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DETERMINATION OF BIOACTIVE CONSTITUENTS OF *Azima tetracantha* LEAVES EXTRACT USING GC-MS

Science

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ABSTRACT

The aim of the study to determine the bioactive components of *Azima tetracantha* using Perkin-Elmer Gas Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the Azima tetracantha was matched with the National Institute of Standards and Technology (NIST) library. GC/MS analysis of *Azima tetracantha* revealed the existence of Twelve compounds were identified in *Azima tetracantha* by GC-MS analysis. The prevailing compounds were Tetradecanoic acid, Pentadecanoic acid, 9,12-Octadecadienoic acid (Z,Z), 1-(+)-Ascorbic acid 2,6-dihexadecanoate Heptadecanoic acid, Octadecanoic acid, Cis--Eicosadienoic acid and Hexadecanoic acid. This study explores the goodness of the *Azima tetracantha* which has a commendable sense of purpose and can be advised as a herbo-mineral drug of pharmaceutical importance. The results of this study offer a platform of using *Azima tetracantha* as herbal drug for various diseases.

KEYWORDS:

Azima tetracantha, GC/MS, Bioactive components.

INTRODUCTION

In many industrialized countries herbal medicines are gaining popularity as alternative and complimentary therapies. In developing countries, communities rely heavily on traditional herbal medicines in order to meet their primary health care needs. The secondary metabolites of plants provides humans with numerous biological active products which has been used extensively as drugs, foods, additives, flavors, insecticides, colorants, fragrances and chemicals Some of the plants are used as food or medicine (Velavan, 2015). These plants exhibit a wide range of biological and pharmacological activities such as anti-cancer, anti-inflammatory, diuretic, oxytocic, laxative, antispasmodic, antihypertensive, anti-diabetic, and anti-microbial functions (Pukalarasan and Kathiravan, 2017).. 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. Hence, Gas chromatography (GC) and Mass spectroscopy (MS) associated with particular detection techniques have become a sophisticated means for analysis of various compounds (Gani, 2003).

Our research is therefore being directed towards elucidating potential sources of ethnomedicinal plants using modern scientific analysis like Gas Chromatography-Mass Spectrometry because developments in biotechnology have enhanced investigation of natural compounds faster with more precision than before, leading to isolation of bioactive compounds with health benefits. In the last few years, gas chromatography mass spectrometry (GC-MS) has become firmly established as a key technological platform for secondary metabolite profiling in both plant and non-plant species (Fernie et al., 2004; Kell et al., 2005).

Secondary metabolites are an important source with a variety of structural arrangements and properties. It has been shown that in vitro screening methods could provide the needed preliminary observations necessary to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations. The aim of this study is to determine the organic compounds present in the active fraction of Azima tetracantha with the aid of GC-MS Technique, which may provide an insight in its use in traditional medicine.

MATERIAL AND METHODS

Collection of plant materials

The mature Azima tetracantha leafs were collected in May 2013 from Kodaikanal, Dindugal district, Tamil Nadu, India. The leafs were identified and authenticated by Botanist, Prof. Dr. S. John Britto, Director, The Rapinat Herbarium, St. Josephs College, Tiruchirappalli, Tamil Nadu, India.

Preparation of leaf extract

The dried leaves were pulverized well with mortar and pestle to make a powder. Twenty grams of powder sample was mixed into 100 ml of ethanol and the mixture was kept in 24 hrs. After 24 hrs the leaf extract was filtered with Whatman No. 1 filter paper. The filtrate was stored at 4◦C for further use.

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

RESULTS AND DISCUSSION

The GC-MS spectrum confirmed the presence of various components with different retention times as illustrated in Figure1. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from 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 are based on Dr.Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA.

GC-MS Analysis

Free radicals play a crucial role in the development of tissue damage in pathological events. The extraction method presented is simple, rapid and inexpensive, with reduced solvent consumption. GC-MS method used for the analysis of the obtained extracts can be an interesting tool for testing the amount of some active principles in herbs used in cosmetic, drugs, pharmaceutical or food industry. The *Azima tetracantha* subjected to GC-MS investigation. It is evident from the table 1 that all fractions have a complex chemical composition. Some of the GC-MS peaks remained unidentified, because of lack of authentic samples and library data of corresponding compounds. Twenty 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, Pentadecanoic acid, 9,12-Octadecadienoic acid (Z,Z), 1-(+)-Ascorbic acid 2,6-dihexadecanoate Heptadecanoic acid, Octadecanoic acid, Cis--Eicosadienoic acid and Hexadecanoic acid. The biological activities of prevailing compounds are summarized in table 2. The ethanolic leaf extract obtained from *Azima tetracantha* were subjected to chemical analysis by GC-MS method which confirmed the presence of phytocompounds which are responsible for pharmacological activities.

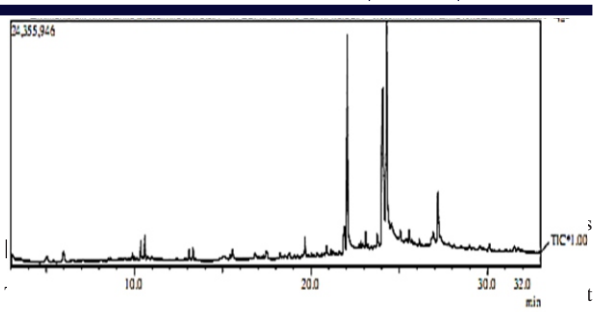

Figure 1: Chromatogram obtained from the GC/MS with *Azima tetracantha*

Table 1 shows the components identified in *Azima tetracantha* (GC MS study)

Peak#	R. Time	Molecular formula	Molecular weight	Area%	Name of the compounds
1	5.979	C12H26	170	1.22	Dodecane
2	9.884	C11H10	142	0.45	Naphthalene, 1-methyl-
3	10.359	C13H26	182	1.17	1-Tridecene
4	10.594	C14H30	198	1.51	Tetradecane
5	13.099	C15H30	210	0.72	1-Pentadecene
6	13.311	C15H32	212	0.76	Pentadecane
7	15.416	C17H34	238	0.24	1-Heptadecene
8	15.560	C20H42	282	0.88	Eicosane
9	17.499	C12H14O4	222	1.00	Diethyl Phthalate
10	19.652	C14H28O2	228	1.16	Tetradecanoic acid
11	20.883	C15H30O2	242	0.62	Pentadecanoic acid
12	21.137	C23H36O4	376	0.34	Phthalic acid, butyl undecyl ester
13	21.893	C20H38O2	310	3.30	cis-13-Eicosenoic acid
14	22.053	C38H68O8	652	18.63	l-(+)-Ascorbic acid 2,6-dihexadecanoate
15	22.821	C22H44O3S	388	0.37	Sulfurous acid, cyclohexylmethyl pentad
16	22.917	C16H30O	238	0.50	cis-9-Hexadecenal
17	23.100	C17H34O2	270	1.24	Heptadecanoic acid
18	23.235	C26H54O3S	446	0.30	Sulfurous acid, 2-ethylhexyl octadecyl es
19	23.740	C20H40O	296	1.04	2-hexadecen-1-OL, 3,7,11,15-Tetra
20	23.808	C19H38O2	298	0.31	Octadecanoic acid
21	24.033	C18H34O2	282	13.53	Octadec-9-enoic acid
22	24.073	C18H34O2	282	15.27	Octadec-9-enoic acid
23	24.277	C24H42O7	442	24.79	L-Ascorbic acid, 6-octadecanoate
24	25.060	C18H32O2	280	0.81	9,12-Octadecadienoic acid (Z,Z)
25	25.564	C19H38O2	298	1.03	Nonadecanoic acid
26	26.133	C35H68O5	568	0.44	Hexadecanoic acid
27	26.817	C23H44O2	352	0.67	22-Tricosenoic acid
28	26.928	C15H28O2	240	1.44	Cyclopentadecanone, 2-hydroxy-
29	27.187	C20H40O2	312	5.57	Icosanoic Acid
30	30.105	C21H40O3	340	0.65	Glycidol stearate

Table 2 Bioactivity of components identified in *Azima tetracantha* by GC-MS

S.no	Name of the Compounds	Biological activity**
1.	Tetradecanoic acid	Antioxidant, cancer preventive, hypercholesterolemic, nematicide, lubricant, cosmetic.
2.	Pentadecanoic acid	Antioxidant
3.	9,12-Octadecadienoic acid (Z,Z)	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.	Octadecanoic acid	5-α reductase inhibitor, hypo cholesterolemic, suppository, cosmetic, lubricant, surfactant & softening agent, perfumery, propecic, flavour.
7.	Cis--Eicosadienoic acid,	Antiinflammatory, antioxidant, antiarthritic, anticoronary.
8.	Hexadecanoic acid	Antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, anti-androgenic, flavour, hemolytic-5-α reductase inhibitor.

for various ailments by traditional practioners. The present study aimed at identifying the nature of the components responsible for their antioxidant activity. This study clearly shows that GC-MS is a powerful technique enabling fast separation and characterization of bioactive metabolites. The high sensitivity of this technique helps in characterization of active compounds in Azima tetracantha

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