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Gas Chromatography Coupled With Mass Spectroscopy for The Isolated Lipoidal matters of *Manilkara hexandra.*

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ABSTRACT

Manilkara hexandra family Sapotaceae is a small to medium sized evergreen tree. It is native to South Asia and Tropical Countries. *M. hexandra* cultivated in Egypt was studied for the saponifiable and unsaponifiable matters content for its leaves and fruits by GC/MS analysis for the first time. The results showed that the leaves contains unsaponifiable matters as hydrocarbons (89.1%) and sterols by (2.7%) while the saponifiable matters are saturated fatty acids (53.5%) and unsaturated fatty acids (22.8%). The fruits of *M. hexandra* contains unsaponifiable matters as hydrocarbons (82.5%) and sterols (6.4%) while the saponifiable matters shows twenty compounds of which saturated fatty acids (42.8%) and unsaturated fatty acid (41.5%).

Keywords: *Manilkara hexandra*, Sapotaceae, Leaves, Fruits, Saponifiable Matters, Unsaponifiable Matters, Hydrocarbons, Fatty acids, Sterols, GC/MS analysis.

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INTRODUCTION

Manilkara hexandra (common name is Rayan/ Khirni) is a small to medium sized evergreen tree. It is native to South Asia and Tropical Countries and widely distributed in South, North and Central India [1-2]. *Manilkara hexandra* is cultivated in Egypt in the Zoo garden, Giza, Egypt [3]. Leaves, fruits, roots and barks of *M. hexandra* are well known for their medicinal values [4-5]. There are no reports dealing with lipoidal matters of *Manilkara hexandra* so this study focuses on the lipoidal matters of leaves and fruits of *M. hexandra*.

MATERIALS AND METHODS

Plant Material

The leaves and fruits of *Manilkara hexandra* (Roxb.) Dubard were collected in June 2014 from the Zoo garden, Giza, Egypt. The plant was identified by Dr. Reem samir Botany department, Faculty of Science, Cairo University. A voucher specimen was deposited at Faculty of Pharmacy, Helwan University.

Methods

Preparation of the Unsaponifiable Matter

100 gm of air dried leaves and fruits of *M. hexandra* were extracted with petroleum ether. About 1 gm of the dried petroleum ether fraction of the leaves and fruits of *M. hexandra* was subjected to alkaline hydrolysis for saponification. The petroleum ether fraction was refluxed with 50 ml of 10% alcoholic potassium hydroxide for about 6 hrs on a boiling water bath. The major part of the alcohol present was distilled off and the liquid left was diluted with twice its volume of water and then extracted with several portions of chloroform until exhaustion. The combined chloroformic extracts were washed with alcoholic sodium hydroxide (10%) then with distilled water until the wash were free from any alkalinity. The chlorofom extracts were dehydrated over anhydrous sodium sulphate and then the chloroform was distilled off. The residue obtained (represent the unsaponifiable matter) was dark orange yellow in color and semisolid at room temperature [6-7].

Preparation of saponifiable matter (fatty acids)

The alkaline aqueous solution remained after removal of the unsaponifiable matter was acidified with sulphuric acid (10%). The liberated fatty acids were extracted with successive small portions of chloroform. The combined chloroformic extracts were washed with distilled water, till the wash was neutral to litmus paper. The chlorofrom was distilled off and the residue of total fatty acids was dried over anhydrous sodium sulfate. It was semisolid at room temperature and brown in color [6-7].

Preparation of fatty acid methyl esters:

The fatty acids were converted to their methyl esters by refluxing with 50 ml. absolute methanol and 1.5 ml. conc. sulphuric acid for two hrs. The major part of alcohol was distilled off and the residue was solubilized with distilled water and then extracted with several portions of chloroform. The combined chloroformic extracts were washed with distilled water, till the wash was free from any acidity. The chloroformic extract was concentrated and the residue was dried over anhydrous calcium chloride overnight and then kept for further investigation [6-8].

Unsaponifiale matter of both leaves and fruit were analyzed by GC/MS. Electron Impact (EI) mode of ionization, with mass range of 50-700 *m/z*. While the GC/MS analysis of the fatty acid methyl esters carried with mass range 50-500 *m/z*. The components were identified by comparing their retention times and mass fragmentation patterns with published data [9].

RESULTS AND DISCUSSION

The results of the GC/MS analysis of the unsaponifiable matter of *Manilkara hexandra* leaves and fruits are shown in Figure (1&2) and Table (1&2) respectively. The results revealed that *M. hexandra* leaves

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contains about 89.1 % as hydrocarbons (cyclic and A cyclic), 2.7 % sterols and about 8.2 % classified as unidentified compounds. The most abundant compound in the unsaponifiable fraction of lipophilic extract of the leaves of *M. hexandra* was pentadecane. While, in the unsaponifiable fraction of the fruit of *M. hexandra* reaveled the presence of 82.5% hydrocarbons (acyclic and cyclic), sterols (6.4%) and 11.1% unidentified compounds. The *n*-alkanes ranging from Undecane (C_{11}) to Triatetracontane (C_{43}).

Pentadecane is detected in the leaves and fruits extract beside 2- methyl pentadecane, eicosane and n-tetradecane. The concentration of pentadecane in leaves (28.6 %) is higher than in fruits (16.9 %). The steroidal compounds are identified in the leaves extract as 16-Allopregnen-3α-ol-20-one (1.4%) and ethylisoallocholate (1.3). The steroidal compound identified in the fruits extract is lupeol. Lupeol is identified in the fruits only with concentration 6.4%.

Lupeol (3-hydroxylup-20(29)-ene) a pentacyclic triterpene, Lupeol is an immense bioactive compound present in different medicinal plants [10-12]. A wide range of bioactivities and bioassays of lupeol are reviewed [13], which suggest its useful medicinal properties with diversity of action against different diseases. This compound is reported to be antioxidative and anti-inflammatory in nature [14]. It inhibits early responses of tumor growth induced by benzoyl peroxide [15]. It also plays very important role in normalization of lipid profile [16], wound healing activity [17], protective effect in hypercholesterolemia associated with renal damage [18] and suppression of immune factors [19]. Lupeol also reported as hepatoprotective, cardioprotective, antimicrobial and antiprotozoal [20].

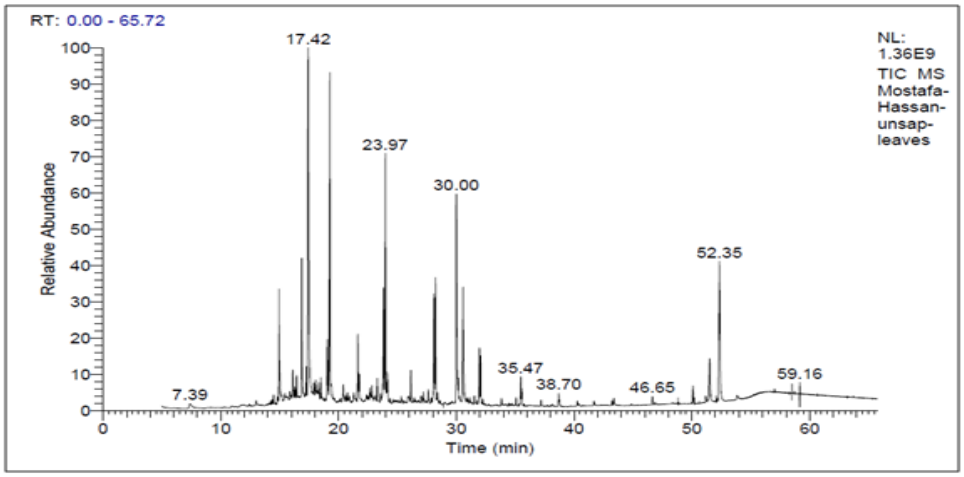

Fig 1: GC chromatogram of the unsaponifiable matter of *M. hexandra* leaves

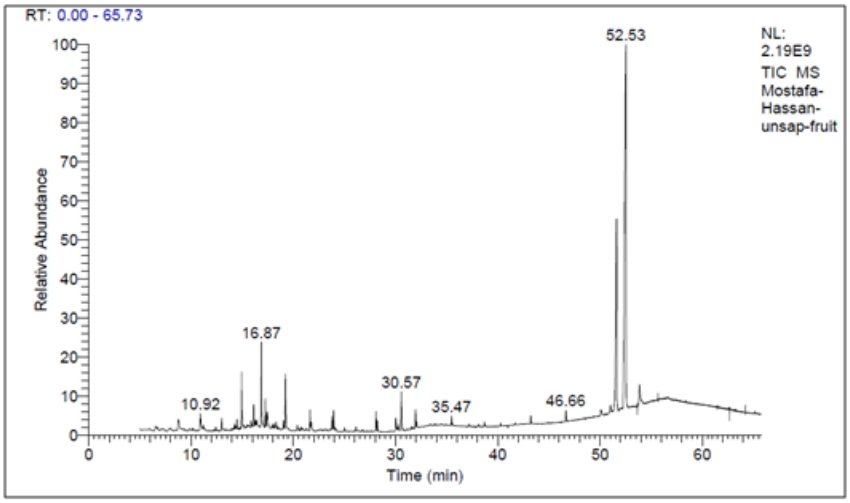

Fig 2: GC chromatogram of the unsaponifiable matter of *M. hexandra* Fruits

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Table 1: GC/MS analysis of the unsaponifiable matter of *M. hexandra* leaves

Identified Compound	RR_t *	M^+	Molecular formula	Percentage
n-Tetradecane	0.78	198	$C_{14}H_{30}$	9.4
4-Decen, (E)	0.94	140	$C_{10}H_{20}$	1.6
2-Methyl pentadecane	0.95	226	$C_{16}H_{34}$	1.7
n-Docosane	0.96	310	$C_{22}H_{46}$	1.7
1-Dodecene	0.98	168	$C_{12}H_{24}$	6.1
n-Pentadecane	1	212	$C_{15}H_{32}$	28.6
n-Hexadecane	1.05	226	$C_{16}H_{34}$	2.2
n-Dodecane	1.12	170	$C_{12}H_{26}$	6.4
4-Ethyl tetradecane	1.18	226	$C_{16}H_{34}$	1.7
3-Methyl heptadecane	1.2	254	$C_{18}H_{38}$	2.2
1-Octadecene	1.23	252	$C_{18}H_{36}$	9.6
n-Eicosane	1.36	282	$C_{20}H_{42}$	2.7
n-Tetracosane	1.47	338	$C_{24}H_{50}$	9.7
n-Hexatriacontane	1.67	506	$C_{36}H_{74}$	3.7
n-Nonacosane	2.6	408	$C_{29}H_{60}$	1.8
16-Allopregnen-3α-ol-20-one	1.3	316	$C_{21}H_{32}O_2$	1.4
Ethylisoallocholate	0.39	436	$C_{26}H_{44}O_5$	1.3
Total hydrocarbon				89.1
Sterols				2.7
Unidentified compounds				8.2

RR_t*: relative retension time relative to pentadecane= 19.25min.

Table 2: GC/MS analysis of the unsaponifiable matter of *M. hexandra* Fruits

Identified compound	RRt*	M^+	Molecular formula	Percentage
2-Methyldecane	0.47	156	$C_{11}H_{24}$	2.4
n-Undecane	0.52	156	$C_{11}H_{24}$	4.6
n-Dodecane	0.65	170	$C_{12}H_{26}$	4.8
4-Methyl-5 propylnonane	0.77	184	$C_{13}H_{28}$	2.8
n-Tetradecane	0.89	198	$C_{14}H_{30}$	11.3
2-Cyclo hexyloctane	0.94	196	$C_{14}H_{28}$	2.3
6-Methyl octadecane	0.95	268	$C_{19}H_{40}$	6.1
Pentadecane	1	212	$C_{15}H_{32}$	16.9
2-Methyl pentadecane	1.08	226	$C_{16}H_{34}$	2.5
Eicosane	1.14	282	$C_{20}H_{42}$	12.1
Docosane	1.29	310	$C_{22}H_{46}$	4.8
Heptacosane	1.42	380	$C_{27}H_{56}$	2.4
Pentacosane	1.67	352	$C_{25}H_{52}$	4.3
n-Nonacosane	1.89	408	$C_{29}H_{60}$	2.6
n-Triatetracontane	2.1	604	$C_{43}H_{88}$	2.6
Lupeol	3.19	426	$C_{30}H_{50}O$	6.4
Total hydrocarbons				82.5
Sterols				6.4
Unidentified compounds				11.1

RR_t*: relative retension time relative to Pentadecane = 16.87 min

The results of the fatty acid (saponifiable matters) analysis of *M. hexandra* leaves and fruit are shown in Table (3&4) and Fig. (3&4) respectively.

The results of *M. hexandra* leaves show that the saturated fatty acids (53.47%) represent higher percentage than that of unsaturated ones (22.85%). Palmitic (35.44%) and Stearic acid (15.14%) represent the major identified saturated fatty acid while Oleic acid (22.39%) represents the major unsaturated fatty acid.

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The fruits of *M.hexandra* show presence of of saturated fatty acids 42.82% and unsaturated fatty acid 41.46%. Palmitic acid (22.27%) represents the major unsaturated fatty acid while the oleic acid (35.47%) represents the major unsaturated fatty acid.

Oleic acid ($C_{18:1}$) is able to prevent/attenuate palmitic acid hepatotoxicity [21] through channeling palmitic acid into triglycerides synthesis, changing the composition of the intracellular fatty acids pool thus protecting the liver cells from palmitic acid-dependent apoptosis [22]. In addition, it has the ability to reduce the inflammatory effects of long-chain saturated fatty acids in human aortic endothelial cells [23]. Oleic acid exerts its anti-inflammatory effect by influencing arachidonic acid metabolism which is a precursor of the eicosanoids pro-inflammatory agents [24].

Stearic acid is a long-chain saturated fatty acid; its behavior is especially unique with respect to its effects on serum cholesterol levels [25]. It possesses an antioxidant effect which mediates its neuroprotective effects [26].

Linoleic acid (C18:2) represent the family of *ω*-6 fatty acids. It possesses antioxidant and anti-inflammatory activities, the anti-inflammatory effect are through inhibition of COX-I and COX-II enzymes [27]. There is some similarity in the fatty acid content of the fruit with that of the leaves in the presence of oleic, stearic, palmitic, Adrenic and Arachidic acids.

The study includes the investigation of the unsaponifiable and saponifiable matters of *Manilkara hexandra* (Roxb.) Dubard leaves and Fruits by GC/MS could be helpful in authentication of the plant. This is the first record for the GC/MS analysis of *Manilkara hexandra* growing in Egypt.

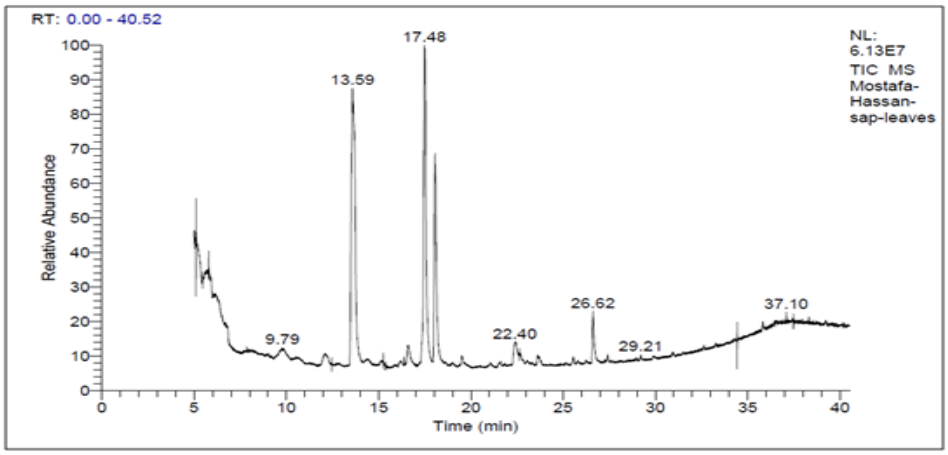

Figure 3: GC Chromatogram of Saponifiable Matters of *M. hexandra* leaves

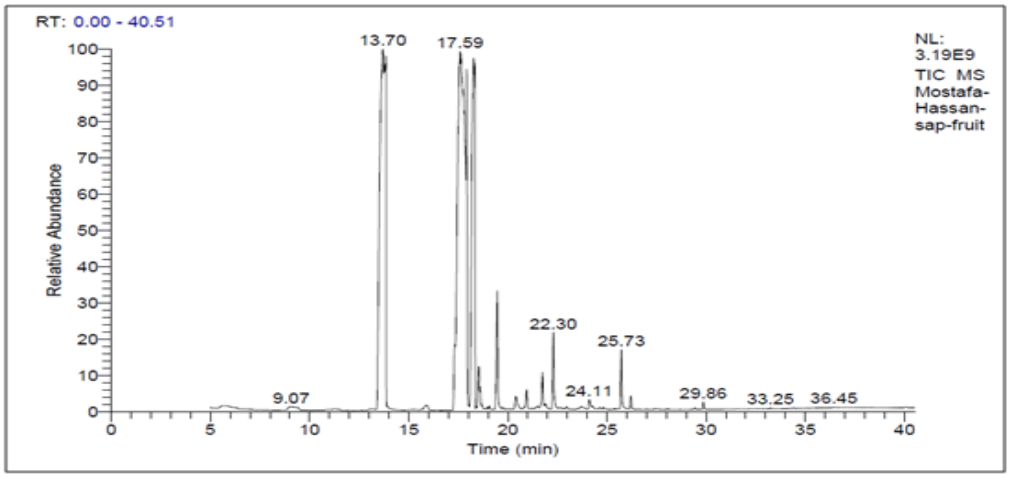

Figure 4: GC Chromatogram of Saponifiable Matters of *M. hexandra* Fruits

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Table 3: GC/MS analysis of the saponifiable matter of *M. hexandra* leaves

Identified compound		RR_t*	M^+	Percentage
Arachidic acid	**C(20:0)**	0.33	374	2.89
Palmitic acid	**C(16:0)**	0.77	270	35.44
Adrenic acid	**C(22:4)**	0.95	330	0.46
Oleic acid	**C(18:1)**	**1**	294	**22.39**
Stearic acid	**C(18:0)**	1.03	398	15.14
Saturated fatty acid				53.47
Unsaturated fatty acid				22.85
Unidentified compound				23.68

RR_t*: relative retension time relative to Oleic acid=17.51 min

Table 4: GC/MS analysis of the saponifiable matter of *M. hexandra* Fruits

Identified compound		RR_t*	M^+	Percentage
Isopalmitic acid	C(16:0)	0.66	270	0.12
Capric acid	C(10:0)	0.69	186	0.08
Palmitic acid	C(16:0)	1	270	22.27
Lauric acid	C(12:0)	1.16	284	0.13
Myristic acid	C(14:0)	1.16	242	0.1
Rumenic acid	C(18:2)	1.26	294	0.32
Palmitelaidic acid	C(18:1)	1.29	296	35.47
Elaidic acid	C(18:1)	1.3	296	3.27
Stearic acid	C(18:0)	1.33	298	17.8
Linoleic acid	C(18:2)	1.35	295	.07
Linolelaidic acid	C(18:2)	1.39	294	0.31
Adrenic acid	C(18:4)	1.5	346	0.08
Linolenic acid	C(18:3)	1.57	292	0.06
Gadoleic acid	C(20:1)	1.58	282	1.43
Palmitoleic acid	C(16:1)	1.6	254	0.1
Arachidic acid	C(20:0)	1.63	326	1.07
Oleic acid	C(18:1)	1.67	282	0.25
Erucic acid	C(22:1)	1.87	352	0.13
Behinic acid	C(23:0)	1.91	354	1.1
Lignoceric acid	C(18:0)	2.17	382	0.15
Saturated fatty acid				42.82
Unsaturated fatty acid				41.46
Unidentified compound				15.72

RR_t*: relative retension time relative to palmitic acid =13.71

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