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Research Article

GAS CHROMATOGRAPHY AND MASS SPECTROSCOPIC ANALYSIS OF BIOACTIVE CONSTITUENTS IN *MANGIFERA INDICA* LEAVES EXTRACT

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ABSTRACT

The aim of this study was to carry out for identification of bioactive compounds from ethanolic extract of *Mangifera indica* leaves by Gas chromatography and Mass spectroscopy (GC-MS). GCMS analysis of ethanolic 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 of *Mangifera indica* leaves extract revealed the presence of various compounds like Heptadecane, Tetradecane, Heptadecane,1-Hexadecyne, 6-Dodecanone, Hexadecanoic acid, Hexadecane,Pentadecane, 1-Pentadecanol, 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl and 9,12,15-Octadecatrienoic acid, methyl ester. These findings support the traditional use of *Mangifera indica* leaves in various disorders.

Keywords: Gas chromatography and Mass spectroscopy, *Mangifera indica* leaves, Phytochemistry

1. INTRODUCTION

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 [1]. Plants have been an important source of medicine with qualities for thousands of years. Plants are used medicinally in different countries, and they are the source of many potent and powerful drugs. Mainly on traditional remedies such as herbs for their history, they have been used as popular folk medicines [2]. 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 [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, amino acids, proteins, purines and pyrimidines of nucleic acids, chlrophyll's etc. Secondary constituents are the remaining plant chemicals such as alkaloids (derived from amino acids), 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].

Gas Chromatography Mass Spectroscopy (GC-MS) a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification of biochemical components of medicinal plants [7]. The chosen medicinal plant namely as *Mangifera indica leaves* belongs to Anacardiaceae Family. *Mangifera indica leaves* it is widely distributed from the Indian subcontinent through Southeast Asia to northern Australia. he aim of this study is to determine the bioactive compounds present in *Mangifera indica* leaves with the aid of GC-MS Technique.

2. MATERIAL AND METHODS

2.1. Plant materials

The *Mangifera indica* leaves were collected from Thanjavur district, Tamil Nadu, India. The plant were identified and authenticated by Dr. S. John Britto, The Director, the Rapinat Herbarium and center for molecular systematics, St. Joseph's college Trichy-Tamil Nadu. India. A Voucher specimen has been deposited at the Rabinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil nadu, India.

2.2. Preparation of extracts

The collected *Mangifera indica* leaves were washed several times with distilled water to remove the traces of impurities from the leaves. Then examined carefully old, infected and fungus damaged portion of the leaves were removed. Healthy leaves were spread out in a plain paper and shade dried at room temperature for about 10 days and ground in to fine powder using mechanical grinder. The powder was extracted with ethanol for 24 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The *Mangifera indica* leaves extract was stored in refrigerator until used

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 μl was employed (split ratio of 10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C /min, to 200°C, then 5°C/min to 280°C, ending with a 9min isothermal at 280°C. 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.

2.4. Identification of components

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technique, WILEY7 having more than 65 000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the WILEY7 library. The name, molecular weight, molecular formula and structure of the component of the test material were ascertained. 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 GC MS solution ver. 2.53.

3. RESULTS AND DISCUSSION

Gas chromatography – mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample [8]. In the last few years, GC-MS has become firmly established as a key technological platform for secondary metabolite profiling in both plant and non-plant species [9]. 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 [10]. 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.

Twenty compounds were identified in *Mangifera indica* leaves 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 Heptadecane, Tetradecane, Heptadecane,1-Hexadecyne, 6-Dodecanone, Hexadecanoic acid, Hexadecane, Pentadecane, 1-Pentadecanol, 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl and 9,12,15-Octadecatrienoic acid, methyl ester. The biological activities of selected compounds were listed (Table 2) are based on Dr.Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA [11].

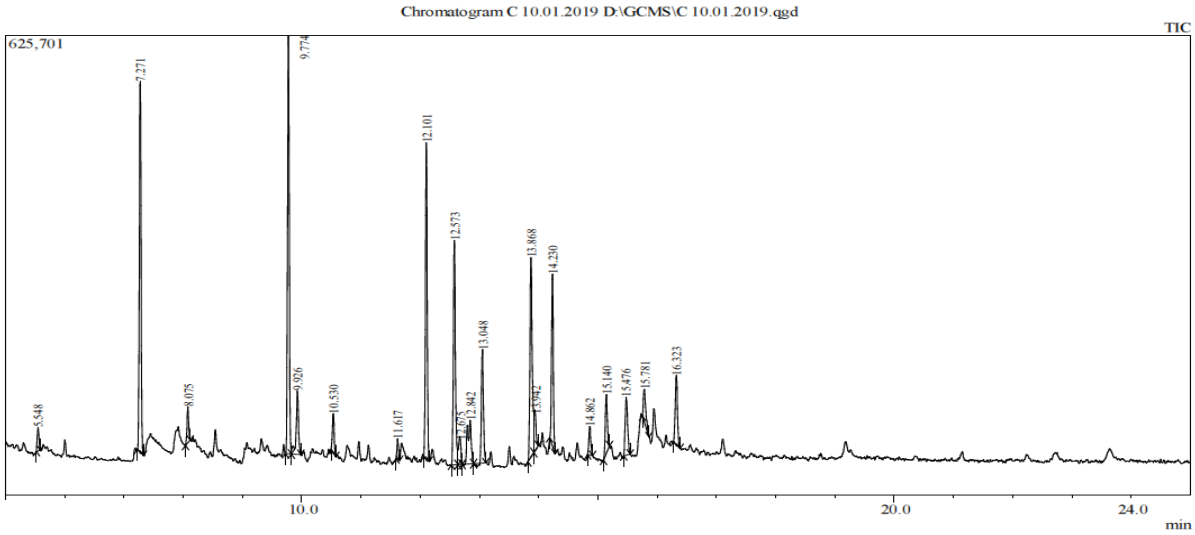

Fig. 1: GC-MS Chromatogram of *Mangifera indica* leaves extract

Table 1: Identification of bioactive compounds in ethanolic extract of *Mangifera indica* leaves using GC MS

Peak#	R.Time	Area %	Molecular formula	Molecular weight	Name of the compound
1	5.548	0.86	$C_{16}H_{14}$	86	Butane, 2,2-dimethyl-
2	7.271	14.09	$C_{17}H_{36}$	240	Heptadecane
3	8.075	1.35	$C_{14}H_{30}$	198	Tetradecane
4	16.323	15.88	$C_{17}H_{36}$	240	Heptadecane
5	16.323	3.22	$C_{15}H_{12}O_4$	256	2-(Benzyloxy)Carbonyl] Benzoic Acid
6	16.323	1.49	$C_{16}H_{34}$	226	Hexadecane
7	16.323	0.63	C_9H_{20}	128	Hexane, 2,4,4-Trimethyl
8	16.323	12.04	$C_{17}H_{36}$	240	Heptadecane
9	16.323	10.16	$C_{16}H_{30}$	222	1-Hexadecyne
10	16.323	1.34	$C_{12}H_{18}O$	178	6-Dodecanone
11	16.323	3.47	$C_{16}H_{30}$	222	1-Hexadecyne
12	16.323	4.81	$C_{16}H_{32}O$	240	Oxirane, tetradecyl
13	16.323	10.09	$C_{16}H_{32}O_2$	256	Hexadecanoic acid
14	16.323	1.66	$C_{16}H_{34}$	226	Hexadecane
15	16.323	6.88	$C_{15}H_{32}$	212	Pentadecane
16	16.323	1.19	$C_{17}H_{36}$	240	Heptadecane
17	16.323	2.70	$C_{15}H_{32}O$	228	1-Pentadecanol
18	15.476	3.13	$C_{20}H_{40}O$	296	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-
19	15.781	1.67	$C_{19}H_{32}O_2$	292	9,12,15-Octadecatrienoic acid, methyl ester,
20	16.323	3.33	$C_{15}H_{32}$	212	Pentadecane

The identified compounds possess many biological properties. Among the identified phytochemicals, hexadecanoic acid is suggested as Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic, 5-Alpha reductase inhibitor [12, 13]. 9,12,15- Octadecatrienoic acid, methyl ester, (Z,Z,Z)- is a polyenoic fatty acid compound and it may be acts as an anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, anti-histaminic, anti-arthritic, anticoronary, anti eczemic, anti-acne, 5-alpha reductase inhibitor and anti-androgenic [14]. Similar results were observed by [15, 16].

Pentadecane act as sugar-phosphatase inhibitor, chymosin inhibitor and antibacterial activities. 2-Hexadecen-1-Ol, 3, 7, 11, 15-Tetramethyl possess antimicrobial, sedatives and anesthetics activities. Hexadecane possess possible antifungal, antibacterial and antioxidant activities. Tetradecane possess antimicrobial, antifungal and nematicidal activities. Heptadecane has potential antioxidant activity [17].

Several other compounds were also detected through GC/MS chromatogram having notable medicinal property. The above said compounds found in the ethanol extract of *Mangifera indica* leaves are being used for the pharmacological work. Thus this type of GC-MS analysis is the first step towards understanding the nature of active principles in the medicinal plants

The investigation concluded that the stronger extraction capacity of ethanol 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 2: Biological activity of Phytocomponents identified in ethanolic extract of *Mangifera indica* leaves

S. No	Compound Name	Biological activity**
1.	Heptadecane	Antioxidant
2.	Hexadecanoic acid	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic, 5-Alpha reductase inhibitor
3.	Pentadecane	Suger-phosphatase inhibitor, Chymosin inhibitor, Antibacterial
4.	2-Hexadecen-1-Ol, 3,7,11,15-Tetramethyl	Antimicrobial, Sedatives and Anesthetics
5.	Hexadecane	Antifungal, Antibacterial, Antioxidant
6.	Tetradecane	Antimicrobial, Antifungal, Nematicidal
7.	Heptadecane	Antioxidant
8.	9,12,15-Octadecatrienoic acid, methyl ester,	Hypocholesterolemic, Nematicide, Antiarthritic, Hepatoprotective, Anti androgenic, 5-Alpha reductase inhibitor, Antihistaminic, Anticoronary, Insectifuge, Antieczemic, Anticancer.

***Duke's. Phytochemical and Ethnobotanical Databases, https://data.nal.usda.gov/ dataset/dr-dukes-phytochemical-and-ethnobotanical-databases, 2019*

4. CONCLUSION

The investigation concluded that the stronger extraction capacity of ethanol could have been produced number of active constituents responsible for many biological activities. From this study it can be concluded that *Mangifera indica* may serve as a new potential source of nanoparticle preparation due to the presence of numerous important bioactive compounds.

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