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Chemical Investigation of Lipoidal Matter of *Ficus craterostoma*

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ABSTRACT

Objectives: This study aimed to investigate for the first time the lipoidal matter of *Ficus craterostoma* Mildbr. & Burret family (Moraceae) growing in Egypt. **Methods**: The identification of saponifiable and unsaponifiable matter of *F. craterostoma* leaves were carried out by gas chromatography coupled with mass spectroscopy (GC/MS). **Results**: Saponification of *n*-hexane extract of *F. craterostoma* leaves yielded 55% and 11% for unsaponifiable matter and fatty acids, respectively. The content of saturated fatty acids (66.09%) identified is higher than unsaturated fatty acids (22.23%); where palmitic acid (59.71%) and linolenic acid (11.59%) are the main saturated and unsaturated fatty acids identified, respectively. Hexacosane (29.36%) is the main hydrocarbon, while a cholesterol derivative (0.3%) is the only sterol identified.

Keywords: *Ficus craterostoma, Hydrocarbons, Fatty acids, GC/MS*

INTRODUCTION

The Moraceae, often called the mulberry family or fig family, is a family of flowering plants comprising about 38 genera and over 2000 species. Most of these plants are widespread in tropical, subtropical regions and few in temperate climates[1]. *Ficus* constitutes one of the most illustrious genus in mulberry family (Moraceae), with more than 1000 species of trees and shrubs in the tropical and sub-tropical regions worldwide[2]. It is one of the most diverse plant genera in regard to its growth habit with both deciduous and evergreen free-standing trees, stranglers, climbers, creepers and small shrubs[3].

In Egypt, many *Ficus* species are widely distributed in streets, gardens, parks and outside the canal banks. Many researches on *Ficus* have focused on its edible part (fruits) followed by aerial roots and barks while the leaves are rarely studied compared with other parts[4]. *Ficus* species were traditionally used in African folk medicine in the treatment of many illnesses such as convulsions and respiratory disorders [5]. Also, they were reported to have hypotensive, antidiabetic, mild laxative, antirheumatic and digestive activities[6]. Externally, they have been used to treat postulous, eczema, cure tinea and leprosy, treat cracks in the soles of the feet, and as a dressing to boils[7,8]. A variety of chemical constituents, such as triterpenes of different types, sterols, flavonoids, coumarins, alkaloids and other miscellaneous compounds have been reported in the genus *Ficus*[9,10].

F. craterostoma is a member of Moraceae; deciduous or evergreen trees and shrubs, distributed mostly in tropical and subtropical regions[11,12]. Since nothing has been reported concerning the study of lipoidal matter of *F. craterostoma* together with its valuable importance have encouraged the authors to undertake this study.

MATERIALS AND METHODS

Apparatus

Agilant 6890 gas chromatograph (Faculty of Agriculture, Cairo University, Egypt) equipped with an Agilant mass spectrometric flame ionization detector (FID), thermo scientific capillary column (5% Phenyl Polysil Phenyl-Siloxane); (30 m X 0.25 mm X 0.25 µm film thickness), using helium at 1ml/min flow rate. The injector and detector temperature were 250°C while ion source temperature was 180°C. The ionization voltage was kept 70eV during the experiment.

Plant material

Ficus craterostoma Mildbr & Burret leaves were collected from the Botanical Garden of Mohamed Ali's Museum in the Experimental Station of Medicinal Plants, Giza, Egypt during April 2013. The plant was kindly identified by Dr. Ibrahim Ahmed El-Garf, Associate Professor of Plant Taxonomy, Faculty of Science, Cairo University, Giza, Egypt. Voucher specimens were kept in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Helwan University, Egypt.

Authentic reference materials

Hydrocarbons, triterpenes and sterols

Octodecane, pentatriacontane, eicosane, hexacosane, tetracosane, heneicosane, hexatriacontane, phytol, isophytol, squalene, *β*-amyrin, cholesterol and cholesterol derivatives were obtained from Principal Laboratory, Faculty of Agriculture, Cairo University, Egypt.

Fatty acids

Tridecanoic, myristic, stearic, linoleic, linolenic, palmitic acids were obtained from Principal Laboratory, Faculty of Agriculture, Cairo University, Egypt.

Preparation of lipoidal matter

About 100 g of the air-dried powdered *Ficus craterostoma* leaves were exhaustively extracted with *n*-hexane. The solvent was evaporated at 40°C under reduced pressure to yield 10 g lipoidal matter[13].

Saponification of lipoidal matter

Two grams of the lipid sample was saponified by refluxing with 50 ml alcoholic potassium hydroxide (10%) for two hours. After cooling and dilution with water, the unsaponifiable matter was extracted with diethylether, dried over anhydrous sodium sulfate and evaporation of ether afforded (1.1 g) residue of unsaponifiable matter (USM). The soapy aqueous layer of saponifiable matter was acidified with 2N HCL and the liberated fatty acids were extracted with ether, and then washed with water until neutralization, dried over anhydrous sodium sulfate followed by evaporation of ether afforded total fatty acids (TFA) residue (0.22 g) [14].

Preparation of fatty acid methyl esters

The free fatty acids (0.22 g) were subjected to methylation by refluxing with 100 ml of absolute methanol and 5 ml sulphuric acid for one hour then extracted with diethyl ether, dried over anhydrous sodium sulfate followed by evaporation to give fatty acid methyl esters[15].

Identification of USM and TFA

The unsaponifiable matter and total fatty acid methyl esters are subjected to GC/MS analysis. The identification of hydrocarbons, sterols and fatty acids were carried out by comparing the relative retention times of the peaks with those of the pure available authentics. Quantitative estimation was done by peak area measurement using a computing integration. The results are shown in **Tables 1, 2** and **Figures . 1, 2**

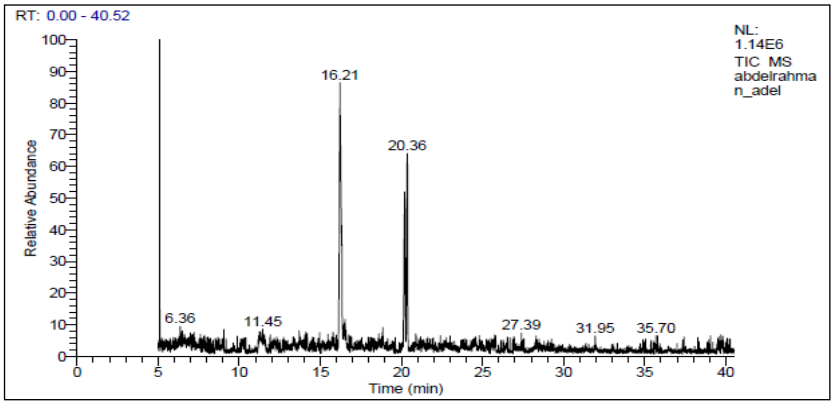

Figure 1. GLC chromatogram of saponifiable matter of *Ficus craterostoma* leaves

Table 1: Result of GC/MS analysis of the fatty acid methyl esters of the saponifiable fraction of the *n*-hexane extract of *Ficus craterostoma* leaves

RRT* (min)	Compound	Area %
0.69	Tridecanoic acid C13:0	2.74
0.70	Tetradecanoic acid (Myristic acid) C14:0	2.56
1	Hexadecanoic acid (Palmitic acid) C16:0	59.71
1.25	9,12Octadecadienoic acid (Linoleic acid) C18:2	10.64
1.26	9,12,15 Octadecatrienoic acid (Linolenic acid) C18:3	11.59
1.29	Octadecanoic acid (Stearic acid) C18:0	1.08
Saturated fatty acid		66.09%
Unsaturated fatty acid		22.23%
Unidentified compounds		11.68%

*RRT: Relative retention time to palmitic (Rt = 16.21 min)

RESULTS

Saponification of *n*-hexane extract of *F. craterostoma* leaves yielded 55% and 11% for unsaponifiable matter and fatty acids, respectively. The content of saturated fatty acids (66.09%) identified is higher than unsaturated fatty acids (22.23%); where palmitic acid (59.71%), linolenic acid (11.59%) are the main identified saturated and unsaturated fatty acids, respectively are shown in table1, fig.1. Total identified hydrocarbons were 10 compounds, representing 72.29% of the total identified unsaponifiable compounds of the plant extract, mainly attributed to hexacosane (29.36%) and hexatriacontane (25.65%). Four terpenes were identified constitute (18.44%) of total unsaponifiable compounds, mainly attributed to phytol (12.41%) while a cholesterol derivative (0.3%) is the only sterol identified (**Table 2, Fig. 2**).

A wide range of bioactivities of palmitic acid are reviewed which suggest its useful medicinal properties with diversity of action against different diseases. This compound is reported to have antibacterial, antifungal[16], antioxidant[17], anti-inflammatory, hypocholesterolemic effects[18].

Linolenic acid is an immense bioactive compound having antimicrobial[16], antimalarial[19], antidiabetic, anticancer, antiarteriosclerotic effects and used in treatment of obesity[20].

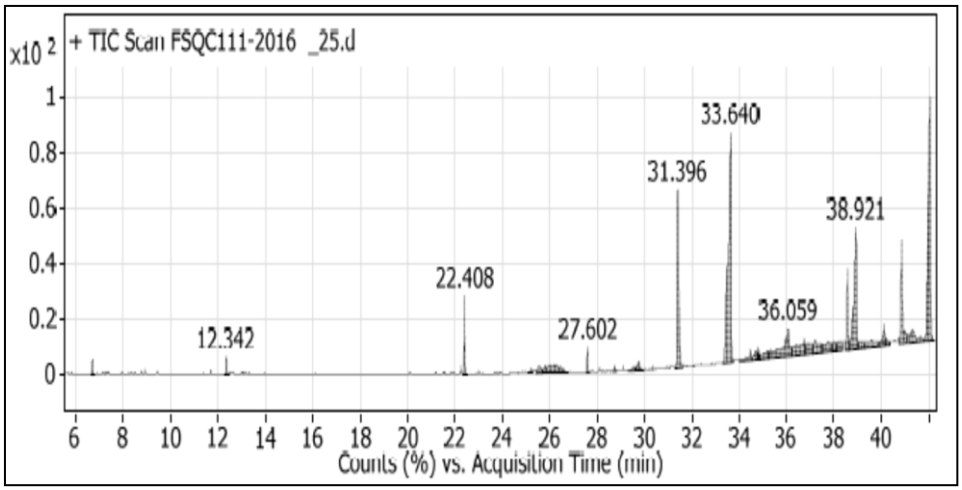

Figure 2. GLC chromatogram of unsaponifiable matter of *Ficuscraterostoma* leaves

Table 2. Result of GC /MS analysis of the unsaponifiable fraction of the *n*-hexane extract of *Ficus craterostoma* leaves

RRT* (min)	Compound	Molecular formula	Area %
0.265	1,3-dimethyl benzene	C_8H_{10}	**0.16**
0.759	Octadecane	$C_{18}H_{38}$	**0.71**
0.767	Pentatriacontane	$C_{35}H_{72}$	**0.16**
0.835	1,1'-(2-propyl-1,3-propanediyl)bis-cyclohexane	$C_{18}H_{34}$	**0.20**
0.885	Eicosane	$C_{20}H_{42}$	**0.84**
1	**Hexacosane**	$C_{26}H_{54}$	**29.36**
1.146	Tetracosane	$C_{24}H_{50}$	**3.12**
1.156	**Heneicosane**	$C_{21}H_{44}$	**11.74**
1.249	n-Hexatriacontane	$C_{36}H_{74}$	**25.65**
0.933	**Phytol**	$C_{20}H_{40}O$	**12.41**
0.864	**Isophytol**	$C_{20}H_{40}O$	**0.17**
1.214	**Squalene**	$C_{30}H_{50}$	**5.50**
1.242	**β-Amyrin**	$C_{30}H_{50}O$	**0.36**
1.033	**8,24-dien-3-ol, 4-methyl-, (3.beta.,4.alpha.)-cholesterol**	$C_{28}H_{46}O$	**0.3**
Total hydrocarbons			**72.29%**
Total sterols			**0.3%**
Total terpenes			**18.44%**

*RRT: Relative retention time to hexacosane (Rt = 33.640 min).

CONCLUSION

The investigation of lipoidal matter of *Ficus craterostoma* leaves was carried out by GC/MS analysis that revealed the presence of bioactive lipoidal compounds that make the plant have great potential as a source for natural health product. This is the first record for lipoidal investigation of *Ficus craterostoma* leaves growing in Egypt.

Conflict of Interest

The authors declare that they don't have any conflict of interest.

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