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Phytochemical screening by LC-MS analysis and *invitro* anti- inflammatory activity of *Marselia quadrifolia* plant extract

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Abstract: The plant *Marselia quadrifolia* traditionally claimed to be useful in treatment of the cutaneous affections such as inflammation, wounds etc. The aim of the present study was to identify the phytoconstituents using Liquid chromatography - Mass spectroscopy analysis. In-vitro anti-inflammatory activity of *Marsilea quadrifolia* methanolic extract. The phytoconstituents were identified by Liquid Column Mass Spectrometry (LC-MS) analysis. The anti-inflammatory activity was further analyzed by using the Albumin Denaturation Assay and Membrane Stabilization Assay. Extract also showed *In-vitro* anti-inflammatory activity by inhibiting the heat induced albumin denaturation and Red Blood Cells membrane stabilization with its IC50 value of 208.275±2.78 and 261.09±9.56µg/ml. Thus this study indicates the vast potential of the methanolic extract of *Marsilea quadrifolia* as a medicinal drug especially in anti-inflammation. The leaves of *Marsilea quadrifolia* is used to retard the inflammation. From LC-MS report, it was concluded that flavonoids and related polyphenols present in the extract of *Marsilea quadrifolia* may be responsible for the anti-inflammatory activity.
Keywords: *Marsilea quadrifolia,* Anti-inflammatory, LC-MS, Phytochemical evaluation.

Introduction:

Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants which is characterized by redness, swelling and pain. According to the Medilexicon's inflammation is A fundamental pathologic process consisting of a dynamic complex of histologically apparent cytological changes, cellular infiltration, and mediator release that occurs in the affected blood vessels and adjacent tissues in response to an injury or abnormal stimulation caused by a physical, chemical, or biologic agent. Chronic inflammation can cause many diseases including some cancers rheumatoid arthrities,arthrosclerosis and heart attack[1]. Nowadays herbal based drug have increased manifold worldwide, so the researcher interested to discovering new drug from the plant. The plant *Marselia quadrifolia (M. quadrifolia)* is a creeping perennial herb with a slender long branching rhizome belongs to the family marsileaceae. It is widely distributed in India, China, Japan and north America. Based on the ethnomedicinal information, the *Marsilea quadrifolia* leaves are used in the treatment of hypertension, sleep disorders and headache. Entire fresh plant is used in the treatment of cough and convulsion. A juice of the the leaves is used for diuretic snakebite and febrifuge. The plant also posses anti-inflammatory, diuretic, depurative and refrigerant property[2,3] Previously reported pharmacological activity of *M. quadrifolia are* anticonvulsant, antioxidant[4] and antibacterial[5], antidiabetic[6],muscle relaxant, Alzeimars disease[7], analgesic and ant diarrheal[8]. The phytoconstituents reported on *M. quadrifolia* such as marsilin (1-triacontanol-cerotate), 3-hydoxy-tri-acontan-11-one, beta-sitosterol, flavonol-O-mono-and-diglycoside[9]. Only few phytochemicals were reported till

now from the plant, so in this present work, the phytochemical screening using LC-MS are performed to identify the more phytoconstituents in the methanolic extract of *M. quadrifolia* and evaluate its anti inflammatory potential by some invitro assay model.

Materials and Methods:

Collection of plants

The whole plant of *M. quadrifolia* was collected from the ponds of Erode. The leaves of the plants were washed, dried and coarsely powdered. The freshly collected plants were authenticated by Dr. M.Palanisamy Botanical Survey of India Coimbatore, the voucher specimen were stored in the Department of pharmaceutical Technology, BIT Campus,Anna UIniversity-Tiruchirappalli, India.

Preparation of extract[10]

Fresh plant of *M. quadrifolia* were collected and shade dried at room temperature, pulverized and 500g of leaves were exhaustively extracted with 100% methanol in Soxhlet apparatus for 72 hrs. The extracts were concentrated in rotary vacuum evaporator. The residue was dried in a desiccator and stored at 4^0C untill usage. The percentage yield of methanolic extract was 14.75%

Phytochemical studies

The methanolic extract of extracts of leaves of *M. quadrifolia* were subjected to qualitative chemical tests to detect the presence of various class of phyto-constituents.

Liquid Chromatography -Mass Spectrometry

UHPLC system was equipped with an autosampler and the employed column was a Waters nanoAcquityHSS T_3, 1.8 µm, 100 µm × 100 mm. The mobile phases were water 0.1 % formic acid (A) and 90 % acetonitrile in water 0.1 % formic acid (B) at a flow rate of 500 µL min^{-1}. The LC conditions were 5 % B during 0–3 min, a linear increase from 5 to 20 % B during 3–25 min, from 20 to 40 % B during 25–40 min and from 40 to 50 % B during 40–55 min, finally from 50 to 95 % B during 55–63 min followed by 15 min of maintenance. A Thermo Electron LTQ-Orbitrap XL mass spectrometer equipped with a nano electrospray ion source (ThermoFisher Scientific, Bremen, Germany) and operated under Xcalibur 2.1 version software, was used in positive ionization mode for the MS analysis using data-dependent automatic switching between MS and MS/MS acquisition modes.[11]

Invitro Evaluation of Anti-Inflammatory Activity

Inhibition of Albumin Denaturation

The reaction mixture was consisting of test extract at different concentrations and 1% aqueous solution of bovine albumin fraction. pH of the reaction mixture was adjusted using small amount of 1N HCl. The samples were incubated at 37˚C for 20 min and then heated at 57˚C for 20 min. After cooling the samples, the turbidity was measured spectrophotometrically at 660 nm.[12]

Percent inhibition of protein denaturation was calculated as follows:

$$\text{Percentage inhibition} = \frac{(\text{Absorbance control} - \text{Absorbance sample})}{\text{Absorbance control}} \times 100$$

Membrane Stabilization Assay

Preparation of Red Blood cells

(RBCs) suspension

Fresh whole human blood (10 ml) was collected and transferred to the centrifuged tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline.

Heat induced hemolysis

1 ml of methanolic extract of *M. quadrifolia* at different concentration (100-400 µg/ml) mixed with 10% RBC's suspension, instead of drug only saline was added to the control test tube. Aspirin was taken as a standard drug. All are taken in a centrifuge tube. The reaction mixture were incubated in a water bath at 56^{o}C for 30 min. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. [13,14]

$$\text{Percentage inhibition} = \frac{(\text{Absorbance control} - \text{Absorbance sample})}{\text{Absorbance control}} \times 100$$

Results and Discussion

The preliminary phytochemicals were identified in the methanolic extract of *M. quadrifolia* using various chemical tests. The presence of phytochemicals are shown in teble1. It showed glycosides, saponins and fats and oil are not identified in the methanolic extract.

Table 1: Preliminary screening of methanolic extract of *Marsilea quadrifolia*

Name of the Phytochemical	Methanolic extract of *M. quadrifolia*
Carbohydrates	++
Alkaloides	++
Steroides	++
Glycosides	++
Saponins	--
Flavonoids	--
Tannins	++
Proteins and amino acids	++
Fats and oils	++
Triterpenes	--
	++

++ Sign indicates presence and – sign indicates the absence of the phytochemical constituents

LCMS Reports

The LC-MS chromatogram of methanolic extract of *Marsilea quadrifolia* was showm in fig(1) and mass spectrum of the detected compounds was shown in (fig2-9). It was observed that the different peaks was obtained at different retention times. In this the highest peak is at the retention time of 2.9 followed by, 3.6, 3.3 belonging to the compound β-sitosterol, Quercetin and chlorogenic acid.

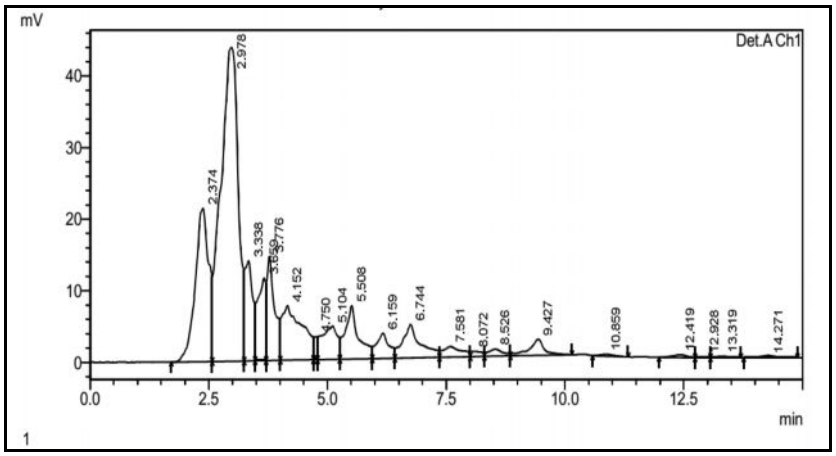

Fig 1: LC-MS chromatogram of methanolic extract of *M. quadrifolia*

Table 2 : Important compound identified from the methanolic extract of *M. quadrifolia* by LC-MS

Peak	Retention time	Name of the compound	Molecular weight	Arrea %
2	2.9	Betasitosterol	414.71	39.46
4	3.6	Quercetin	302.23	4.86
5	3.7	Tridecyliodide	310.26	5.75
3	3.3	Chlorogenic acid	354.31	5.74
9	5.5	2,3,7,8 tetrachlorodibenzofuran	305.97	5.12
8	5.1	Propionylglycine di TBDMS	150.72	3.69
6	4.1	Pentachlorophenylacetate	308.37	7.93

With the standard reference graphs, the compounds are elucidated using the molecular weight. The highest peak at the particular retention time is found out and the compounds with the highest peak are given in Table(2). The compound β-sitosterol is a plant sterol which exhibit excellant antiinflammatory and cholesterol lowering activity. [15]The one more important phytoconstituents identified are Quercetin, it is a bioflavonoid chemically it comes under flavonol group. It exhibit wide variety of pharmacological activity such as antioxidant, antiinflammatory, anticancer, atherosclerosis, and hypertension.[16] Chlorogenic acid is also a bioflavonoid which exhibit the phamacological activity like antioxidant, antidiabetic and anti obesity.

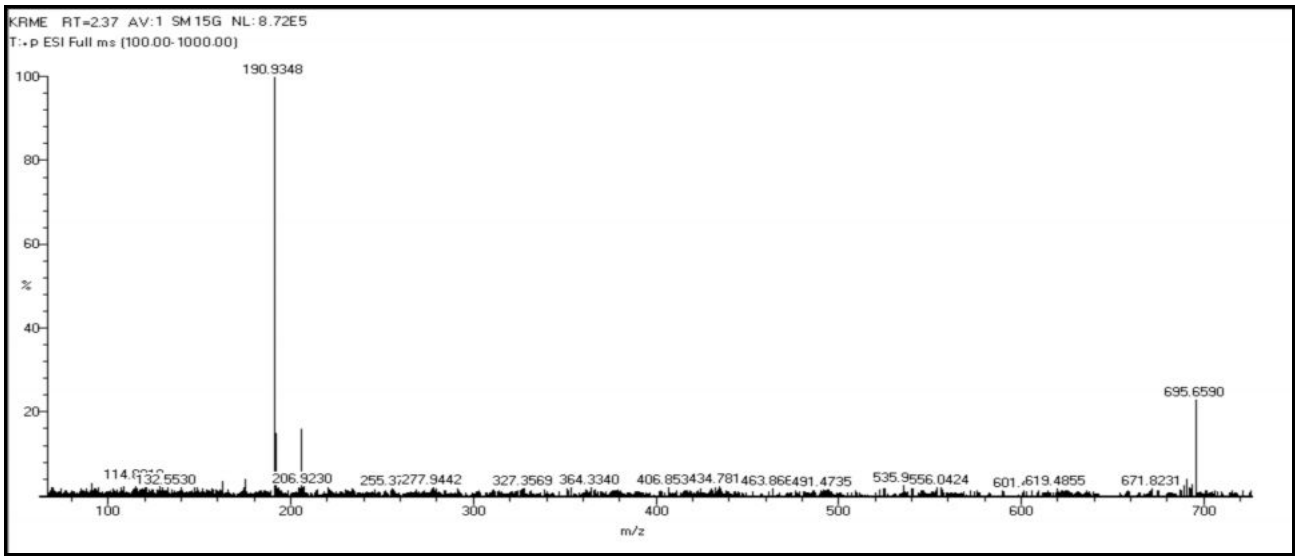

Fig2: Mass spectrum at the retention time 2.3

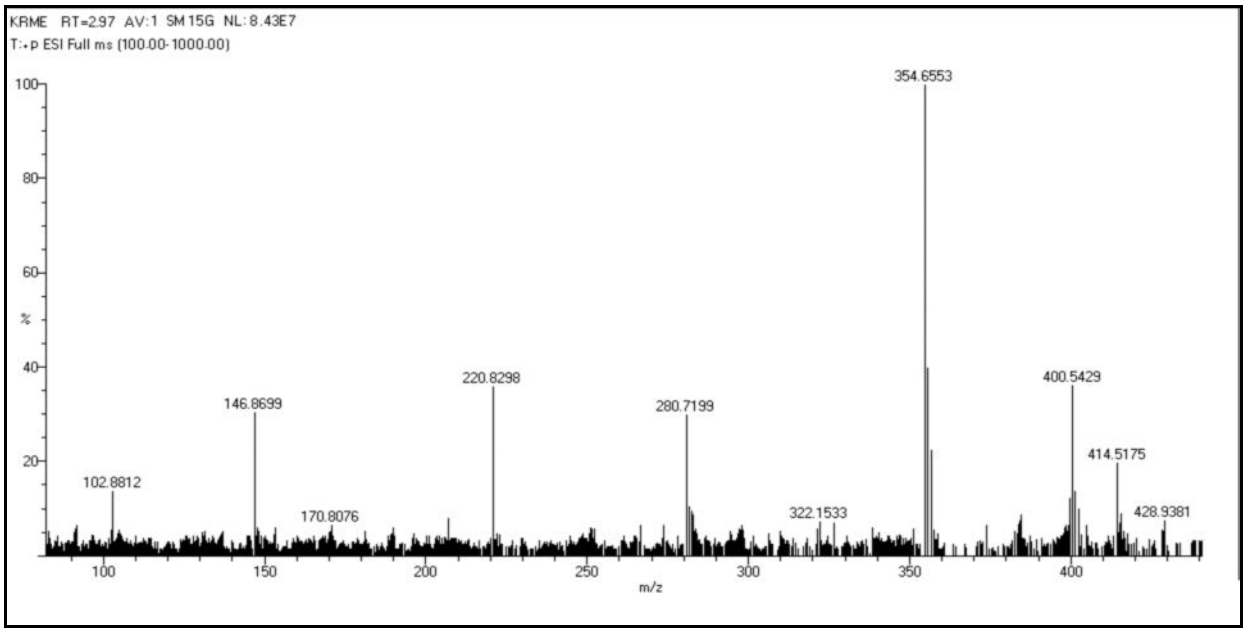

Fig 3: Mass spectrum at the retention time 2.9

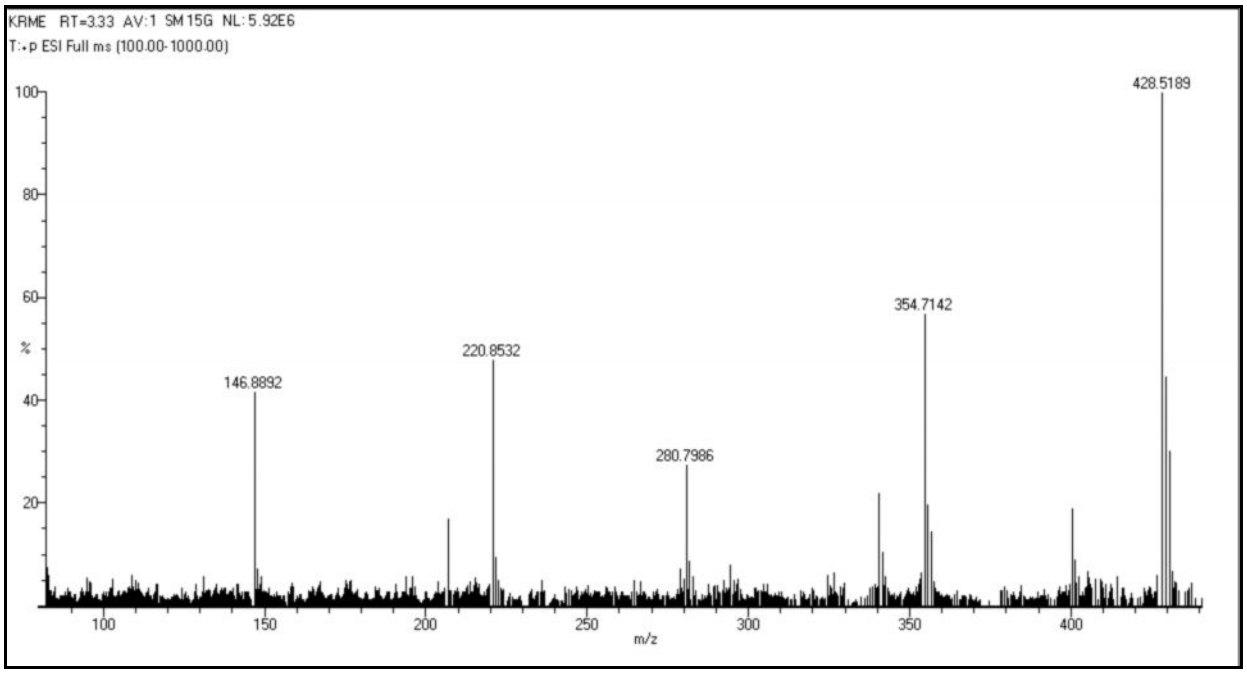

Fig 4 : Mass spectrum at the retention time 3.3

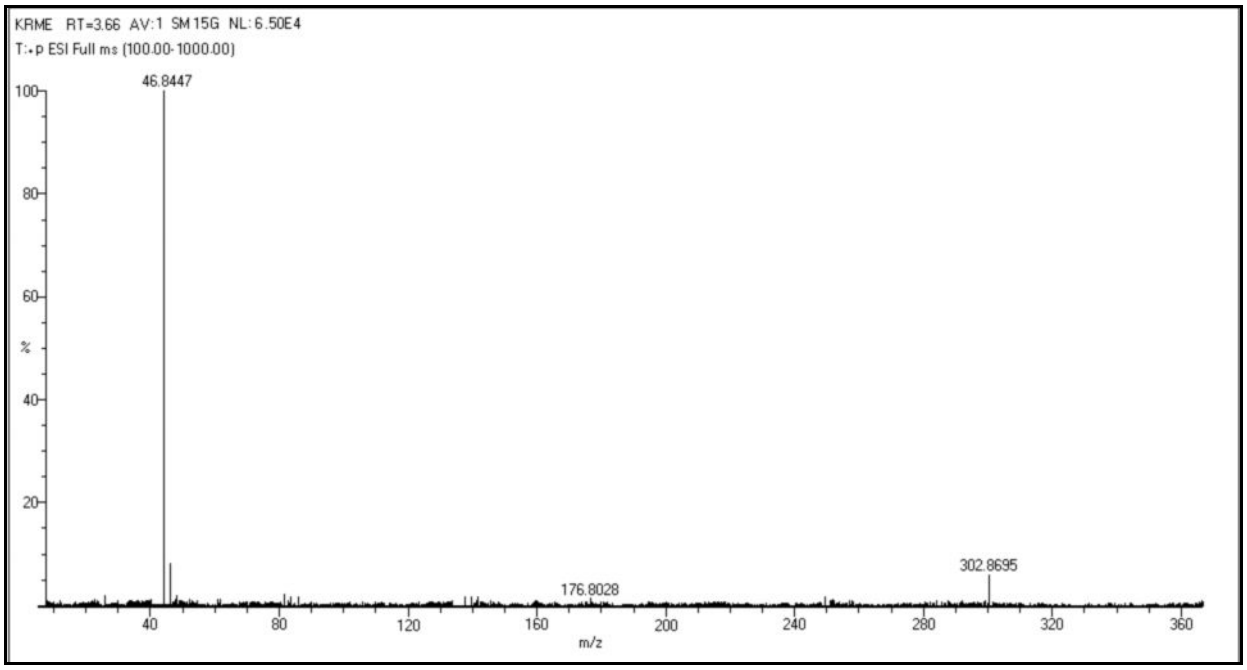

Fig 5: Mass spectrum at the retention time 3.6

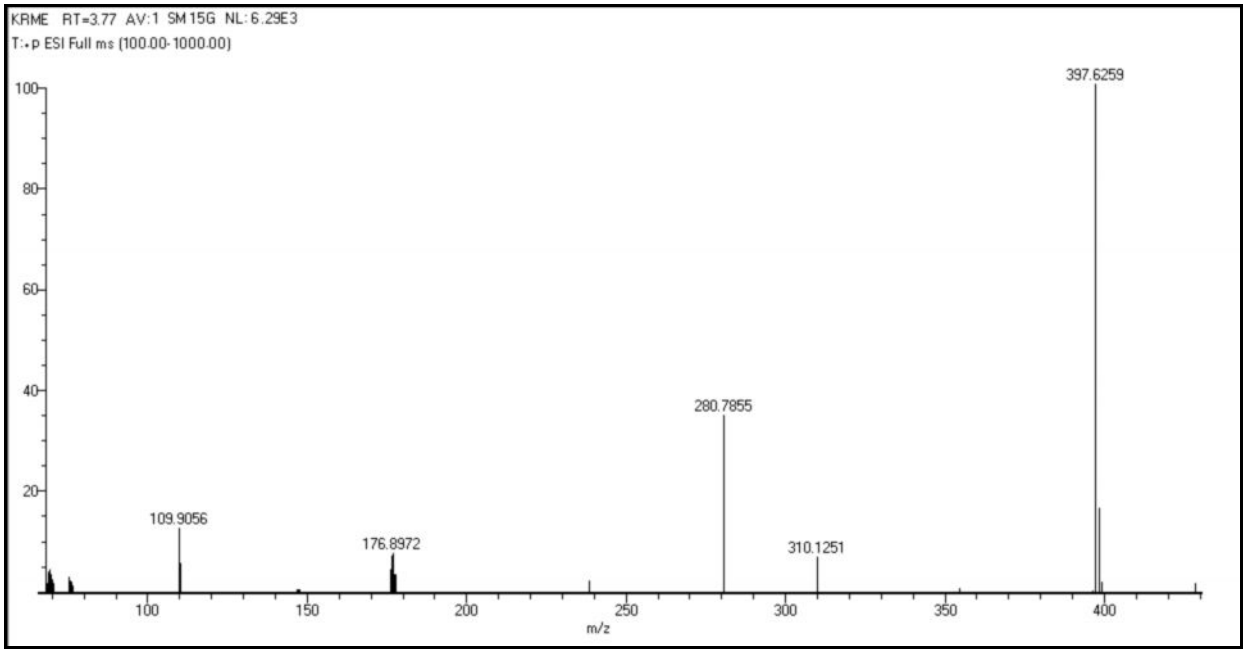

Fig 6: Mass spectrum at the retention time 3.7

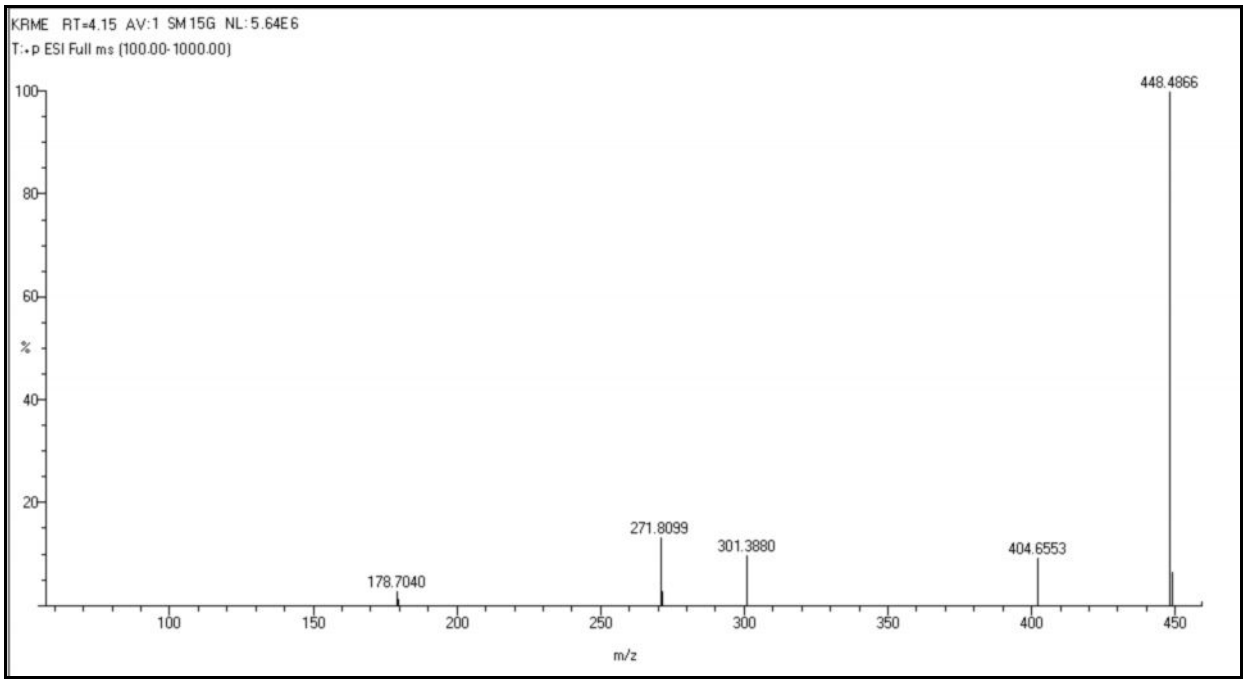

Fig 7: Mass spectrum at the retention time 4.1

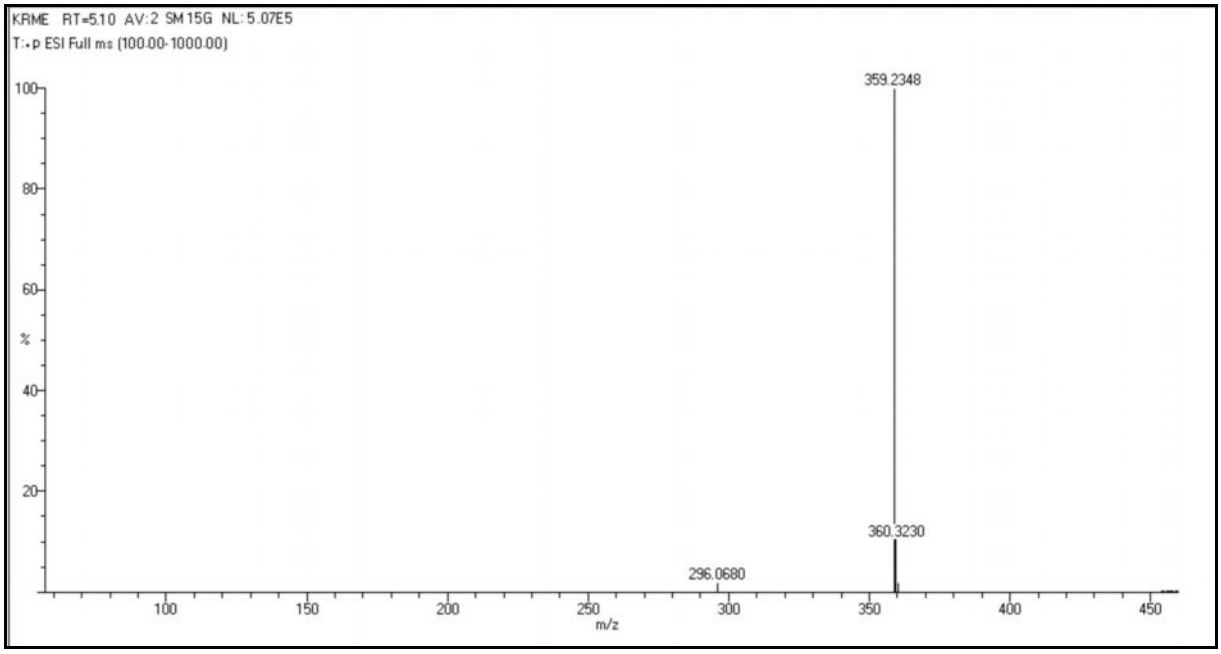

Fig 8: Mass spectrum at the retention time 5.1

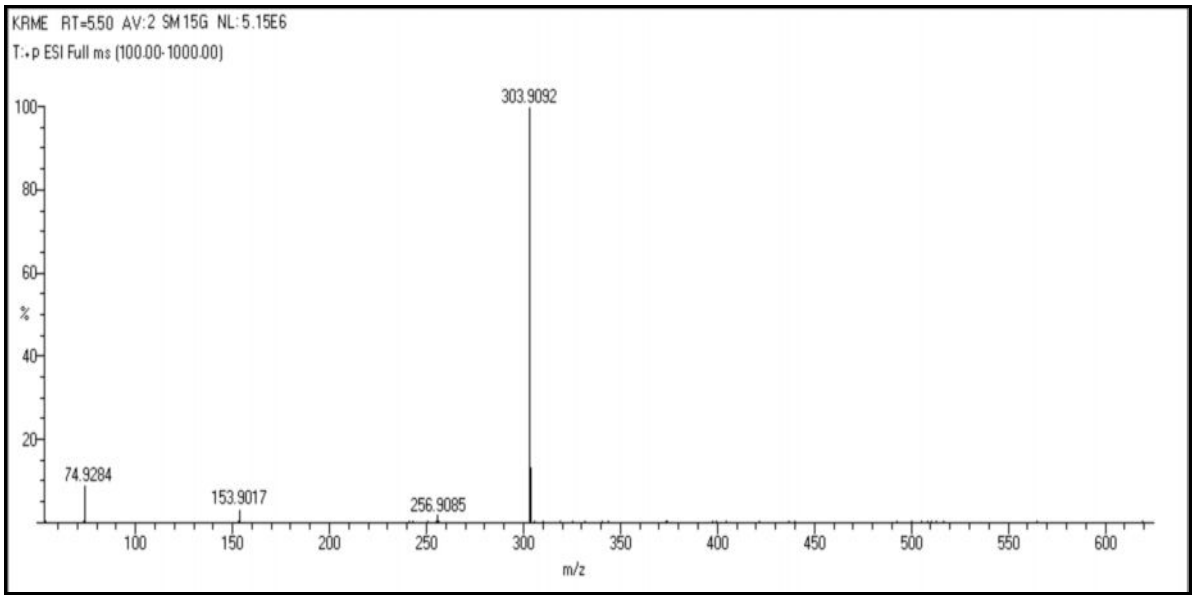

Fig 9: Mass spectrum at the retention time 5.5

***Invitro* Antiinflammatory Activity**

The *invitro* antiinflammatory activity of *M. quadrifolia* at different concentration was performed by albumin denaturation assay and the results were shown in table (3). From the results it was observed that the percentage inhibition of inflammation increased with increased concentration. The higher % inhibition was obtained at the concentration of 400(µg/ml) found to be 76.4±0.85 ,it was comparable with aspirin standard (75.89±0.56) at 200(µg/ml). The observed IC_{50} value was 208.28 (µg/ml) at correlation coefficient (r^2) of 0.9935.

Table 3: Inhibition of albumin denaturation assay of methanolic extract of *M. quadrifolia*

Samples	Concentration (µg/ml)	%Inhibition
Methanolic extract of *M. quadrifolia*	100	33.6±1.13
	200	48.8±0.74
	300	65.4±1.13
	400	76.4±0.85
Co-relation coefficient®	-	0.9935
IC50 value	-	208.27±2.78
Aspirin	100	67.45±0.64
	200	75.89±0.56

The *invitro* antiinflammatory activity of *M. quadrifolia* at different concentration was performed by membrane stabilization assay and the results were shown in table (4). From the results it was observed that the percentage inhibition of inflammation increased in the concentration dependant manner. The higher % inhibition was found to be 75.74±1.08 at 400(µg/ml),it was comparable with aspirin standard (73.92±0.75) at 200(µg/ml). The observed IC_{50} value was 261.0±9.56(µg/ml) at correlation coefficient (r^2) of 0.9935.

The potent antiinflammatory activity of methanolic extract of *Marsilea quadrifolia* due to the presence of bio flavonoids(chlorogenic acid) , phytosterols(β-sitosterol) and flavonol (Quercetin).[15,16]

Table 4: Membrane stabilization assay of methanolic extract of *M. quadrifolia*

Samples	Concentration(µg/ml)	% inhibition
Test extract of Marselia quadrifolia	100	22.60±0.68
	200	39.49±0.90
	300	56.69±1.32
	400	75.74±1.08
Co-relation coefficient®	-	0.990
IC50 value	-	261.09±9.56
Aspirin standard	100	72.56±0.58
	200	73.92±0.75

Conclusion

Marsilea quadrifolia is an aquatic fern of the family Marsileaceae. The active phyto-constituents present in the plant were the potential source for new drug and therapeutic leads. The results of this study revealed that methanolic extract of *Marsilea quadrifolia* contains pharmacologically active substances with anti-inflammatory activity and it showed most effective inhibition of cox receptor. The LC-MS analysis have brought light for the presence of phytosterols and bioflavonoid. Therefore the crude extracts of *Marsilea quadrifolia* leaf could be new sources of development of new plant based therapy for management of several diseases.

References

1. Garima Verma, Akranth Marella,MD, Shaquiquzzaman and Md. Mumta Alam., Immunomodulatory responses in gastrointestinal tract injury and recovery, Acta Biochemica Bolonica.,2013,60(2):143-149.
2. O. Longman, Indian medicinal plants, vol. 4 (Orient Longman Pvt. Ltd., Chennai, India., 1997, pp. 5-9
3. C. P. Khare, Encyclopedia of Indian Medicinal Plants, Rational western theraphy, Ayurvedic and other Traditional Uses, Botany (Springer, London, 2004) p. 303
4. T. Mathangi1*and P. Prabhakaran2., DPPH Free Radical Scavenging Activity of the Extracts of the Aquatic Fern *Marsilea quadrifolia* Linn. International J. pharmacy and Pharmaceutical Sciences., 2013, 2(10): 534-536.
5. Ripa, F.A., Nahar, L., Haque, M., Islam, M.M., Antibacterial, Cytotoxic and Antioxidant Activity of Crude Extract of Marsilea Quadrifolia. European J. Scientific Research., 2009, 33(1): 123-129
6. Dongare SS, Maske AP, Patil SM, Umbare RP and Mate GS, Antidiabetic Activity of Marsilea quadrifolia linn in Alloxan-Diabetic Rats, Research J.Pharmacognosy and Phytochemistry., 2009, 1(1):15-17
7. Ashwini G, Pranay.P, Thrinath.G, Karnaker Reddy. Giri Prasad.V. Pharmacological evalution of Marsilea qudrifolia plant extracts against Alzheimer's disease. International J. Drug Development & Research., 2012; 4.
8. Nahar, Laizuman; Ripa, Farhana Alam; Zahan, Ronok; Bulbul, Israt Jahan; Parvej, Ibrahim. Evaluation of Analgesic and Antidiarrhoeal Effects of Methanolic Extract of Marsilea quadrifolia in rat, J. Pharmacy Research.,2011, 4 (12): 4374.
9. Asha M,Tripathy S, Madhuri Y,Priliminary screening of *Marsilea quadrifolia* extracts for their antianxiety potential, International J. Pharmacy and Pharmaceutical Sciences.,2013 5 (3): 137-139.
10. JB Harborne, Phytochemical methods, A guide to Modern Technique of Plant Analysis, Chapman and Hill, London, 1992, 279.
11. Angela Mari, David Lyon, Lena Fragner, Paola Montoro, Sonia Piacente, Stefanie Wienkoop, Volker Egelhofer, and Wolfram Weckwerth,Phytochemical composition of *Potentilla anserina* L. analyzed by an integrative GC-MS and LC-MS metabolomics platform, Metabolomics., 2013.9(3): 599–607.
12. Mizushima Y and Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. J of Pharma Pharmacol. 1968; 20:169-173.
13. Sadique J, Al-Rqobahs WA, Bughaith and EI-gindi AR., The bioactivity of certain medicinal plants on the stabilization of RBC membrane system. Fitoterapia., 1989,60:525-532.

14. Shinde UA, Phadke AS, NariAM, Mungantiwar AA, Dikshit VJ and Saraf MN. Membrane stabilization activity - a possible mechanism of action for the anti- inflammatory activity of cedrus deodara wood oil, Fitoterapia., 1999,70:251-257.
15. Loizou S[1], Lekakis I, Chrousos GP, Moutsatsou P ,Beta-sitosterol exhibits anti-inflammatory activity in human aortic endothelial cells. Mol Nutr Food Res. 2010 Apr;54(4):551-8.
16. Parul Lakhanpal, Deepak Kumar Rai, Quercetin: A Versatile Flavonoid. Internet Journal of Medical Update, Vol. 2, No. 2, Jul-Dec 2007.

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