

Research Article



Analysis of Bioactive Constituents from Organic Crude Ethanol Extracts from the Local Medicinal Plant of *Cassytha Filiformis* L (Lauraceae) by Gas Chromatography-Mass Spectrometry

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ABSTRACT

To characterize the chemical constituents of whole plant (except root) of *Cassytha filiformis* (L.) using GC-MS. The shade dried leaves powder was extracted with ethanol by using Soxhlet extractor. The Clarus 500 GC used in the analysis employed a column packed with Elite-1 (100% dimethyl poly siloxane, 30 nm × 0.25 mm ID × 1 μm df) and the components were separated using Helium (1 mL/min) as the carrier gas. The 2 μL sample extract injected into the instrument was detected by Turbo gold mass detector (Perkin Elmer) with the aid of the Turbo mass 5.1 software. The GC-MS analysis provided different peaks determining the presence of six different phytochemical compounds namely 3,7,11,15-tetramethyl-2-hexadecen-1-ol (8.86%), hexanedecanoic acid, methyl ester (8.72%), hexanedecanoic acid, ethyl ester (12.32%), 7-octadecenoic acid, methyl ester (24.44%), ethanol, 2-[9-octadecenyloxy]-[z] (11.59%), and 9,12,15-octadecatrienoic acid, ethyl ester, [z,z,z]- (34.05%). The bioactive compounds in the ethanolic extract of *cassytha filiformis* (L.) have been screened using this analysis. Isolation of individual phytochemical constituents may proceed to find a novel drug.

Keywords: Bioactive compounds, Ethanol extract, GC-MS, Phytochemical constituents.

INTRODUCTION

Natural products have played an important role in the development of drugs and drug leads for various diseases including cancer.¹ Even today compounds from plants continue to play a major role in primary health care as therapeutic remedies in many developing countries.² Plant materials remain an important resource to combat serious diseases in the world. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries. Plants still represent a large untapped source of novel compounds that might serve as leads for the development of novel drugs³ and they are an important source of new biochemical substances with potential therapeutic effects.⁴ The modern methods describing the identification and quantification of active constituents in plant material may be useful for proper standardization of herbal and its formulations. GC-MS is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds etc.⁵

Cassytha filiformis (Common name: love-vine, Vernacular name: Kothan) is a creeper plant in the family Lauraceae, traditionally used in treatment of several diseases (cancers, human birthing issues, hypertension, pains and fever).⁶ This species actually is a vine resembling the common parasitic dodder which is so prevalent in many parts of California and other subtropical and tropical areas of the world. Its species are also widely used as a medicinal plant.^{7,8} A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. With this background the present

study was aimed to identify the phytoconstituents in *cassytha filiformis* (L.) by using GC-MS analysis.

MATERIALS AND METHODS

Collection and preparation plant material

The fresh plants of *Cassytha filiformis* (L.) were collected from Kumarai Village in Cuddalore district of Tamilnadu, India during January to December 2012 and authenticated by director of the Rapinat Herbarium and Centre for Molecular Systematic, St. Joseph's college (campus), Trichirappalli, Tamilnadu, India. The sample were washed thoroughly in running tap water to remove soil particles and adhered debris and finally washed with sterile distilled water. The whole plants were shade dried and ground into fine powder. The powdered materials were preserved in airtight containers until use.

Extraction procedure

The powdered sample of *cassytha filiformis* (100g) were extracted with ethanol (500ml, 46 h) at temperature between 55-60°C by using Soxhlet extractor. The solvent was evaporated by rotavapor (Yamato Rotary Evaporator, Model RE-801) to obtain viscous semi solid masses. The semi dry ethanol crude extract was suspended in water and it analyzed by GC-MS, it had led to the identification and characterization of nine different organic compounds, representing 4.09% of the total extract from plant samples. The crude extracts were filtered separately through Whatman No. 41 filter paper to obtain dust free plant crude extract. The residue was re-extracted twice follow the same and filtered. The combined extracts were concentrated and dried by using rotary evaporator under vacuum.



GC-MS analysis

The Clarus 500 GC used in the analysis employed a fused silica column packed with Elite-1 (100% dimethyl poly siloxane, 30 nm × 0.25 nm ID × 1µm df) and the components were separated using Helium as carrier gas at a constant flow of 1 mL/min. The µL sample extract injected into the instrument was detected by the Turbo gold mass detector (Perkin Elmer) with the aid of the Turbo mass 5.1 software. During the 36th minute GC extraction process, the oven was maintained at a temperature of 110°C with 2 minutes holding. The injector temperature was set at 250°C (mass analyzer).

The different parameters involved in the operation of the Clarus 500 MS, were also standardized (Inlet line temperature: 200°C; Source temperature: 200°C). Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 450 Da.

Identification of components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology having more than 62,000 patterns. 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.

Table 1: Components detected in the plant of ethanol extract of *Cassythia filliformis* (L.)

Compound Name	Molecular Formula	MW	RT	Peak Area	% Peak Area
3,7,11,15-tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O ₂	296	15.8	10131552	8.86
hexanedecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	16.25	9970368	8.72
hexanedecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284	16.92	14073536	12.32
7-octadecenoic acid, methyl ester	C ₁₉ H ₃₆ O ₄	296	17.95	27915312	24.44
ethanol,2-[9-octadecenyloxy]-,[z]	C ₂₀ H ₄₀ O ₂	438	18.1	13241552	11.59
9,12,15-octadecatrienoic acid, ethyl ester,[z,z,z]-	C ₂₀ H ₃₄ O ₂	306	18.6	38907072	34.05

MW: Molecular Weight, RT: Retention Time

Table 2: Activity of Phyto-Components identified in the ethanol extracts of the plant of *Cassythia filliformis* (L.)

Name of the compound	Molecular formula	Nature of compound	**Activity
3,7,11,15-tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O ₂	Terpene alcohol	Antimicrobial, Anti-inflammatory.
hexanedecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	Palmitic acid methyl ester	Antioxidant, Hypochlolesterolemic, Nematicide, Pesticide, Antiandrogenic, Flavor, Haemolytic, 5-Alpha reductase inhibitor.
hexanedecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	Palmitic acid ethyl ester	Antioxidant, Hypochlolesterolemic, Nematicide, Pesticide, Antiandrogenic, Flavor, Haemolytic, 5-Alpha reductase inhibitor.
7-octadecenoic acid, methyl ester	C ₁₉ H ₃₆ O ₄	Fatty acid methyl ester	Antibacterial.
ethanol,2-[9-octadecenyloxy]-,[z]	C ₂₀ H ₄₀ O ₂	-	No activity reported.
9,12,15-octadecatrienoic acid, ethyl ester,[z,z,z]-	C ₂₀ H ₃₄ O ₂	Linoleic acid ethyl ester	Hypochlolesterolemic, Nematicide, Antiarthritic, Heptaprotective, Antiandrogenic, 5-Alpha reductase inhibitor, Anticoronary, Insectifuge, Antieczemic, Antiacene.

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

RESULTS

The results pertaining to GC-MS analysis led to the identification of number of compounds from the GC fractionations of the ethanolic extract of *Cassythia filliformis*. These compounds were identified through mass spectrometry attached with GC. The compound prediction is based on National Institute Standard and Technology Database. The results of the present study were tabulated in Table 1. The gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in the plant. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The results revealed that the presence of 3,7,11,15-tetramethyl-2-hexadecen-1-ol (%), hexanedecanoic acid, methyl ester(%), hexanedecanoic acid, ethyl ester (%), 7-octadecenoic acid, methyl ester(%), ethanol,2-[9-octadecenyloxy]-,[z](%), 9,12,15-octadecatrienoic acid, and ethyl ester,[z,z,z](%). The spectrum profile of GC-MS confirmed the presence of six major components with the retention time 15.8, 16.25, 16.92, 17.95, 18.1, and 18.6 respectively (Figure: 1).The individual fragmentation of the components were illustrated in (Figure 2A-2F).



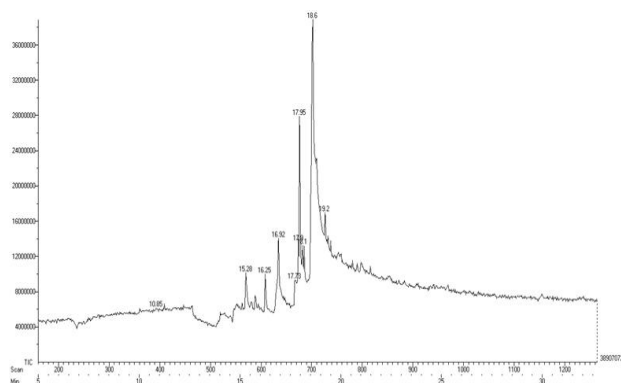


Figure 1: GC-MS Chromatogram of ethanolic extract of the *Cassytha filiformis* (L.)

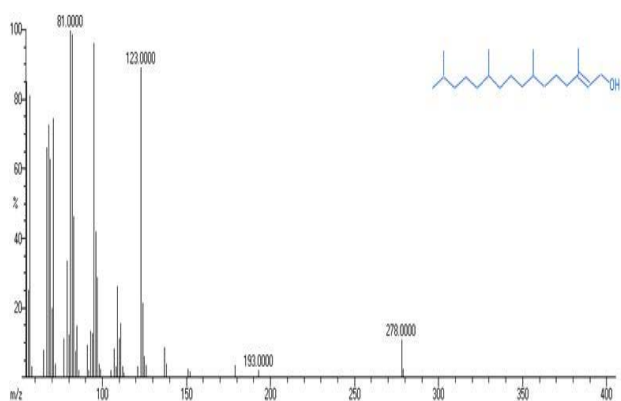


Figure 2A: Mass spectrum of 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol. (RT: 15.8)

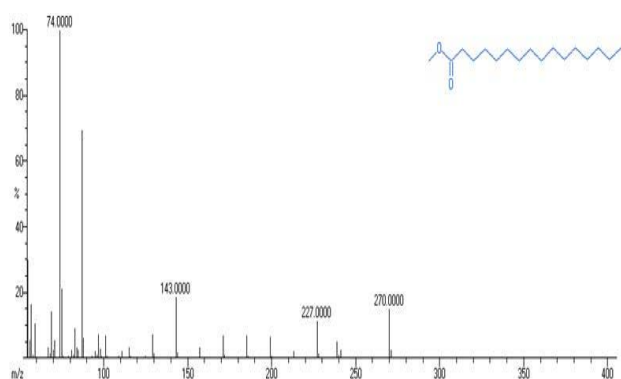


Figure 2B: Mass spectrum of hexanedecanoic acid, methyl ester. (RT: 16.25)

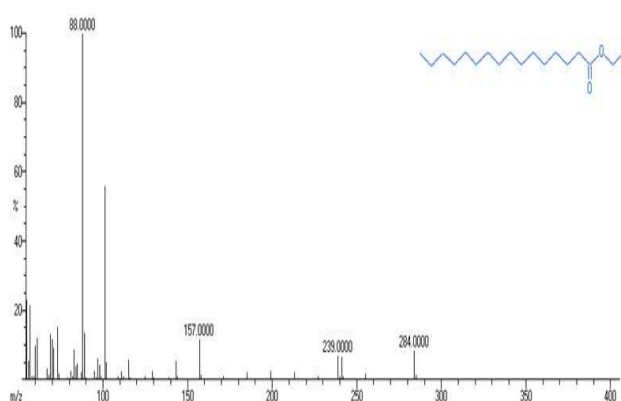


Figure 2C: Mass spectrum of hexanedecanoic acid, ethyl ester. (RT: 16.92)

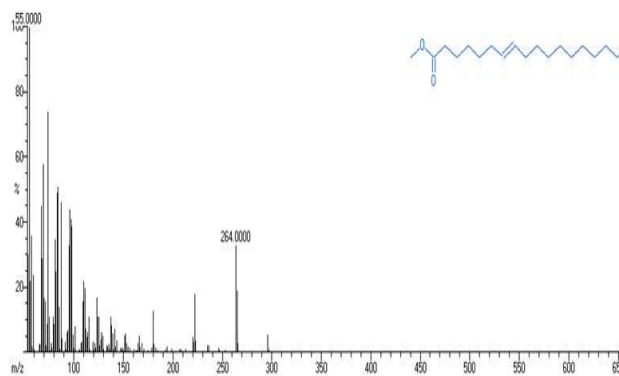


Figure 2D: Mass spectrum of 7-octadecenoic acid, methyl ester. (RT: 17.95)

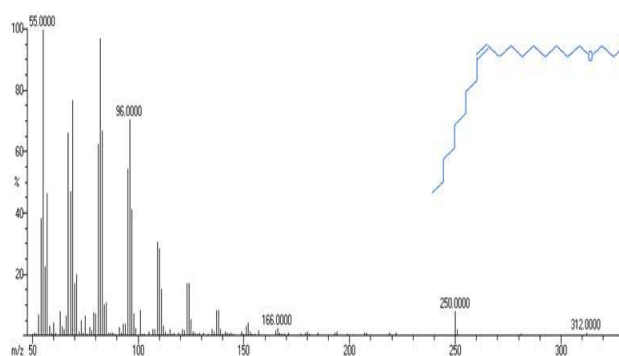


Figure 2E: Mass spectrum of ethanol, 2-[9-octadecenyloxy]-, [z]. (RT: 18.1)

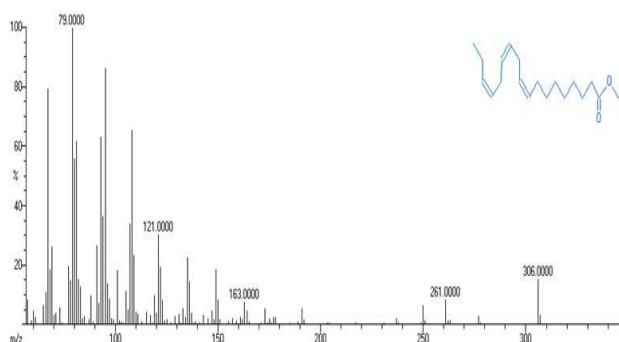


Figure 2F: Mass spectrum of 9, 12, 15-octadecatrienoic acid, ethyl ester, [z,z,z]-. (RT: 18.6)

DISCUSSION

In the present study, the GC-MS analysis of the ethanolic extract of plant of *Cassytha filiformis* (L.) showed the presence of six compounds. In terms of percentage amounts 9, 12, 15-octadecatrienoic acid, ethyl ester, [z,z,z](34.05%)-, 7-octadecenoic acid, methyl ester(24.44%), and hexanedecanoic acid, ethyl ester(12.32%) were predominant in the extract. The compounds of 9, 12, 15-octadecatrienoic acid, ethyl ester, [z,z,z]- and hexanedecanoic acid ethyl ester were major compounds have all shown to have hypochlolesterolemic, nematocidal, antiarthritic, hepatoprotective, antiandrogenic, 5-alpha reductase inhibitor, anticoronary, insectifuge, antieczemic, antiacene. Antibacterial is shown by 7-octadecenoic acid, methyl ester. Antimicrobial and anti-inflammatory are shown by 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol (8.86%).

Antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic, flavor, haemolytic, and 5-alpha reductase inhibitor are shown by hexanedecanoic acid, methyl ester (8.72%). There is growing awareness in correlating the phytochemical compounds and their biological activities.⁹⁻

¹¹ We report the presence of some of the important components resolved by GC-MS analysis and their biological activities. Thus this type of GC-MS analysis is the first step towards understanding the nature of active principles in this medicinal plant and this type of study will helpful for further detailed study.

CONCLUSION

In the present study six chemical compounds have been identified from ethanolic extract of the plant of *casstha filliformis* (L.) by Gas chromatography-Mass spectrometry (GC-MS) analysis. The presence of various bioactive compounds justifies the use of the *casstha filliformis* (L.) for various ailments by traditional practitioners. By isolating and identifying these compounds, new drugs can be formulated to treat different diseases and further plan of study includes isolation and purification of chemical compounds.

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