International Journal for Pharmaceutical Research Scholars (IJPRS)

V-3, I-1, 2014 ISSN No: 2277 - 7873

RESEARCH ARTICLE

GC-MS Evaluation of Bioactive Compounds of *Marsilea quadrifolia* Linn (Aquatic Fern)

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Manuscript No: IJPRS/V3/I1/00035, Received On: 22/01/2014, Accepted On: 30/01/2014

ABSTRACT

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the aquatic fern *Marsilea quadrifolia* was carried. The thirteen bioactive compounds identified are Hexadecanoic acid, ethyl ester (26.88%), Phytol (16.97%), 9,12-Octadecadienoic acid (Z,Z) (12.46%), 1,2-Benzenedicarboxylic acid, diisooctyl ester (8.62%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (6.71%), 2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl (ñ) (6.56%), Octadecanoic acid, ethyl ester (5.69%), 2-Piperidinone, N-[4-bromo-n-butyl (4.53%), Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3a,17a) (4.16%), Z,Z,Z-1,4,6,9-Nonadecatetraene (3.86%), 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (E,E) (1.82%), 10-Undecen-1-al, 2-methyl (1.20%), 5à-Androstan-16-one, cyclic ethylene mercaptole (0.53%).

KEYWORDS

Marsilea quadrifolia, GC-MS Analysis, Phytochemicals

INTRODUCTION

Traditional medical knowledge is important not only for its potential contribution to drug development and market values, but also for the people's healthcare.[1] According to the WHO, 80% of the world's population primarily those of developing countries rely on plant-derived medicines for their healthcare needs.[2]

Phytochemicals are chemical compounds formed during the plants normal metabolic processes. These chemicals are often referred to as "Secondary metabolites" of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids.[3] In addition to these substances, plants contain other chemical compounds.

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These can act as agents to prevent undesirable side effects of the main active substances or to assist in the assimilation of the main substances. Plants have an almost limitless ability to synthesize aromatic substances, mainly secondary metabolites of which 12,000 have been isolated, a number estimated to be less than 10% of the total.[4] These active components serve as molecules of plant defense against attack by microorganisms, insects and herbivores and at the same time also exhibit medicinal properties for treating several ailments. Scientific research has allowed us to discover a wide range of active components, of which the most important, as far as health is concerned, are essential oils, alkaloids, glycosides or heterosides, mucilage and gums and tannins.[5]

From time immemorial herbal products were used for curing diverse type of bacterial, fungal and viral diseases. Natural products, either as pure compounds or as standardized plant

extracts, provide unlimited opportunities for discovery of new drug because of the unmatched availability of chemical diversity. It is the demand of present time to discover new alternative antimicrobial compounds with diverse chemical structure and novel mechanism of action for new and reemerging infectious diseases.[6]

Pteridophytes (ferns and fern allies) are called as reptile group of plants and are one of the earliest groups of vascular plants. Most of the indigenous people are not well aware of the uses of pteridophytes since it is not easily available like flowering plants. Pteridophytes have an important role in the earth's biodiversity.[7]

Marsilea quadrifolia Linn is an aquatic fern which belongs to the family (Marsileaceae) commonly named as Aaraikeerai in Tamil, Neeraral in Malayalam and Cauptiya, Sunsuniya in Hindi. It is an aquatic fern bearing 4 parted leaf resembling '4-leaf clover', and the leaves float in deep water or erect in shallow water or on land. It possesses long stalked petiole with 4 clover like lobes and are either held above the water or submerged. Juice extracted from the leaves is diuretic and febrifuge and also used to treat snake bite and applied to abscesses etc. The plant is anti-inflammatory, diuretic, depurative, febrifuge and refrigerant.[8] The plant is also useful to treat psychopathy, leprosy, haemorrhoids, skin diseases, fever, insominia and febrifuge.[9] However the literature lacks GC-MS analysis of *Marsilea quadrifolia* to identify the different bioactive compounds present in it. Hence the present work was carried out.

MATERIALS AND METHOD

The plant *Marsilea quadrifolia* was collected as whole plant from the shores of the pond and the banks of the fields of Manimuthaar (foot region of Western Ghats), Tirunelveli District, and Tamil Nadu. They were shade dried and pulverized to powder. 50gram of the plant powder is soaked in 500ml of ethanol in a stoppered flask, shaking intermittently for 48 hours. Then the extract was filtered through whatmann No.1 filter paper. The filtrate was evaporated to dryness by vacuum distillation unit and stored.

GC-MS Analysis

GC-MS Analysis of the extract was performed using Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a Elite-5Ms, fused silica capillary column (30mm x 0.25mm X o.25µM df, composed of 5% Diphenyl/95% Dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70eV was used. Helium gas (99.99%) was used as the carrier gas at constant flow rate 1ml/min and an injection volume of 1µl was employed (split ratio of 10:1); Injector temperature 250^{o}C; The oven temperature was programmed from 110^{o}C (isothermal for 2 min.), with an increase of 10^{o}C /min upto 200^{o}C, then 5^{o}C /min to 280^{o}C, ending with a 9 minute isothermal at 280^{o}C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total area. The software adopted to handle mass spectra and chromatogram was Turbomass 5.2. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST)-version Year 2005. The spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test material were ascertained.

RESULTS AND DISCUSSION

GC-MS analysis of *Marsilea quadrifolia* whole plant extract revealed the presence of 13 compounds. The name of the compound with retention time, molecular formula, molecular weight, and concentration was listed in the table-1.

The thirteen compounds were Hexadecanoic acid, ethyl ester (26.88%), Phytol (16.97%), 9,12-Octadecadienoic acid (Z,Z) (12.46%), 1,2-Benzenedicarboxylic acid, diisooctyl ester

Table-1. GC MS analysis of *Marsilea quadrifolia*. The components identified and their activity

No.	RT	Name of the compound	Molecular formula	MW	Peak Area %	Compound nature	**Activity
1	2.68	2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl-, (ñ)-	$C_6H_{10}O_3$	130	6.56	Ketone compound	No activity reported
2	11.08	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	6.71	Terpene alcohol	Antimicrobial Anti-inflammatory
3	12.82	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	26.88	Palmitic acid ester	Antioxidant Hypocholesterolemic Nematicide Pesticide Anti androgenic Flavor Hemolytic 5-Alpha reductase inhibitor
4	14.26	Phytol	$C_{20}H_{40}O$	296	16.97	Diterpene	Antimicrobial Anticancer Antioxidant Diuretic
5	14.96	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	12.46	Linoleic acid ester	Hypocholesterolemic Nematicide Antiarthritic Hepatoprotective Anti androgenic Nematicide 5-Alpha reductase inhibitor Antihistaminic Anticoronary Insectifuge Antieczemic Antiacne Anticancer

6	15.29	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	5.69	Stearic acid ester	No activity reported
7	17.10	Z,Z,Z-1,4,6,9-Nonadecatetraene	$C_{19}H_{32}$	260	3.86	Alkene compound	No activity reported
8	19.05	10-Undecen-1-al, 2-methyl-	$C_{12}H_{22}O$	182	1.20	Aldehyde compound	Antimicrobial Anti-inflammatory
9	19.97	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	8.62	Plasticizer compound	Antimicrobial Antifouling
10	23.66	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (E,E)-	$C_{15}H_{26}O$	222	1.82	Alcoholic compound	Antimicrobial
11	26.92	5à-Androstan-16-one, cyclic ethylene mercaptole	$C_{21}H_{34}S_2$	350	0.53	Steroid	Antimicrobial Anticancer Anti-inflammatory Antiarthritic Antiasthma Hepatoprotective
12	29.24	2-Piperidinone, N-[4-bromo-n-butyl]-	$C_9H_{16}BrNO$	233	4.53	Alkaloid	Antimicrobial Anti-inflammatory
13	30.70	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3á,17á)-	$C_{22}H_{32}O_2$	328	4.16	Steroid	Antimicrobial Anticancer Anti-inflammatory Antiarthritic Antiasthma Hepatoprotective

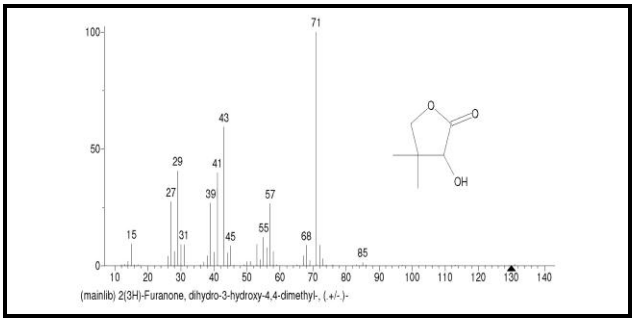
Activity source: **Dr. Duke's Phytochemical and Ethanobotanical Database

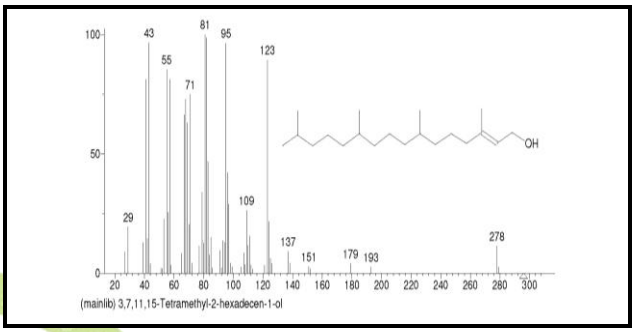
(8.62%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (6.71%), 2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl (n) (6.56%), Octadecanoic acid, ethyl ester (5.69%), 2-Piperidinone, N-[4-bromo-n-butyl (4.53%), Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3a,17a) (4.16%), Z,Z,Z-1,4,6,9-Nonadecatetraene (3.86%), 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (E,E) (1.82%), 10-Undecen-1-al, 2-methyl (1.20%), 5à-Androstan-16-one, cyclic ethylene mercaptole (0.53%).

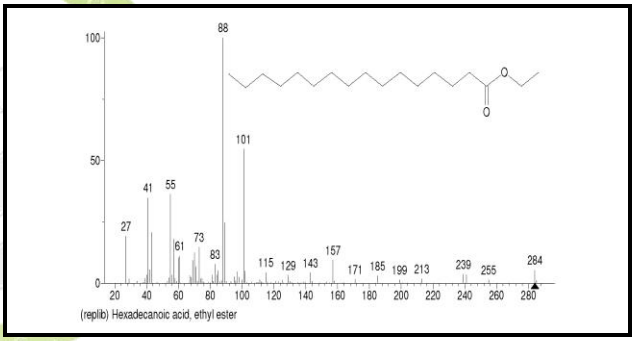
The identified compounds with more percentage like Hexadecanoic acid, ethyl ester (Palmitic acid ester) (26.88%), Phytol (Diterpene) (16.97%), 9,12-Octadecadienoic acid (Z,Z) (Linoleic acid ester) (12.46%) showed a wide range of potent bioactivity. Among the thirteen identified compounds 8 showed Anti-microbial activity, 6 showed Anti-inflammatory, 4 showed Anti-cancer and 2 other showed anti-oxidant and hypocholesterolemic activity. It was also observed that activity for 3 compounds have not been reported, a thrust area which has to be worked out.

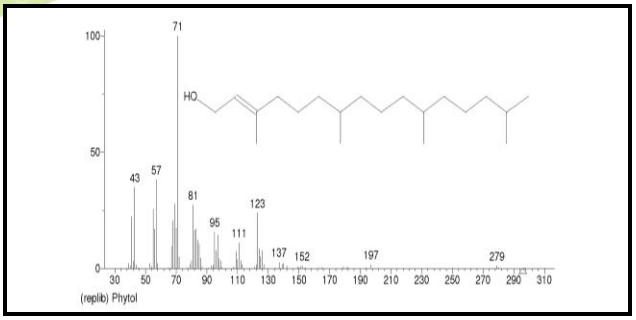
Fatty acids always occur in plants. Fatty acids in plants (Hexadeconoic acid, Octo decadienoic acid etc.) react with alcohols in an esterification reaction to form esters.[10] Unsaturated fatty acids are important to every cell in the body for normal growth, especially of the blood vessels and nerves and to keep the skin and other tissues youthful and supple through their lubricating quality.[11] These are nutrients which are invaluable for the production and movement of energy throughout the body, regulation of transportation of oxygen and are vital in maintaining the integrity of cell structure as well as the unique ability to lower cholesterol levels of the blood.[12]

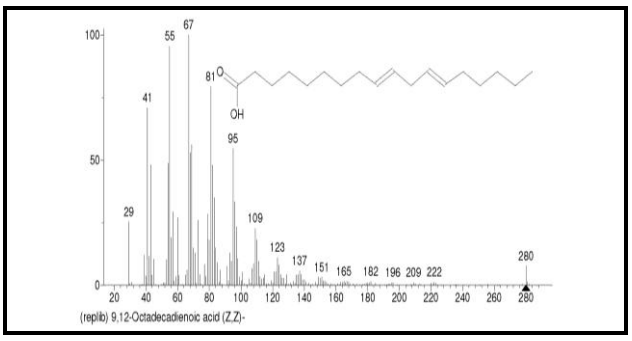
Steroids are abundant in nature; many derivatives of steroids have physiological activity.[13] Steroid hormones control sexual development and fertility in the human body[14]. Many steroids are used in medicine in the treatment of cancer, arthiritis or allergies and in birth control.[13,14] Detection of steroids in *Marsilea quadrifolia* may also be used in fertility therapy.

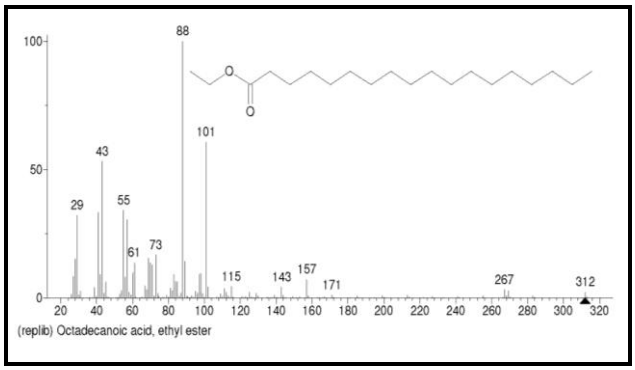


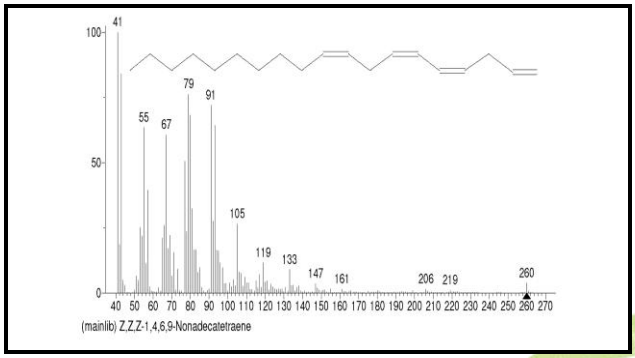


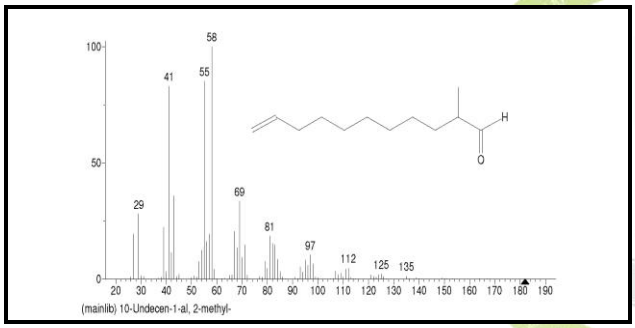


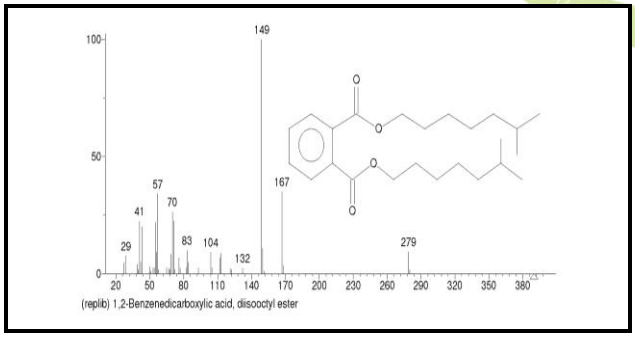


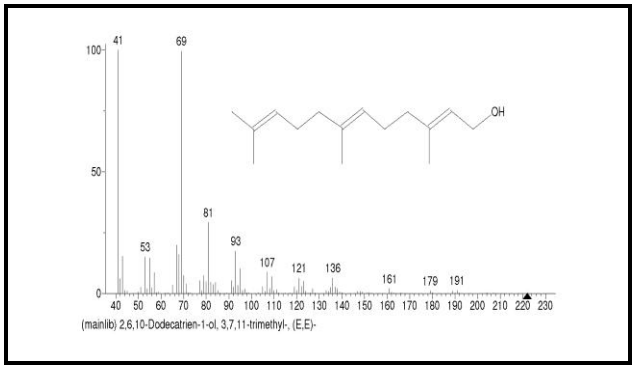


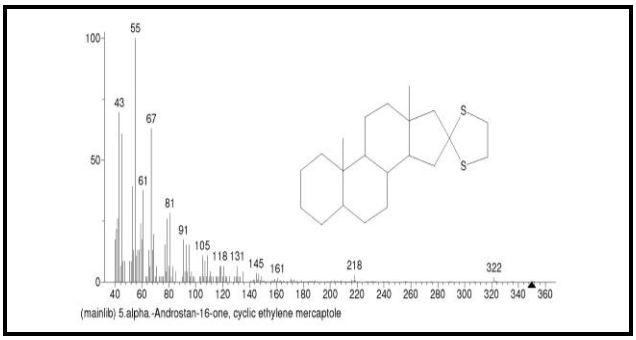


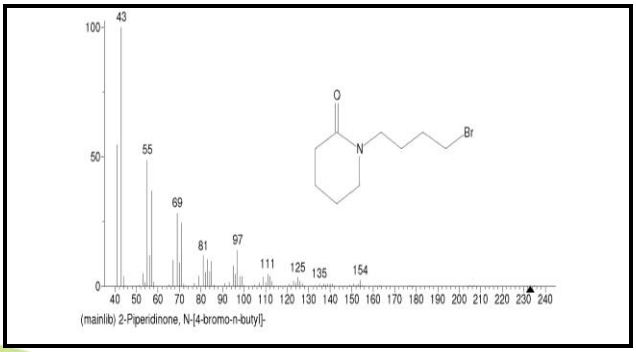


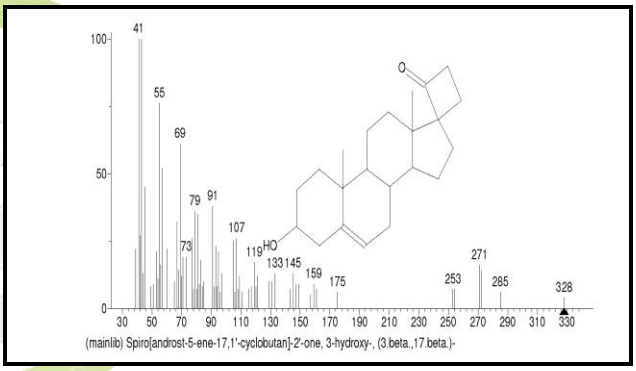












Phytols are the precursor for the manufacture of synthetic forms of Vitamin E and Vitamin K. Plants use phytol and its metabolites as chemical deterrents against predation.[15] Phytol acts as effective adjuvants and also increases the titers of all major ImmunoglobulinG (IgG) subclass and is also capable of inducing specific cytotoxic effector Tcell responses.[16]

Alkaloids have marked physiological activity. Pure isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agent for its analgesic, anti-spasmodic and anti-bacterial properties. For example Quinine (alkaloid) extracted from Cinchona is used to treat malaria.[17]

Thus from the present GC MS study it is evident that *Marsilea quadrifolia* is a potent medicinal fern of pharmaceutical importance.

ACKNOWLEDGEMENT

We would like to thank UGC for their financial help to carry out this work. We also thank Mr. S. Kumaravel, Senior Scientist, Indian Institute of Crop Processing Technology, Tanjavore, and Tamilnadu for providing all the facilities and support to carry out the work.

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