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Research Article

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CHARACTERIZATION OF BIOACTIVE COPMOUNDS IN ETHANOLIC EXTRACT OF *Mangifera indica* L KERNELS USING GC-MS TECHNIQUE

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ABSTRACT

Plants have been an important source of medicine with qualities for thousands of years. Phytochemicals are the chemicals extracted from plants. These organic chemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. GC-MS method used for the analysis of the obtained extract can be an interesting tool for testing the amount of some active principles in herbs used in various industries. The aim of this study was to carry out for identification of bioactive compounds from the *Mangifera indica* kernels by Gas chromatography and Mass spectroscopy (GC-MS). GCMS analysis of ethanol 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 4H-Pyran-4-One, 2,3-Dihydro-3,5-Dihydroxy-6-Methyl, 3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol, N-Hexadecanoic Acid, 9,12,15-Octadecatrienoic Acid, Methyl Ester, Phytol Isomer, 9,12-Octadecadienoic Acid, 2-Hexadecen-1-Ol, N-Hexadecanoic Acid and 1,2Benzenedicarboxylic Acid, Dioctyl Ester in the ethanolic extract of *Mangifera indica*. These findings support the traditional use of *Mangifera indica* in various disorders.

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INTRODUCTION

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 (Sathyaprabha et al., 2010). 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 (Mathekaga and Meyer, 1998). 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 (Harborne, 198).

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, chlorophyll'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) (Liu, 2004). 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 (Hamburger M and Hostettmann, 1991). 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 (Roberts, Xia, 1995).

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 (Ronald Hites, 1997). The chosen medicinal plant namely as *Mangifera indica* kernels belongs to Anacardiaceae family. *Mangifera indica*. is widely distributed in India, Nepal and Bhutan. The aim of this study is to determine the organic compounds present in the *Mangifera indica* extract with the aid of GC-MS Technique.

2. MATERIAL AND METHODS

2.1 Plant materials: The *Mangifera indica* L. Kermal were collected from Poyyundarkottai, (Plate-II) Thanjavur District, Tamilnadu, India. The collected Kermalwas carefully identified with the help of the Flora of Tamilnadu Carnatic by Mathew K.M (1983). The Kermalwere identified and authenticated by Dr. Jgadeesan, Department of /environmental and Herbal science, Tamil University, Thanjavur, Tamil Nadu, India. A voucher specimen has been deposited at the Herbarium, Tamil University, Thanjavur, Tamilnadu, India, for future reference.

2.2 Preparation of extracts: The powder was extracted with Aqueous, ethanol and methanol for 24 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The *Mangifera indica* kernel extract

(MIKE) 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 polydioxane), 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

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 (Kell et al., 2005). 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 (Fernie et al., 2004). 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 (De-Fatima et al., 2006). 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* 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 4H-Pyran-4-One, 2,3-Dihydro-3,5-Dihydroxy-6-Methyl, 3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol, N-Hexadecanoic Acid, 9,12,15-Octadecatrienoic Acid, Methyl Ester, Phytol Isomer, 9,12-Octadecadienoic Acid, 2-Hexadecen-1-Ol, N-Hexadecanoic Acid and 1,2Benzenedicarboxylic Acid, Dioctyl Ester. . The biological activities of identified 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.

Among the identified phytochemicals hexadecanoic acid is suggested to be a fatty acid ester and it may employed as antioxidant, antimicrobial, flavor, hypocholesterolemic agent and larvicidal activities (Bodoprost and Rosemeyer, 2007; Falodun et al., 2009). 1, 2-benzenedicarboxylic acid, diisooctyl ester is a plasticizer compound and acts as antimicrobial and antifouling agent (Heinonen et al., 1998).

Compounds like n-hexadecanoic acid, 12-octadecanoic acid, dodecanoic acid, tetradecanoic

acid, 1,2-Benzenedicarboxylic acid, dibutyl ester, hexadecanoic acid, ethyl ester and 9,12-octadecadienoic acid (Z,Z) were identified in the ethanolic leaf extract of *Vitex altissima*, a Verbenaceae member (Sathish et al., 2012). Likewise, hexadecane, dodecanoic acid, nonadecane, eicosane, tetradecanoic acid, oleic acid, heptacosane, 9,12- octadecenoic acid, ethyl ester; n-hexadecanoic acid; 1,2-benzenedicarboxylic acid and 9-octadecenoic acid (Z)-ethyl ester were reported in *Clerodendrum inerme* and *C. phlomidis* leaves (Anandhi and Ushadevi, 2013; Balaji and Kilimozhi, 2014).

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.

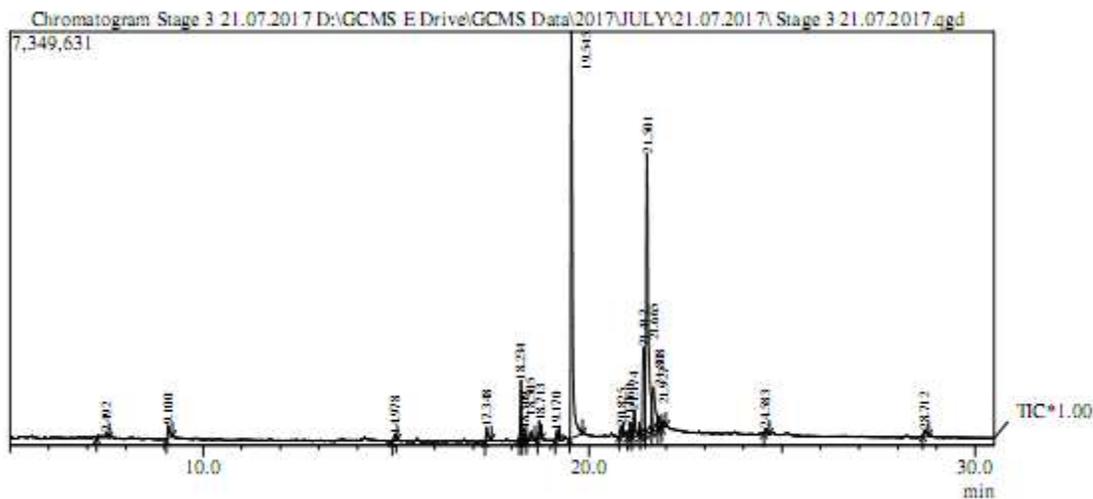


Fig.1: GC MS

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

Peak#	R.Time	Area%	Height%	Molecular formal	Molecular Weight	Name of the compound
1	7.492	0.82	0.45	C ₁₀ H ₁₄	134	Benzene, 1,4-Diethyl-
2	9.100	1.38	1.26	C ₆ H ₈ O ₄	144	4H-Pyran-4-One, 2,3-Dihydro-3,5-Dihydroxy-6-Methyl
3	14.978	0.64	0.64	C ₁₁ H ₁₆ O ₂	180	2(4H)-Benzofuranone, 5,6,7,7a-Tetrahydro-4,4,7a-Trimethyl-, (R)-
4	17.348	1.39	1.30	C ₁₅ H ₃₀ O ₂	242	Pentadecanoic Acid
5	18.234	3.78	5.79	C ₂₀ H ₄₀ O	296	2-Hexadecen-1-Ol, 3,7,11,15-Tetramethyl
6	18.309	1.17	1.08	C ₁₁ H ₂₄ O	172	2-Isopropyl-5-Methyl-1-Heptanol
7	18.505	0.76	0.84	C ₂₀ H ₄₀ O	296	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol
8	18.713	1.20	1.74	C ₁₆ H ₃₂ O	240	Oxirane, Tetradecyl
9	19.170	0.70	0.97	C ₂₅ H ₅₀ O ₂	382	Tetracosanoic Acid, Methyl Ester
10	19.545	33.21	38.99	C ₁₆ H ₃₂ O ₂	256	:N-Hexadecanoic Acid
11	20.825	0.79	1.00	C ₁₇ H ₃₆ O	256	1-Hexadecanol, 2-Methyl-
12	21.060	1.08	1.32	C ₁₉ H ₃₂ O ₂	292	9,12,15-Octadecatrienoic Acid, Methyl Ester
13	21.174	2.32	2.50	C ₂₀ H ₄₀ O	296	Phytol Isomer
14	21.412	8.92	8.35	C ₁₈ H ₃₂ O ₂	280	9,12-Octadecadienoic Acid
15	21.501	31.68	26.81	C ₁₉ H ₃₂ O ₂	292	9,12,15-Octadecatrienoic Acid, Methyl Ester
16	21.665	6.98	4.20	C ₁₈ H ₃₆ O ₂	284	Octadecanoic Acid
17	21.808	1.34	1.09	C ₂₀ H ₃₆ O ₂	308	Ethyl (9z,12z)-9,12-Octadecadienoate
18	21.927	0.65	0.65	C ₁₆ H ₃₂ O ₂	256	N-Hexadecanoic Acid
19	24.583	0.45	0.46	C ₁₀ H ₁₄ O	150	4,7-Methano-5h-Inden-5-One, Octahydro-
20	28.712	0.75	0.56	C ₂₄ H ₃₈ O ₄	390	1,2-Benzenedicarboxylic Acid, Dioctyl Ester
		100.00	100.00			

Table.2: Biological activity of phytochemicals identified in the ethanol kernel extract of *Mangifera indica*

Peak	R.Time	Area %	Name of the compound	Compound Nature	Activity**
1	9.100	1.38	4H-Pyran-4-One, 2,3-Dihydro-3,5-Dihydroxy-6-Methyl	Flavonoid fraction	Antimicrobial, Anti inflammatory
2	18.375	1.55	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol	Terpene alcohol	Antimicrobial, Anti-inflammatory
3	19.545	33.21	N-Hexadecanoic Acid	Palmitic acid	Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor Antioxidant, Hypocholesterolemic
4	21.060	1.08	9,12,15-Octadecatrienoic Acid, Methyl Ester	Fatty acid ester compound	Antiinflammatory,Hypocholesterolemic, Cancer preventive, Hepato protective, Nematicide,Insectifuge Antihistaminic, Antiarthritic,Anticoronary, Antieczemic Antiacne, 5-Alpha reductase inhibitor Antiandrogenic,
5	21.174	2.32	Phytol Isomer	Diterpene	Precursor for the manufacture of synthetic forms of vitamin E and vitamin K1
6	21.412	8.92	9,12-Octadecadienoic Acid	Linoleic acid	Hypocholesterolemic,5-Alpha reductase inhibitor, Antihistaminic, Insectifuge, Antieczemic, Antiacne
7	21.204	0.79	2-Hexadecen-1-Ol	Acyclic diterpene alcohol	Precursor for the manufacture of synthetic forms of vitamin E and vitamin K1. used in the fragrance industry and used in cosmetics, shampoos, toilet soaps, household cleaners, and detergents
8	21.927	0.65	N-Hexadecanoic Acid	Palmitic acid	Antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavor, hemolytic, 5-Alpha reductase inhibitor
9	28.712	0.75	1,2Benzenedicarboxylic Acid, Dioctyl Ester	phthalate ester	Used as Softeners, Used in preparation of perfumes and cosmetics, Used as plasticized vinyl seats on furniture and in cars, and clothing including jackets, raincoats and boots. Used in textiles, as dyestuffs, cosmetics and glass making.

**Duke's. Phytochemical and Ethnobotanical Databases, www.ars-gov/cgi-bin/duke/, 2013.

CONCLUSION

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.

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