

GC-MS and FT-IR analysis of bioactive compounds on methanol extract of *Ipomoea aquatica*

* B Anbu Jothi, B Geetha

P.G. and Research Department of Zoology, V.O Chidambaram College, Tuticorin, Tamil Nadu, India

Abstract

Ipomoea aquatica is one of the medicinally important plants belonging to the family convolvulaceae. Traditionally use of *Ipomoea aquatica* treat to diabetics. The present investigation deals with GC-MS determination of methanol extract FT-IR analysis of the above said plant. Eight phytochemical constituents have been identified by comparing the chromatogram, peak value of unknown compound with entries in NIST database, Neophytadiene, Hexadecanoic acid, Linolenic acid, 9,12,15 – Octadecatrienoic acid methyl ester (z,z,z), prevailing compounds in the methanol extract of *I.aquatica*. The presence of various bioactive compounds confirms the application of *I.aquatica* for various treatment.

Keywords: GC-MS, FT-IR, *Ipomoea aquatica*, 9, 12, 15 - Octadecatrienoic acid methyl ester (z, z, z)

Introduction

In recent years the use of plants in the management and treatment of diseases has gained considerable importance. Plants and fruits are considered as one of the main sources of biologically active compounds. An estimate of the world health organization (WHO) states that around 85-90% of the world's population consumes traditional herbal medicines [15]. Many metabolites, usually with unique and complex structures. Many metabolites have been found to possess interesting biological activities and find application, such as pharmaceuticals, insecticide, fungicide, dyes, flavors and fragrances.

A knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances, etc [3]. In addition, the knowledge of the chemical constituents of plants would further be folkloric remedies.

Ipomoea aquatica is a member of the Morning Glory Family, Convolvulaceae, which contains 500 species. *I.aquatica* and *I.fistula* are the only aquatic species in the genus, which also includes the sweet potato (*Ipomoea batatas* L.) [4]. *I.aquatica* has been cultivated for its edible shoots and medicinal properties but sometimes, it is considered to be a serious weed. *I. aquatica* is a common emergent aquatic weed that can grow freely over the water surface or over marshy ground. Named for its beautiful flowers, a single stem can grow up to 20 meters in length with profuse branching, and its apical shoot may advance 10 cm per day under suitable condition. Morning glory basically a vine, which may be forms dense masses of tangled vegetation, thus developing impenetrable canopies over the water surface, restricting light penetration into the depths.

Gas chromatography has a very wide field of applications, But, its first and main area of use is in the separation and analysis of multi component mixtures such as essential oils, hydrocarbons and solvents [2]. Intrinsically, with the use of the flame ionization detector and the electron capture detector (while have very sensitivities) gas chromatography can

qualitatively determine materials present at very low concentrations. It follows, that the second most important application area is in pollution studies, forensic work and general trace analysis. Because of its simplicity, sensitivity and effectiveness in separating components of mixtures, gas chromatography is one of the most important tools in chemistry. It is widely used for quantitative and qualitative analysis of mixture for the purification of components, and for the determination of such thermo chemical constants as heats of solution and vaporization vapor pressure and activity coefficients [14].

2. Materials and methods

2.1 Collection of the Plant Material

Ipomoea aquatica leaves were collected from the maramangalam village in Thoothukudi district, India. The plant specimen was identified and authenticated in botanical survey of India, Coimbatore, Tamil Nadu.

2.2 Preparation of leaf powder

Leaves were collected, Collected leaves were washed with tap water after removing manually in edible parts, and shade dried and pulverized to powdered in a mechanical grinder and the powder stored in an air tight container at room temperature.

2.3 Preparation of extract for GC-MS

Required quantity of plant powder was weighed and transferred to round bottom flask and treated with methanol in a soxlet apparatus. The process lasted 5-8 hours and maintained with 60°C. The extract was collected and evaporated to dryness by using vacuum distillation unit. This extract was then subjected to GC-MS analysis.

3. Fourier Transform Infrared (FT-IR) spectroscopic analysis

The FT-IR spectra of the samples were determined using a Fourier Transform Infrared Spectrometer (Thermo Scientific Nicolet iSS) IDI Transmission Accessory. Approximately 2mg of the solid sample of *I.aquatica* was ground with

200mg of dried Pottassium bromide (KBr) to form a very homogeny fine powder and the powder was compressed into a thin pellet by using 15 tones Hydraulic press. KBr pellet held in KBr pellet holder was then recorder for FT-IR measurement in the wave number range from 4000 to 400cm⁻¹ using 16 scans [8].

4. GC-MS Analysis

The methanol treated plant extract was subjected to the GC-MS analysis. One micro liter of sample was injected into the gas chromatography (GC -Thermo, trace ultra 5.0, Thermo MS DSQ II). Separation of compound was achieved using DB 5-MS column, (30mts×0.25mm×0.25micro m).The oven temperature was elevated from 70° C to 260° C at 6°C/min. The carrier gas Helium was passed through at a flow rate of 1.0ml/min. The run time was 3.15 to 43.02min. The compounds were identified based on their molecular mass.

5. Identification of components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The Name, Molecular weight and structure of the components of the test materials were ascertained.

6. Results

6.1 FT-IR Spectroscopy studies

The FT-IR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The functional group identification is based on the FT-IR peaks attributed to the stretching and bending liberations. The outcome of FT-IR functional groups were represented in table 1. The FT-IR spectrum profile of *Ipomoea aquatica* powder was illustrated in Figure.1.

Table 1: FT-IR spectroscopic data of *Ipomoea aquatica* leaf

S. No	Functional group	Stretching frequency	Compound name
1	N-H Stretching	3405.75	Pyrroles
2	C-H Stretching	2920.56	Alkanes (-CH ₂ -)
3	C-H Stretching	2850.95	Alkanes (-CH ₂ -)
4	N-H Stretchi	1647.72	Secondary Amines
5	NO ₂ Stretching	1544.87	Tertiary nitro compounds
6	CH ₃ deformation	1384.45	Alkanes
7	S=O Stretching	1320.12	Sulphur compounds
8	-C ^{=O} -O-R Stretching	1255.05	Aliphatic esters,(CH ₃ COOR)
9	S=O Stretching	1155.75	Sulfones
10	S=O Stretching	1051.63	Sulfonic acid
11	C-H deformation	919.94	Mono substituted alkenes
12	C-Cl Stretching	618.99	Halides
13	C-Br Stretching	532.06	Bromides
14	C-I Stretching	445.08	Iodides

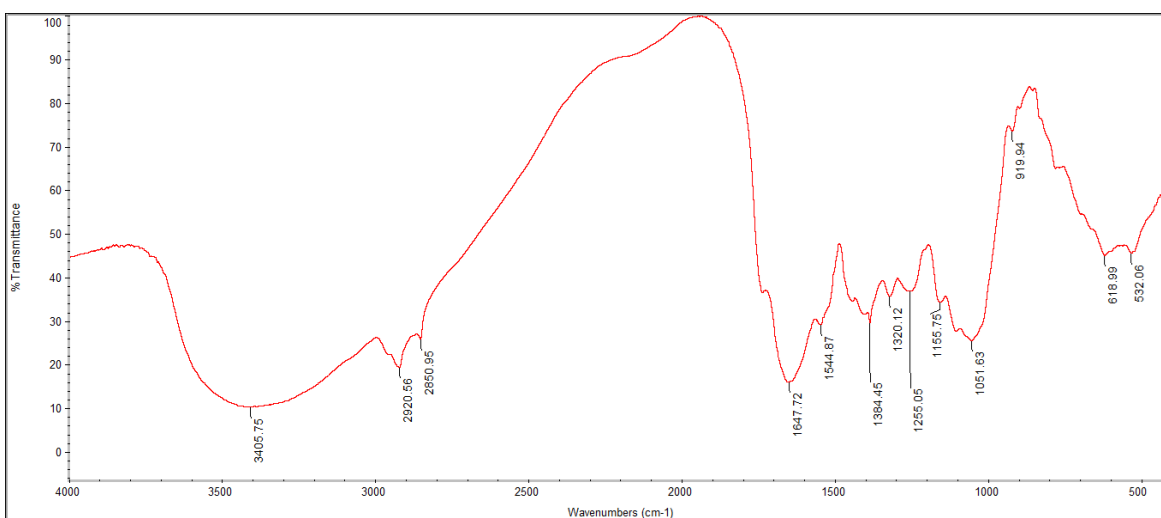


Fig 1: FT-IR spectrum of *Ipomoea aquatica* leaf

6.2 GC-MS Analysis

GC-MS is one of the best techniques to identify the active principles (phytochemicals) present in the plant extract. The chromatogram of the GC-MS analysis of *Ipomoea aquatica* leaf revealed the presence of ten compounds. The active principles with their retention time (RT), molecular formula,

molecular weight (Mw) and concentration (%) in the methanol extract of *Ipomoea aquatica* are presented in table 2.

The prevailing compounds in methanol extract of the *Ipomoea aquatica* were identified by GC-MS. A number of 10 spectral peaks were detected in GC-MS analysis of

Ipomoea aquatica. These peaks were detected at retention time (RT) 4.55, 11.56, 18.65, 22.87, 25.19, 27.23, 32.82 and 37.68 (Table I). Among these 10 peaks, 8 peaks were identified by mass spectroscopy (MS); These compounds are Benzyl-2,3,4-tri-o-(3-methylbutyl)-a,L-arabinopyranoside(60.10%), 2-Methyl-2-tert-butoxypropane-1,3-diol (4.09%), Neophytadiene(0.35%),(1S,3R)-7'-Methoxy-3,4' dimethylspiro(cyclohexane-1,2'-(1 H)-

quinolone)(6.43%),2-Hexadecen-1-ol,3,7,11,15-tetramethyl-,[R-[R*R*-(E)]-(CAS)(7.56%),9,12,15-Octadecatrienoic acid, methyl ester,(z, z, z)(5.41%), Hexadecanoic acid 2-hydroxy 1 (hydroxymethyl) ethyl ester(1.31%), Linolenic acid,2-(hydroxy -1- (hydroxymethyl) ethyl ester (z, z, z) (0.72%). Table 3 listed the major phytochemicals and its biological activities which contribute to the medicinal activities of *Ipomoea aquatica*.

Table 2: Components identified in the extract of leaf of *Ipomoea aquatica*

S.NO.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1	4.55	Benzyl-2, 3, 4-tri-o-(3methyl butyl)-a,L-arabinopyranoside.	C ₂₇ H ₄₆ O ₅	450	60.10
2	11.56	2-Methyl-2-tert-butoxypropane-1,3-diol	C ₈ H ₁₈ O ₃	162	4.09
3	18.65	Neophytadiene	C ₂₀ H ₃₈	278	0.35
4	22.87	(1S,3R)-7'-Methoxy-3,4' dimethylspiro(cyclohexane-1,2'-(1 H)- quinoline)	C ₁₇ H ₂₃ NO	257	6.43
5	25.19	2-Hexadecen-1-ol,3,7,11,15-tetramethyl-,[R-[R*R*-(E)]-(CAS)	C ₂₀ H ₄₀ O	296	7.56
6	27.23	9,12,15-Octadecatrienoic acid,methyl ester,(z,z,z)	C ₁₉ H ₃₂ O ₂	292	5.41
7	32.82	Hexadecanoic acid 2-hydroxy-1-(hydroxymethyl)ethyl ester	C ₁₉ H ₃₈ O ₄	330	1.31
8	37.68	Linolenic acid,2-(hydroxy -1-(hydroxymethyl) ethyl ester (z,z,z)	C ₂₁ H ₃₆ O ₄	352	0.72

Table 3: Activity of components identified in the methanol extract of leaf of *Ipomoea aquatica*

1	Benzyl-2,3,4-tri-o-(3-methylbutyl)-a,L-arabinopyranoside	C ₂₇ H ₄₆ O ₅	Bile acid	Used in digestive activity
2	2-Methyl-2-tert-utoxypropane-1,3-diol	C ₈ H ₁₈ O ₃	Aromatic compound	Antimicrobial, Preservative
3	Neophytadiene	C ₂₀ H ₃₈	Fatty acid	Antipyretic, analgesic, and anti-inflammatory, antimicrobial, antioxidant & antibacterial.
4	(1S,3R)-7'-Methoxy-3,4' dimethylspiro(cyclohexane-1,2'-(1 H)-quinoline)	C ₁₇ H ₂₃ NO	Levorphanol	Fungicidal activity
5	2-Hexadecen-1-ol,3,7,11,15-tetramethyl-,[R-[R ^c ,R ^c -(E)]-(CAS)	C ₂₀ H ₄₀ O	Terpene alcohol	Antimicrobial,anticancer,antiinflammatory, Diuretic.
6	9,12,15-Octadecatrienoic acid,methyl ester,(z,z,z)	C ₁₉ H ₃₂ O ₂	Polyenoic fatty acid	Antiinflammatory, insectifugehypocholesterolemic Cancer, preventive, nematocide, hepato protective, insectifuge, antihistaminic, antieczemic, antiacne,5Alphareductaseinhibitor,antiandrogenic,antiarthritic,anticoronary
7	Hexadecanoic acid 2-hydroxy-1(hydroxymethyl)ethyl ester	C ₁₉ H ₃₈ O ₄	Fatty acid Ester compound	No activity reported
8	Linolenic acid,2-(hydroxy-1-(hydroxymethyl)ethyl ester(z, z, z)	C ₂₁ H ₃₆ O ₄	Linolenic acid	Antiinflammatory, Hypocholesterolemic Cancer preventive, Hepatoprotective, Nematocide, Antihistaminic Antieczemic, Antiacne, Antiarthritic, Anticoronary.

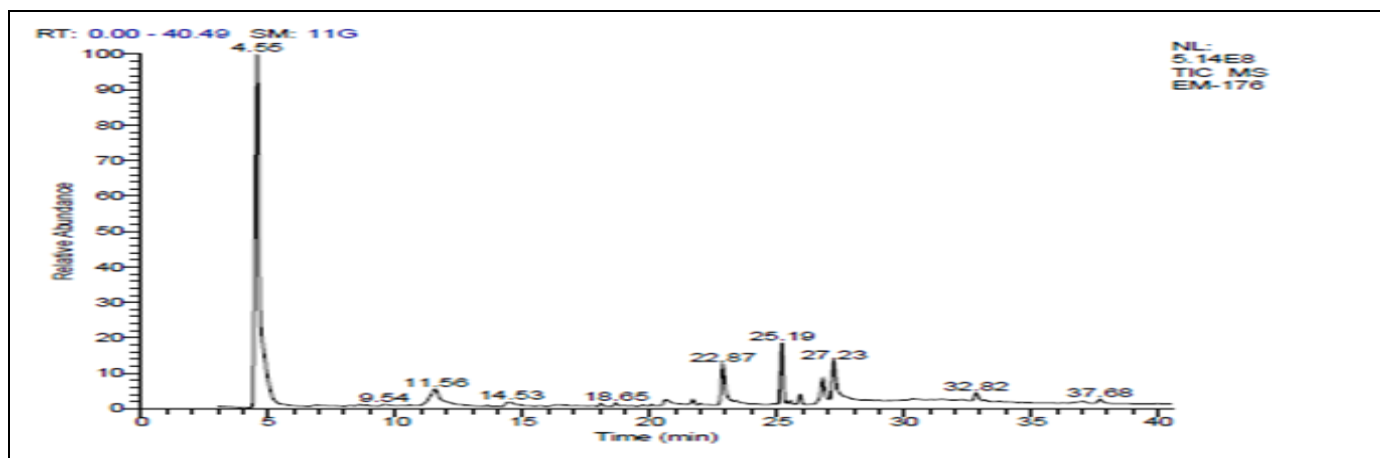


Fig. 4: GC-MS chromatogram of the methanol extract of leaf of *Ipomoea aquatica*

7. Discussion

Spectral differences are the objective reflection of componential differences. The FT-IR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. By using the macroscopic fingerprint characters of FT-IR spectrum, we can trace the constituents in plant powders, identify the medicinal materials true or false and even evaluate the qualities of medicinal materials. So, FT-IR spectrum reflecting objectively the panorama of chemical constituents in complex system is a most credible method to validate and identify the mix substance systems such as traditional medicine and herbal medicine [9].

The FT-IR analysis revealed the presence of different function groups from N-H stretching, pyrroles (3405.75cm^{-1}), C-H stretching, alkanes (2920.56cm^{-1}), another C-H stretching, alkanes (2850.95cm^{-1}), N-H deformation, secondary amines (1647.72cm^{-1}), NO_2 stretching, tertiary nitro compound (1544.87cm^{-1}), CH_3 deformation, alkanes (germinal dimethyl, isopropyl, tert butyl sym. CH_3 bending) (1384.45cm^{-1}), S=O Stretching, sulphur compound (1320.12cm^{-1}), $\text{-C} \diagup \diagdown \text{O} \text{-R}$ Stretching, aliphatic esters (1255.05cm^{-1}), S=O Stretching, sulfones (1155.75cm^{-1}), another S=O Stretching, sulfonic acids (1051.63cm^{-1}), C-H deformation, monosubstituted alkanes (919.94cm^{-1}), C-Cl Stretching, Halides (618.99cm^{-1}), C-Br Stretching, Bromides (532.06cm^{-1}) and the least peak value is C-I Stretching, Iodides (445.08cm^{-1}). From the FT-IR spectra, it is clearly indicated that, although they show substantial overlap of each absorption spectrum of various components, each band represents an overall overlap of some characteristic absorption peaks of functional groups in the sample. Therefore the FT-IR analysis on *I. aquatica* displayed novel phytochemical markers as useful analytical tool to check not only the quality of the powder but also to identify the medicinally important plants.

GC-MS is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino acid nitro compounds. [10]. In the present study, 10 compounds have been identified from methanol extract of the leaves of *I. aquatica* by Gas Chromatography-Mass Spectrometry (GC-MS) analysis.

Among the identified phytochemicals, Benzyl-2,3,4-tri-*o*-(3-methylbutyl)-*a*, L-arabinopyranoside had the property of digestive activity (5) already reported that chemical compound present the digestive activity of *Cucumis trigonus* plant extract. 2-Methyl-2-tert-butoxypropane-1, 3-diol Antimicrobial, Preservative. Neophytadiene activity of Antipyretic, analgesic, and anti-inflammatory, antimicrobial, antioxidant & antibacterial. Neophytadiene has earlier been reported as a component in various extract plant leaves in an antibacterial [11], antipyretic, analgesic, and anti-inflammatory, antimicrobial, antioxidant [12]. It is a fatty acid-related compound and plays an important role in competitive inhibition of lipoxygenase or cyclooxygenase in an inflammation reduction, resulting in decreased production of prostaglandins and leukotrienes [6]. (1*S*, 3*R*)-7'-Methoxy-3, 4'-dimethylspiro (cyclohexane-1,2'-(1*H*)-quinoline) Fungicidal activity. 2-Hexadecen-1-ol, 3, 7, 11, 15-tetramethyl-, [R-[R*, R*(E)]]-(CAS) Antimicrobial, anticancer, anti-inflammatory, Diuretic activity in the extract of *Cynodon dactylon* [7]. 9,12,15-Octadecatrienoic acid, methyl ester, (z,z,z)

Antiinflammatory, insectifuge, hypocholesterolemic Cancer, preventive, nematocidal, hepatoprotective, insectifuge, antihistaminic, antieczemic, antiacne, 5 α Alphareductase inhibitor, antiandrogenic, antiarthritic, anticoronary activity of ethanolic extract from the whole plant of *Saccharum spontaneum* Linn [1]. Hexadecanoic acid 2-hydroxy-1-(hydroxymethyl) ethyl ester Antioxidant and anti-inflammatory. Linolenic acid, 2-(hydroxy-1-(hydroxymethyl) ethyl ester (z,z,z), compound nature of linolenic acid activity anti-inflammatory hypocholesterolemic cancer preventive, hepatoprotective, nematocidal, antihistaminic, antieczemic, antiacne, antiarthritic, anticoronary [13] has been reported that this activity of *Sesuvium portulacastrum*

Thus this type of GC-MS analysis is the first step towards understanding the nature of active principles in the medicinal plants. The presence of various bioactive compounds justifies the use of this plant for various treatment. It might be recommended as a plant of phyto-pharmaceutical importance. However, isolation of individual photochemical constituents and subjecting it to biological activity will definitely give fruitful results. Therefore, it could be concluded that, *I. aquatica* contain various bioactive compounds.

8. Conclusion

This GC-MS Analysis report strongly supported to the *Pomoea aquatica* is one of the best medicinal plant.

9. Acknowledgment

We would like thank to the Mr. S Sivakumar, Senior Scientist, The South Indian Textile Research Association, Coimbatore, Tamil Nadu, India for providing all the facilities and support used to carry out the work.

10. References

1. Amutha iswarya devi, kottai muthu A. Gas chromatography-mass spectrometry analysis of bioactive constituents in the ethanolic extract of *Saccharum spontaneum* linn. International Journal of Pharmacy and Pharmaceutical Sciences. ISSN- suppl 2, 2004; 6:0975-1491
2. Andrew Marston. Role of advances in chromatographic techniques in photochemistry. *Phytochemistry*, 2000; 68:2786-2798.
3. Betz JM, Gay ML, Mossoba MM, Adams S, Portz BS Chirl. Gas chromatographic determination of ephedrine of ephedrine-type alkaloids in dietary supplements containing Ma' Hua'ng. Journal of the Association of Official Analytical Chemists International. 1997; 80(2):303-15.
4. Cook CDK. Origin, autecology, and spread of some of the world's most troublesome aquatic weeds. In: A.H. 1990; 31-38.
5. Gopalakrishnan S, Kalaiarasi T. Identification of chemical compounds from the fruits of *Cucumis trigonus* roxb. by gc-ms analysis International Journal of Phytopharmacy Research Article. 2012; 2(5):122-128.
6. James MJ, Gibson RA, Cleland LG, Amer J. Clinical Nutrition. 2000; 71:343S-348S.

7. Jananie RK, Priya V, Vijayalakshmia K. Determination of Bioactive Components of *Cynodon dactylon* by GC-MS Analysis New York Science Journal. 2011; 4(4).
8. Kareru PG, Keriko JM, Gachanja AN, Kenji GM. Direct Detection of *Triterpenoid saponins* in Medicinal Plants. Afr J Tradit Complement Altern Med. 2008; 5:56-60.
9. Liu H, Sun S, Lv G, Chan KKC. Study on Angelica and its different extracts by Fourier transform infrared spectroscopy and two – dimensional correlation IR spectroscopy. Spect Acta Part A, 2006; 64:321-326.
10. Muthulakshmi A, Margret JR, Mohan VR. GC-MS Analysis of Bioactive components of *Feronia elephantum* Correa (Rutaceae). *J App Pharm Sci*. 2012; 2:69-74.
11. Olena Konovalova1, Evgenia Gergel, Vitaliy Herhel. The Pharma Innovation – Journal. 2013; 2(6):7-12.
12. Palic R, Stojanovic G, Alagic S, Nikolic M, Lepojevic Z. Prilep. Flavour Fragr. J. 2002; 17:323-326.
13. Sheela D, Uthayakumari F, Gc-ms analysis of bioactive constituents from coastal sand dune taxon – *Sesuvium portulacastrum* (L.) Bioscience discovery, jan. issn: 2229-3469 (print), 2013; 4(1):47-53.
14. Thanga Krishnakumari S, Muthukumarasamy S, Mohan V R. GC-MS Analysis of ethanol extract of *Sarcostemma secamone* (L) Bennet(Asclepiadaceae); *Science Research Reporter*, 2(3): 187-191.
15. WHO Report, World Health Organization, Geneva, WHO/EDM/ TRM/ 2002; 21:19.