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GC-MS DETERMINATION OF BIOACTIVE COMPOUNDS IN *AZIMA TETRACANTHA* LEAVES

[1]S. Vasthi Gnana Rani and [2]K. Murugaiah*

[1]Research Scholar, Enathi Rajappa College, Pattukkottai, Thanjavur District, Tamil Nadu, S. India.

[2]Departments of Biochemistry, Enathi Rajappa College, Pattukkottai, Thanjavur District, Tamil Nadu, S. India.

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***Correspondence for Author**
K. Murugaiah
Departments of Biochemistry, Enathi Rajappa College, Pattukkottai, Thanjavur District, Tamil Nadu, S. India.

ABSTRACT

The aim of this study was to carry out for identification of bioactive compounds from methanolic extract of *Azima tetracantha* leaves by Gas chromatography and Mass spectroscopy (GC-MS). GCMS analysis of methanolic extract was done by standard protocol using the equipment Perkin-Elmer Gas Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. The GC-MS analysis revealed the presence of various compounds like Tetradecanoic acid $$ myristic acid, Pentadecanoic acid ,9,12-Octadecadienoic acid (Z,Z)- $$ cis-,1-(+)-Ascorbic acid 2,6-dihexadecanoate, Heptadecanoic acid, Oleic acid $$ 9-octadecenoic acid (Z), Octadecanoic acid, methyl, Cis--Eicosadienoic acid and Hexadecanoic acid in the methanolic extract of *Azima tetracantha*. Hence, the *Azima tetracantha* may have chemopreventive, anticancer, anti-microbial activity, antioxidant and antiinflammatory activity due to the presence of secondary metabolites in the methanolic extract. These findings support the traditional use of *Azima tetracantha* in various disorders.

Keyword: Gas chromatography and Mass spectroscopy, *Azima tetracantha*, Phytochemistry.

1. INTRODUCTION

Plants are used medicinally in different countries, and they are the source of many potent and powerful drugs. Plants have been an important source of medicine with qualities for

thousands of years. Mainly on traditional remedies such as herbs for their history, they have been used as popular folk medicines [1]. It has been shown that *in vitro* screening methods could provide the needed preliminary observations necessary to elect crude plant extracts with potentially useful properties for further chemical and pharmacological investigations.[2]

Phytochemistry or plant chemistry has developed in recent years as a distinct discipline, somewhere in between natural product organic chemistry and plant biochemistry and is closely related to both. It is concerned with the enormous variety of organic substances that are elaborated with and accumulated by plants and deals with the chemical structures of these substances, their biosynthesis, turn over and metabolism, their natural distribution and their biological function.[3]

Phytochemicals are the chemicals extracted from plants. These organic chemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents include the common sugars, aminoacids, proteins, purines and pyrimidines of nucleic acids, chlrophyll's etc. Secondary constituents are the remaining plant chemicals such as alkaloids (derived from aminoacids), terpenes (a group of lipids) and phenolics (derived from carbohydrates) [4]. Plant produces these chemicals to protect itself but recent research demonstrates that emphasizes the plant source of most of these protective, disease-preventing compounds. A true nutritional role for phytochemicals is becoming more probable every day as research uncovers more of their remarkable benefits [5]. Within a decade, there were a number of dramatic advances in analytical techniques including TLC, UV, NMR and GC-MS that were powerful tools for separation, identification and structural determination of phytochemicals.[6]

Azima tetracantha. (Family: *Salvadoraceae*) commonly known as Mulsanku in India. The leaves are found to contain azimine, azcarpine, carpine and isorhamnitine-3-O-rutinoside etc.,[7-9] Friedelin, lupeol, glutinol and β-sitosterol were isolated from the petroleum ether extract of the leaves of *Azima tetracantha*.[10] The seeds of this plant have been found to possess novel fatty acids along with other fatty acids.[11] A. tetracantha leaf powder was assessed for its anti-inflammatory activity.[12] The ethanolic leaf extract of Azima tetracantha Lam. was investigated for hypoglycemic and hypolipidemic activity in alloxan-induced diabetic albino rats.[13, 14] The aim of this paper is to determine the organic compounds present in the *Azima tetracantha* extract with the aid of GC-MS Technique, which may provide an insight in its use in tradition medicine.

2. MATERIAL AND METHODS

2.1 Plant materials

The mature *Azima Tetracantha* leaf were collected in January 2015 from Tamil University in Thanjavur, Tamil Nadu, India. The *Azima Tetracantha* were identified (EP 001) and authenticated by Botanist, Dr. S. John Britto Department of The Rapinat Herbarium and Centre For Molecular Systematics, Thiruchirappalli.

2.2 Preparation of extracts

The *Azima tetracantha* were first washed well and dust was removed from the plant. The plants were washed several times with water to remove the traces of impurities from the plant. Then the plants were dried at room temperature and coarsely powdered. The powder was extracted with 70% methanol for 48 hours using soxlet apparatus. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in desiccator until used. The extract contained both polar and non-polar phytocomponents of the plant material used. The percentage yield was 3.86% (4g gives 300mg extract)

2.3 GC –MS analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column Elite-1 fused silica capillary column (30 x 0.25mm ID x 1µMdf, composed of 100% Dimethyl polydiloxane), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 µI was employed (split ratio of 10:1) injector temperature 250 ºC; ion-source temperature 280 ºC. The oven temperature was programmed from 110 ºC (isothermal for 2 min), with an increase of 10 ºC/min, to 200ºC, then 5ºC/min to 280ºC, ending with a 9min isothermal at 280ºC. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 36min. min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a TurboMass Ver 5.2.0.

3. RESULTS AND DISCUSSION

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives. Most are secondary metabolites, of which at

least 12,000 have been isolated, a number estimated to be less than 10% of the total. These substances serve as plant defense mechanisms against, insects and herbivores. Flavonoids exhibit several biological effects such as anti-inflammatory, anti-fungal, anti-hepatotoxic and anti-ulcer actions.[15]

3.1 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. The biological activities listed (Table 2) are based on Dr.Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA.

3.2 GC-MS ANALYSIS

Thirty compounds were identified in *Azima tetracantha* by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Table 1 and Fig 1). The prevailing compounds were Tetradecanoic acid $$ myristic acid, Pentadecanoic acid ,9,12-Octadecadienoic acid (Z,Z)- $$ cis-,1-(+)-Ascorbic acid 2,6-dihexadecanoate,Heptadecanoic acid,Oleic acid $$ 9-octadecenoic acid (Z),Octadecanoic acid, methyl, Cis--Eicosadienoic acid and Hexadecanoic acid.

The investigation concluded that the stronger extraction capacity of methanol could have been produced number of active constituents responsible for many biological activities. So that those might be utilized for the development of traditional medicines and further investigation needs to elute novel active compounds from the medicinal plants which may be created a new way to treat many incurable diseases.

Table 1: Shows the components identified in methanolic extract of *Azima tetracantha.* (GC MS study)

Peak#	R. Time	Area%	Name	Molecular formula	Molecular weight
1	5.979	1.22	Dodecane $$ n-Dodecane $$ Adakane 12	$C_{12}H_{26}$	170
2	9.884	0.45	Naphthalene, 1-methyl- $$ .alpha.-	$C_{11}H_{10}$	142

			Methy		
3	10.359	1.17	1-Tridecene $$ n-Tridec-1-ene $$ 1-$$	$C_{13}H_{26}$	182
4	10.594	1.51	Tetradecane $$ AI3-04240 $$ BRN	$C_{14}H_{30}$	198
5	13.099	0.72	1-Pentadecene $$ Pentadecene,1- $$ Pen	$C_{15}H_{30}$	210
6	13.311	0.76	Pentadecane $$ BRN 1698194	$C_{15}H_{32}$	212
7	15.416	0.24	1-Heptadecene $$ Hexahydroaplotaxene	$C_{17}H_{34}$	238
8	15.560	0.88	Eicosane $$ Icosane $$ icosane	$C_{20}H_{42}$	282
9	17.499	1.00	Diethyl Phthalate $$ 1,2-Benzenedicarbo	$C_{12}H_{14}O_4$	222
10	19.652	1.16	Tetradecanoic acid $$ Myristic acid	$C_{14}H_{28}O_2$	228
11	20.883	0.62	Pentadecanoic acid $$ Pentadecylic acid	$C_{15}H_{30}O_2$	242
12	21.137	0.34	Phthalic acid, butyl undecyl ester	$C_{23}H_{36}O_4$	376
13	21.893	3.30	cis-13-Eicosenoic acid	$C_{20}H_{38}O_2$	310
14	22.053	18.63	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652
15	22.821	0.37	Sulfurous acid, cyclohexylmethyl pentad	$C_{22}H_{44}O_3S$	388
16	22.917	0.50	cis-9-Hexadecenal$$ 9-hexadecenal, (Z)	$C_{16}H_{30}O$	238
17	23.100	1.24	Heptadecanoic acid $$ potassi	$C1_7H_{34}O_2$	270
18	23.235	0.30	Sulfurous acid, 2-ethylhexyl octadecyl es	$C_{26}H_{54}O_3S$	446
19	23.740	1.04	2-hexadecen-1-OL, 3,7,11,15-TETR	$C_{20}H_{40}O$	296
20	23.808	0.31	Octadecanoic acid, methyl es	$C_{19}H_{38}O_2$	298
21	24.033	13.53	Octadec-9-enoic acid $$ (9E)-9-Octadece	$C_{18}H_{34}O_2$	282
22	24.073	15.27	Octadec-9-enoic acid $$ 9-OCTA	$C_{18}H_{34}O_2$	282
23	24.277	24.79	L-Ascorbic acid, 6-octadecanoate$$L-A	$C_{24}H_{42}O_7$	442
24	25.060	0.81	9,12-Octadecadienoic acid (Z,Z)- $$ cis-	$C_{18}H_{32}O_2$	280
25	25.564	1.03	Nonadecanoic acid $$ AI3-36442	$C_{19}H_{38}O_2$	298
26	26.133	0.44	Hexadecanoic acid, 2-hydrox	$C_{35}H_{68}O_5$	568
27	26.817	0.67	22-Tricosenoic acid	$C_{23}H_{44}O_2$	352
28	26.928	1.44	Cyclopentadecanone, 2-hydroxy-	$C_{15}H_{28}O_2$	240
29	27.187	5.57	Icosanoic Acid $$ Arachinsa	$C_{20}H_{40}O_2$	312
30	30.105	0.65	Glycidol stearate $$ Glycidyl octadecano	$C_{21}H_{40}O_3$	340

Table 2: Activity of phyto-components identified in the methanolic extracts of the *Azima tetracantha.* by GC-MS.

S.no	Compound name	Biological activity**
1.	Tetradecanoic acid $$ myristic acid	Anti oxidant, cancer preventive, hypercholesterolemic, nematicide, lubricant, cosmetic.
2.	Pentadecanoic acid	Anti oxidant
3.	9,12-Octadecadienoic acid (Z,Z)- $$ cis-	Anticoronary, Antialopecic, Antiarteriosclerotic,Antiarthritic, antianaphylactic, Antieczemic, Cancer preventive, antiprostatic ,hepatoprotective, Hypocholesterolemic, Metastatic, Nematicide
4.	1-(+)-Ascorbic acid 2,6-dihexadecanoate	Antioxidant, antiscorbutic, antiinflammatory, antinociceptive, anti- mutagenic, wound healing property.
5.	Heptadecanoic acid	Antioxidant, antifungal, surfactant
6.	Oleic acid $$ 9-octadecenoic acid (Z)	5-α reductase inhibitor, allergenic, α-reductase inhibitor, anti inflammatory, anti androgenic, cancer preventive, anemiagenic, anti alopecic, anti leukotriene-D4, choleretic, dermatitigenic, hypocholestrolemic, insectifuge, perfumery, propecic, flavour.
7.	Octadecanoic acid, methyl es	5-α reductase inhibitor, hypo cholesterolemic, suppository, cosmetic, lubricant, surfactant & softening agent, perfumery, propecic, flavour.
8.	Cis--Eicosadienoic acid,	Antiinflammatory, antioxidant, antiarthritic, anticoronary.
9.	Hexadecanoic acid	Antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, anti androgenic, flavour, hemolytic-5-α reductase inhibitor.

**Source: Dr.Duke's phytochemical and ethnobotanical databases [Online database].

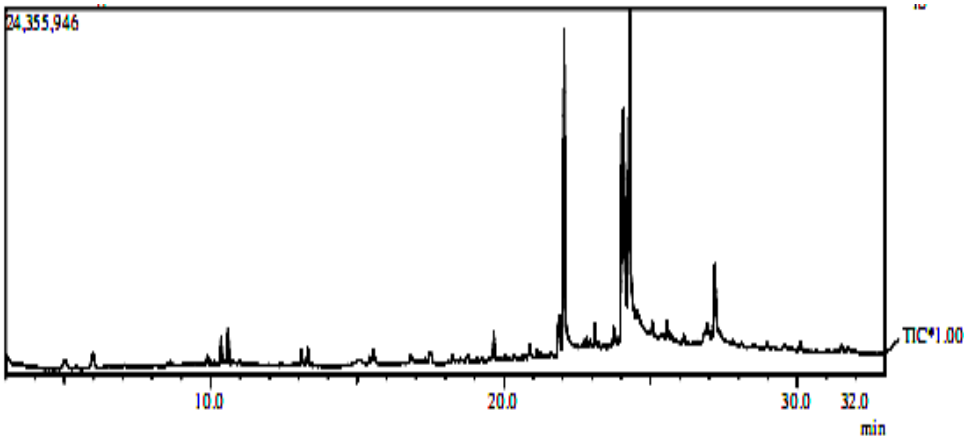

Figure 1: Chromatogram obtained from the GC/MS with the extract of *Azima tetracantha*

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