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GC–MS analysis of fatty acids of *Malvaviscus arboreus* leaves

Omnia H. Abdelhafez[1], John R. Fahim[2*], Usama R. Abdelmohsen[1,2], Samar Y. Desoukey[2]

[1] Department of Pharmacognosy, Faculty of Pharmacy, Deraya University, 61111 New Minia, Egypt
[2] Department of Pharmacognosy, Faculty of Pharmacy, Minia University, 61519 Minia, Egypt

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Abstract

Malvaceae plants have been reported to produce a wide range of structurally varied fatty acids. Therefore, in this work, the fatty acid content of *Malvaviscus arboreus* Cav. leaves was investigated using gas chromatography-mass spectrometry (GC–MS) technique. A total of 17 fatty acids were characterized, with noticeable abundance of saturated acids (50.98%) that were principally dominated by palmitic acid (32.15%). Moreover, a number of mono-, di-, and tri-unsaturated fatty acids were detected constituting 24.92% of the total identified acids, of which 5,12-octadecadienoic acid (7.66%) was the major unsaturated acid identified. As a common feature of several genera of the Malvaceae family, some unusual fatty acids were identified from the saponifiable fraction of *M. arboreus* leaves, namely 9-methyl tetradecanoic, 3-methoxy pentadecanoic, and 9-heptadecynoic acids, that along with tetracosanoic, 9-octadecenoic, 13-octadecenoic, 5,12-octadecadienoic, and 9,11,15-octadecatrienoic acids, were also interestingly reported herein for the first time in the genus *Malvaviscus*.

Key words
Fatty acids, GC–MS, Malvaceae, *Malvaviscus*, Palmitic acid, Saponifiable matter

1. Introduction

Fatty acids are important plant constituents and are commonly known to possess many physiological roles. They primarily occur in bound forms in plants; esterified with glycerol, as fats or lipids. These lipids constitute up to 7% of the dry leaf weight in higher plants, and are important ingredients in mitochondria, green plastids, and cell membranes [1]. These membranes are also rich sources of signaling entities, many of which are biosynthetic derivatives of fatty acids, which can act as intracellular mediators or as extracellular signals, participating in interspecies communication and plant defense mechanisms [2]. Besides leaves, seeds and fruits of many plants also accumulate lipids in substantial amounts, providing a source of stored energy required for germination [1, 3]. On the other hand, plant fatty acids are of wide-ranging nutritional and biological importance for humans, especially those contained within edible species. Additionally, lipids derived from several plant matrices have been gaining reputation in biotechnology, mainly for the development of varied products with therapeutic, cosmetic, or pharmaceutical value [4]. Identification of fatty acid patterns of different plant species has been also reported to be of particular chemotaxonomic value [3].

Malvaceae is a large family of flowering plants with about 4225 species distributed in 244 genera. Plants belonging to this family are well known for their privileged ornamental, economic, food, and ethnomedicinal importance [5, 6]. Long ago, the phytochemistry of Malvaceae plants has attracted considerable attention due to their diverse metabolites that chiefly include mucilages, flavonoids, anthocyanins, phenolic acids, terpenoids, and steroids [6, 7]. These plants have also been shown to produce a number of structurally varied fatty acids; therefore, numerous studies have demonstrated the fatty acid composition of many Malvaceae species, particularly of their seed oils, such as *Malva*, *Hibiscus*, *Gossypium*, and *Sida* spp. [8, 9].

Malvaviscus arboreus Cav. or Sleeping Hibiscus is a Malvaceous plant within the small genus *Malvaviscus* that grows naturally throughout the tropical and subtropical Americas; however, it was cultivated in several other parts of the world as well [10, 11]. Previous studies on Sleeping Hibiscus plant indicated its richness in phenolic metabolites, sterols, terpenoids, and fatty acids. Different extracts from this plant species also revealed cytotoxic, antioxidant, hepatoprotective, and antimicrobial activities [12–14]. In connection with fatty acids, the phospholipid fatty acid profile of *M. arboreus* flowers was previously reported [15], whereas that of the leaves has not been described yet. Thus, in continuation of our research studies on this plant species [12, 13, 16, 17], the saponifiable part of the lipid fraction of *M. arboreus* leaves was extracted and subjected to gas chromatography-mass spectrometry (GC–MS) analysis in order to unveil their fatty acid content.

2. Experimental

2.1. Plant material

Malvaviscus arboreus plants were collected at the flowering period from the campus of Minia University, Egypt, and identified by Prof. Mahmoud A. Hassan, Horticulture Department, Faculty of Agriculture, Minia University. A voucher has been deposited in the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Minia University with the number [Mn-Ph-Cog-027].

2.2. Extraction of fatty acids

The air-dried leaves of *M. arboreus* were extracted with petroleum ether (b.p. 60–80°C) at room temperature and concentrated under vacuum till dryness. The obtained residue (2.5 g) was refluxed for 7–8 h with 50 ml of N/2 alcoholic KOH. Then, the major part of alcohol was evaporated, whereas

**** Correspondence: John R. Fahim***
Tel.: *+2 01227454025****; Fax:*** *+20 862369075*
Email Address: *john.michael@mu.edu.eg*

the left mixture was diluted with distilled water in a separating funnel and extracted with successive volumes of dichloromethane to get rid of the unsaponifiable matter. The remaining aqueous layer (soap) was then rendered acidic by addition of 10% H_2SO_4, followed by extraction of the liberated free fatty acids with dichloromethane. The pooled dichloromethane extracts were thoroughly washed with sufficient amounts of distilled water until the washings were free from acidity. The organic solvent was then distilled off to afford a semisolid residue of the total fatty acids (0.9 g) [18].

2.3. Preparation of fatty acids methyl esters (FAME)

FAME were prepared by refluxing the obtained residue of fatty acids with a mixture of methanol (50 ml) and conc. H_2SO_4 (1.5 ml) for about 2 h. The major part of methanol was then distilled off and the remained solution was mixed with distilled water in a separating funnel and re-extracted with dichloromethane. Dichloromethane extracts were thoroughly washed with distilled water until they became neutral to litmus paper. The organic solvent was evaporated yielding a semisolid yellowish brown residue, which was then dried overnight on $CaCl_2$ [18].

2.4. GC–MS analysis of FAME

The programmed temperature GC–MS analysis of FAME was carried out on Thermo GC-Trace Ultra equipped with a flame ionization detector and a Finnigan SSQ 7000 mass spectrometer. An Rtx-5MS capillary column (30 m × 0.25 mm) of 0.25 µm film thickness was used. Helium was also used as the carrier gas at 0.8 ml/min. The injection volume was l µl, and the initial temperature was 70 °C, and then gradually risen to 220 °C at 3 °C/min. The injector and detector temperatures were 240 ºC. The chromatographic run lasted for 1 h with a split ratio of 1:50. Mass spectra were obtained in the electron impact ionization mode at 70 eV with a mass range of *m/z* 40–500. Compounds were identified by comparing their mass spectra with literature and some databases, such as the National Institute Standard and Technology (NIST) [19, 20].

3. Results and discussion

GC–MS analysis of FAME extracted from *M. arboreus* leaves **(Figure 1)** showed the presence of thirty-eight components, 17 of which representing 75.90% were identified. The amount of saturated fatty acids (50.98%) was nearly as twice as that of the unsaturated acids, which accounted only for 24.92% of the total identified acids **(Table 1)**. The saturated fatty acids' group was dominated by palmitic acid (32.15%), followed by stearic (7.90%), myristic (2.36%), arachidic (2.12%), behenic (1.77%), and margaric (1.06%) acids. On the other hand, 5,12-octadecadienoic (7.66%), elaidic (5.25%), 12,15-octadecadienoic (4.14%), and 13-octadecenoic (2.27%) acids constituted the major proportion of the unsaturated fatty acids, in conjunction with smaller amounts of 9-heptadecynoic (2.11%), linolelaidic (1.20%), linoleic (1.19%), and 9,11,15-octadecatrienoic (1.10%) acids.

While plant seeds contain a broad range of fatty acids, those from leaf tissues are noticeably constant from plant to another, and their fatty acid content is also quite distinctive; consequently, variation in the fatty acid compositions of different plants has long been of important chemotaxonomic interest, and was employed in classification of botanical families into classes and subclasses [3, 21]. In the same context, among the characterized fatty acids herein from *M. arboreus* leaves, palmitic, stearic, myristic, behenic, margaric, arachidic, and linoleic acids were also previously reported from the related species, *Malvaviscus penduliflorus* DC. through GC–MS based analysis of the *n*-hexane extract of its flowers [22]. In line with our current findings, palmitic and stearic acids have also dominated the fatty acid content of *M. penduliflorus* flowers. In another similar GC–MS study, the fatty acid profile of *M. arboreus* flowers was also shown to be predominated by palmitic (34.3%) and linoleic (31.8%) acids, in addition to the presence of stearic (9.4%), 2-hydroxyoctadecanoic (1.8%), margaric (1.3%), and myristic (1.2%) acids [15], which were also detected herein in the leaves. In contrast, the present study on *M. arboreus* leaves represents the first report on the presence of 9-methyl tetradecanoic, 3-methoxy pentadecanoic, tetracosanoic, elaidic, 13-octadecenoic, 5,12-octadecadienoic, 9,11,15-octadecatrienoic, and 9-heptadecynoic acids in the genus *Malvaviscus*. In this context, the occurrence of some unusual fatty acids, exemplified by acetylinic, ethylene-interrupted, cyclopropene, epoxy, methyl-branched, methoxylated, or hydroxylated fatty acids, has been reported from several plant families, including Malvaceae and their related families, Bombacaceae and Sterculiaceae [9, 13, 15, 23–25].

From a biological point of view, several plant-derived fatty acids and their crude mixtures have been reported to exhibit antibacterial, antifungal, anticancer, antioxidant, anti-inflammatory, cardioprotective, and wound healing activities, among many others [3, 26–29]. In addition, the involvement of fatty acids in food and pharmaceutical industries has become of pivotal significance with the purpose of developing new plant-based products with multiple benefits [30]. In this regard, the current report on the fatty acids of *M. arboreus* leaves should be coupled in the future with their possible therapeutic or industrial applications that are related to these fatty metabolites.

4. Conclusion

The fatty acids of *M. arboreus* leaves were analyzed and quantified by GC–MS. The main proportion of the saponifiable fraction was occupied by saturated fatty acids, among which, palmitic acid was the major constituent identified. Additionally, eight of the detected fatty acids were characterized herein for the first time from *Malvaviscus* plants. The identification of such molecules and some other unusual fatty acids from *M. arboreus*, along with the marked abundance of palmitic acid could be of particular chemotaxonomic importance. In this regard, further comprehensive investigation of the possible biological potential as well as the seasonal and/or organ-to-organ variability of fatty acids produced by different species within the genus *Malvaviscus* is strongly recommended.

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Table 1: A list of fatty acids identified as methyl esters from *Malvaviscus arboreus* Cav. leaves.

Peak	IUPAC Name	Common Name	Molecular	R_t[b]	RR_t[c]	Base	Peak
5	Tetradecanoic acid	Myristic acid	$C_{15}H_{30}O_2$	22.06	0.84	74.05	2.36
9	9-Methyl tetradecanoic acid	–	$C_{16}H_{32}O_2$	24.21	0.92	74.05	0.90
13	9-Heptadecynoic acid	–	$C_{18}H_{32}O_2$	25.82	0.98	82.10	2.11
15	**Hexadecanoic acid** [d]	**Palmitic acid**	$C_{17}H_{34}O_2$	26.32	1	74.10	**32.15**
17	Heptadecanoic acid	Margaric acid	$C_{18}H_{36}O_2$	28.19	1.07	74.05	1.06
18	3-Methoxy pentadecanoic acid	–	$C_{17}H_{34}O_3$	28.54	1.08	75.05	0.97
19	5,12-Octadecadienoic acid	–	$C_{19}H_{34}O_2$	29.50	1.12	67.10	7.66
20	9*E*-Octadecenoic acid	Elaidic acid	$C_{19}H_{36}O_2$	29.60	1.12	55.10	5.25
21	13-Octadecenoic acid	–	$C_{19}H_{36}O_2$	29.70	1.13	55.10	2.27
22	Octadecanoic acid	Stearic acid	$C_{19}H_{38}O_2$	30.08	1.14	74.05	7.90
23	(9*E*,12*E*)-Octadecadienoic acid	Linolelaidic acid	$C_{19}H_{34}O_2$	30.38	1.15	67.10	1.20
25	(9*Z*,12*Z*)-Octadecadienoic acid	Linoleic acid	$C_{19}H_{34}O_2$	31.08	1.18	67.10	1.19
26	12,15-Octadecadienoic acid	–	$C_{19}H_{34}O_2$	31.19	1.19	67.10	4.14
28	2-Hydroxy octadecanoic acid	–	$C_{19}H_{38}O_3$	32.26	1.23	97.10	0.81
30	(9*Z*,11*E*,15*Z*)-Octadecatrienoic acid	–	$C_{19}H_{32}O_2$	33.16	1.26	95.10	1.10
31	Eicosanoic acid	Arachidic acid	$C_{21}H_{42}O_2$	33.57	1.28	74.05	2.12
33	Docosanoic acid	Behenic acid	$C_{23}H_{46}O_2$	37.07	1.41	74.05	1.77
35	Tetracosanoic acid	Lignoceric acid	$C_{25}H_{50}O_2$	42.29	1.61	74.05	0.94
Percentage of identified saturated fatty acids							50.98%
Percentage of monounsaturated fatty acids							9.63%
Percentage of polyunsaturated fatty acids							15.29%
Percentage of identified unsaturated fatty acids							24.92%
Percentage of total identified fatty acids							75.90%

[a] The peak numbers of identified fatty acids were only mentioned.
[b] **R_t:** retention time. [c] **RR_t:** relative retention time (relative to the major compound palmitic acid (peak no. 15)).
[d] Bold data denote the major fatty acid identified.

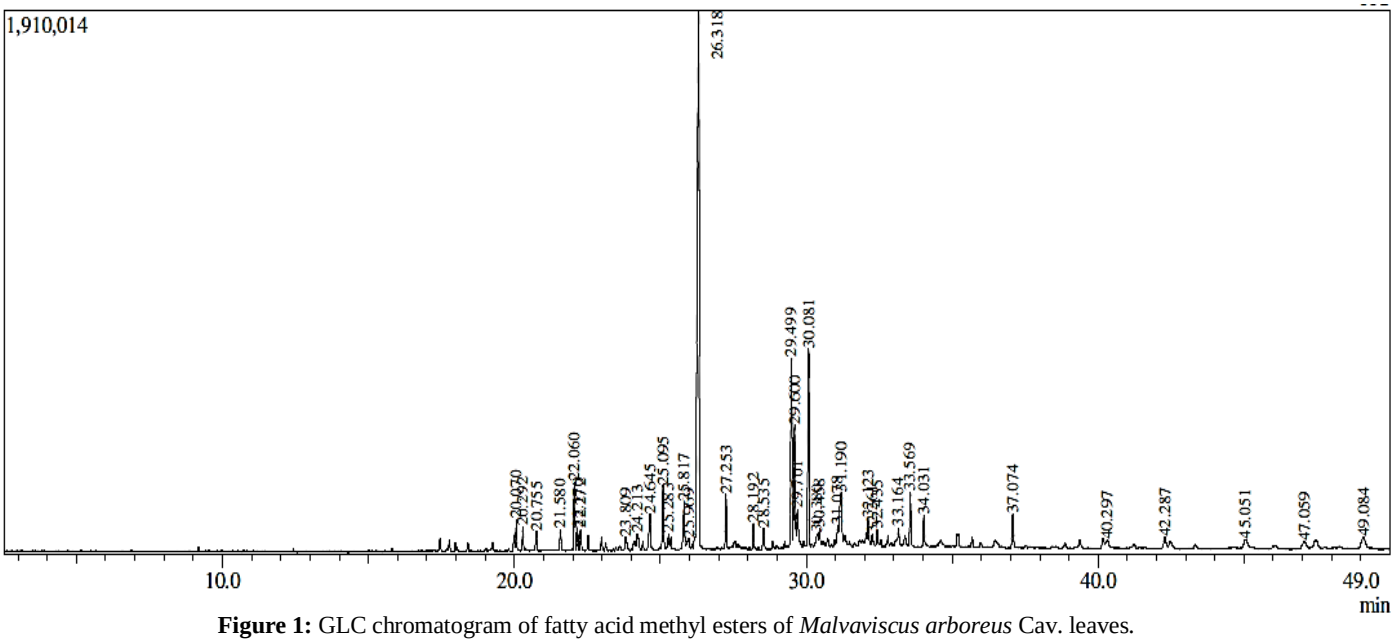

Figure 1: GLC chromatogram of fatty acid methyl esters of *Malvaviscus arboreus* Cav. leaves.

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