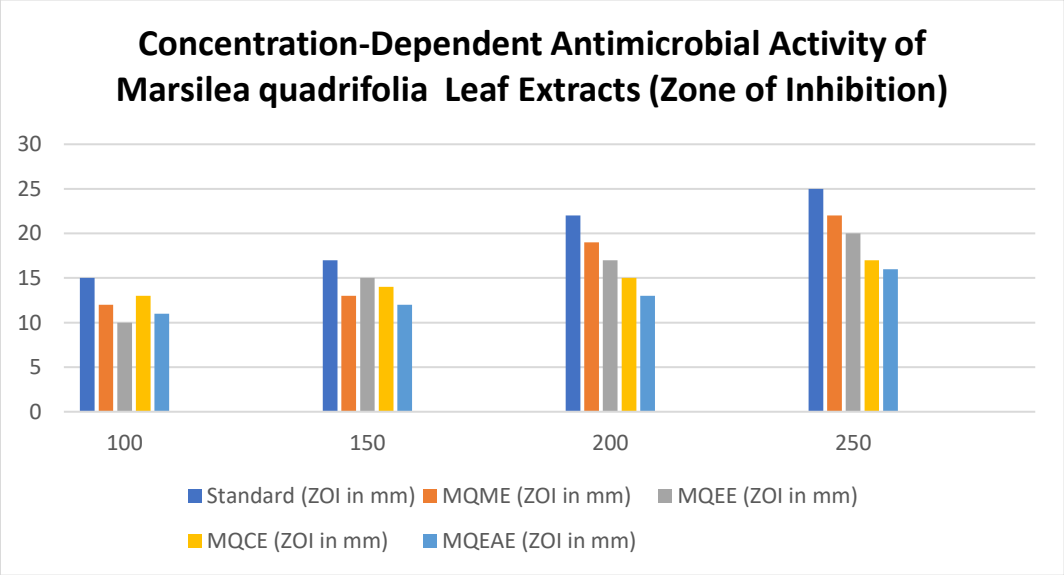


Figure 2: Standard curve

DISCUSSION

The antimicrobial activity assay demonstrated that all tested extracts exhibited a concentration-dependent increase in the zone of inhibition against the selected microbial strains. Among the extracts, MQME showed the highest activity, followed by MQEE, MQCE, and MQEAE, indicating varying levels of bioactive constituents across the fractions. Although the standard drug (ciprofloxacin) exhibited superior inhibitory effects, the plant extracts displayed significant antimicrobial potential, particularly at higher concentrations (200–250 µg/ml). These findings suggest that the extracts possess promising antimicrobial compounds and could serve as a potential natural source for developing alternative antimicrobial agents**.**

Assessment of *in vitro* anti-inflammatory potential:

The goal of the in vitro anti-inflammatory investigation of *Marsilea quadrifolia* leaf extract is to provide scientific support for its customary application in the treatment of inflammation. The body uses inflammation as a natural defense mechanism in reaction to damage, illness, or damaging stimuli. However, several health problems, including autoimmune illnesses, cardiovascular diseases, and arthritis, can be brought on by persistent or severe inflammation.

The egg albumin denaturation assay, a widely used technique based on the idea that many anti-inflammatory drugs reduce heat-induced protein denaturation, can be used to assess the in vitro anti-inflammatory activity of *Marsilea quadrifolia* leaf extract. This assay involves mixing a known quantity of fresh egg albumin with phosphate-buffered saline (pH 6.4) and different plant extract concentrations (e.g., 100, 150, 200, and 250 µg/mL).

A standard solution containing a recognized anti-inflammatory medication (such as aspirin or diclofenac sodium) and a control sample (without extract) are also made for comparison. To cause denaturation, all mixtures

are heated to 70°C for five minutes after being incubated at 37°C for fifteen minutes. A UV-Vis spectrophotometer is used to test each sample's absorbance at 600 nm once it has cooled to room temperature. The following formula is then used to determine the percentage inhibition of protein denaturation:

$$\% \text{ Inhibition} = [(A.\ C - A.\ T)/A.\ C] \times 100.$$

Here, A, C & T accordingly denote Absorption, Control & Test.

Stronger anti-inflammatory potential is indicated by a higher percentage of inhibition. The anti-inflammatory efficacy of *Marsilea quadrifolia* leaf extract can be reliably evaluated using this straightforward, repeatable procedure, which also gives useful preliminary data for additional pharmacological analysis. In the instance of *Marsilea quadrifolia* leaf extract, a noteworthy percentage of inhibition supports its traditional use in the treatment of inflammation-related illnesses while also highlighting its potential as a natural anti-inflammatory drug. Furthermore, these results provide useful baseline information for additional pharmacological assessment through more sophisticated in vivo and clinical investigations. The precise mechanisms of action, therapeutic dose, bioavailability, and safety profile of the extract can all be clarified by such studies.

Observation Table:

Concentration (µg/ml)	% Inhibition of Diclofenac sodium	% Inhibition of MQME	% Inhibition of MQEE	% Inhibition of MQEAE	% Inhibition of MQCE
100	94.65	49.41	45.67	48.43	18.32
150	96.52	50.53	49.81	52.93	25.12
200	98.37	58.27	52.54	55.96	33.09
250	99.24	66.72	61.35	56.40	38.43

Graphical Interpretation of Results:

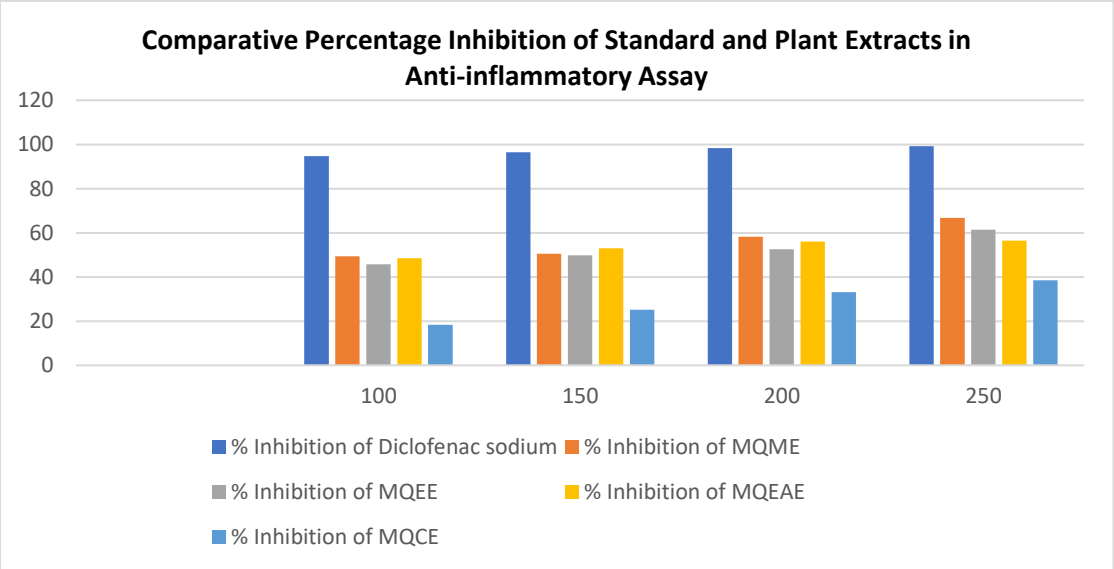


Figure 3: Standard curve

DISCUSSION

For every extract of Marsilea quadrifolia, the anti-inflammatory assay findings showed a definite concentration-dependent rise in percentage inhibition. With a 66.72% inhibition at 250 µg/ml, MQME demonstrated the most anti-inflammatory effect among the studied extracts, outperforming MQEE, MQEAE, and MQCE. The results indicate that the methanolic extract has significant anti-inflammatory potential and may be a promising source of natural anti-inflammatory chemicals, even though the activity was lower than that of normal diclofenac sodium.

CONCLUSION

The current investigation showed that the various *Marsilea quadrifolia* extracts have strong antibacterial and anti-inflammatory properties in a concentration-dependent way. All extracts demonstrated an increase in the zone of inhibition against the investigated microbial strains in the antimicrobial tests as the concentration increased. The methanolic extract (MQME), followed by MQEE, MQCE, and MQEAE, showed the strongest antibacterial activity among the extracts, generating a zone of inhibition that was comparable to that of the conventional medication at higher concentrations. This implies that the methanolic

fraction contains strong bioactive phytoconstituents that have antibacterial properties.

Similarly, all extracts showed significant inhibitory efficacy against protein denaturation in the anti-inflammatory research; their effects were not as strong as those of the common medication diclofenac sodium. With maximum % inhibition at higher concentrations, the methanolic extract once again showed the best anti-inflammatory effect, followed by MQEE and MQEAE, while MQCE showed relatively less activity. The presence of phytochemicals with antibacterial and anti-inflammatory properties, such as flavonoids, phenolics, alkaloids, tannins, and terpenoids, may be responsible for the observed biological activity.

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Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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