ISSN: 2357-0547 (Print)
ISSN: 2357-0539 (Online)

Short Communication / JAPR / Sec. A
Mohammed et al., 2021, 5 (4), 362-370

Journal of Advanced Pharmacy Research

Section A: Natural Products & Metabolomics

Comparative Lipoidal Matter Investigation of Three *Abutilon* Species Aerial Parts

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Submitted on: 06-07-2021; Revised on: 30-07-2021; Accepted on: 02-08-2021

To cite this article: Mohammed, N. M.; Kamal, A. M.; Abdelhady, M. I. S.; Haggag, E. G. Comparative Lipoidal Matter Investigation of Three *Abutilon* Species Aerial Parts *J. Adv. Pharm. Res.* **2021**, *5* (4), 362-370. DOI: 10.21608/aprh.2021.000000000

ABSTRACT

Objectives: In the present study, a comparative evaluation of the lipoidal matter of the aerial parts (leaves and stems) of three *Abutilon* (*A.*) species, *A. pannosum* (Forst.f) Schltdl., *A. hybridum* Hort. (Ex Siebert. & Voss), and *A. hirtum* (Lam.) Sweet (family Malvaceae) has been carried out for the first time. **Methods**: Gas-liquid chromatography coupled with flame ionization detector (GLC/FID) was used for analyzing and identifying the saponifiable and unsaponifiable compounds. **Results:** Saponification of lipoidal matter of the petroleum ether extracts of *A. pannosum*, *A. hybridum*, and *A. hirtum* revealed the presence of 23 compounds tentatively identified in the unsaponifiable matter of *A. pannosum*, 19 in *A. hybridum*, and 22 compounds in *A. hirtum.* Whereas *n*-heneicosane, *β*-sitosterol, *n*-tricosane were the most abundant compounds identified in the unsaponifiable matter of the aerial parts of *A. pannosum*, *A. hybridum* and *A. hirtum*, respectively. In the saponifiable fractions, a total of 14 compounds were specified for *A. pannosum*, 18 for *A. hybridum* and 19 compounds for *A. hirtum*. Palmitic acid was the major identified saturated fatty acid in the three *Abutilon* species accounting for 23.18% for *A. pannosum*, 23.97% for *A. hybridum*, and 19.23% for *A. hirtum*, while oleic acid represents the major unsaturated fatty acid in the three species (17.78, 34.44 and 22.13%, respectively). **Conclusion:** Lipoidal matter investigation by Gas-liquid chromatography coupled with flame ionization detector (GLC/FID) was used for the tentative identification of different bioactive lipoidal constituents as hydrocarbons, sterols, unsaturated and saturated fatty acids from *A. pannosum*, *A. hybridum*, and *A. hirtum.* The results showed that the three *Abutilon* species are worthy candidates for further pharmacological and phytochemical studies. The present study is the first report on the petroleum ether extracts of *A. hybridum*, *A. hirtum* plants and the first report on the aerial parts of *A. pannosum*.

Keywords: *Abutilon pannosum; A. hybridum; A. hirtum; Lipoidal matter; Aerial parts.*

INTRODUCTION

Plants have evolved and adapted over millions of years to produce unique, structurally diverse secondary, bioactive metabolites. Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries. The ingredients present in the plant play a significant role in the identification of crude drugs[1].

Malvaceae (Mallow Family) is a family of flowering plants containing about 243 genera and 4225 species. The plants of this family are mainly herbs, shrubs, trees, and they are widely distributed throughout the world and particularly in tropical regions, mainly in

South America [2-3]. *Abutilon* is one of the important genera of this family, various plants of *Abutilon* species are traditionally used in the treatment of inflammation, piles, gonorrhea, bronchitis, diarrhea, cleaning wounds, and ulcers[4]. The significant importance of the genus is attributed to valuable fibers obtained from different species of the genus and also due to several species is grown as garden ornamentals. Phytochemical studies of the genus revealed the presence of flavonoids, sterols, triterpenes, anthocyanins, and fatty acids[5-6]. *Abutilon pannosum* (Forst.f) Schltdl. is a tomentose undershrub widely distributed in India, North Africa, Asia, and Australia[7-8]. Important phytochemicals have been reported in the petroleum ether extracts of *A. pannosum* leaves like alkaloids, fatty acids, steroidal lipids, and heterocyclic compounds[9]. *A. hirtum* (Lam.) Sweet is a perennial herb or shrub, distributed throughout the tropical and subtropical regions of the Indian subcontinent[10]. *A. hybridum* Hort. (Ex Siebert. & Voss) "Nabob" is evergreen shrubs that originated in tropical regions of South America[11]. *A. pannosum* aerial parts were collected from Saudi Arabia while *A. hybridum* and *A. hirtum* were collected from Egypt.

Gas liquid chromatography (GLC) is a well-established technique for lipid analysis. It is fast, reproducible, and quantitative method. A major advantage of GLC is its sensitivity and specificity; since the mobile phase is gaseous, flame ionization, and nitrogen-specific detectors can be used[12]. The Flame Ionization Detector (FID) is the most common detector paired with gas chromatography instruments for analytical applications. FID is a standard instrument used in industry for measuring hydrocarbon gas concentration. It is mass sensitive, not concentration sensitive; hence, changes in carrier gas flow rate have little effect on the detector response. It uses a flame to ionize organic compounds containing carbon such that after separation of the sample in GC column each analyte passes through a flame fuelled by hydrogen and zero air that ionizes the carbon atoms[13].

As to the best of our knowledge to date, no reported studies carried on the petroleum ether extracts of the aerial parts of these plants. This study intended to identify and compare the lipoidal matter contents of the aerial parts (leaves and stems) of the three *Abutilon* species, *A. pannosum*, *A. hybridum* and *A. hirtum*.

MATERIAL AND METHODS

Plant material

The non-flowering aerial parts of *A. hybridum* and *A. hirtum* were collected in March 2015 from El-Orman Botanical Garden, Giza, Egypt, and the non-flowering aerial parts of *A. pannosum* were collected in February 2015 from Eltaif road, Saudi Arabia. The taxonomic authentication of the three plants was performed by Dr. Thérèse Labib, Head of the Taxonomists at Al-Orman Botanical Garden, Giza, Egypt. Voucher specimens (01Apa, 02Ahy, and 03Ahi/2015) were deposited at the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Helwan University, Helwan, Cairo, Egypt.

Chemicals

Petroleum ether (60-80), 10 % alcoholic potassium hydroxide, diethyl ether, conc. hydrochloric acid, conc. sulphuric acid, methanol, diazomethane, anhydrous sodium sulphate, chloroform were supplied by the Cairo University Research Park (CURP, Faculty of Agriculture, Cairo University, Giza, Egypt). All authentic references of hydrocarbons, sterols, and fatty acids were supplied by the National Research Center (NRC, Cairo, Egypt) for the lipoidal matter analysis.

Investigation of the lipoidal matter

Preparation of the lipoidal matter[14]

The air-dried powder of *A. pannosum*, *A. hybridum* and *A. hirtum* aerial parts (80 g) each were independently exhaustively extracted with petroleum ether (60-80ºC) (5x350 ml). The extracts were filtered and evaporated individually under reduced pressure at 50ºC to give 4.3, 3.2, and 2.8 g of viscous residues of lipoidal matter respectively, which were kept in the dark for further analysis.

Saponification and fractionation of lipoidal matter[15]

The lipoidal matter prepared was individually saponified by refluxing with 50 ml of 10% alcoholic potassium hydroxide solution for 2 h followed by alcohol evaporation, dilution with distilled water, and extraction with ether exhaustively. The collected ethereal extracts were washed with distilled water until the wash was free from alkalinity, dehydrated over anhydrous sodium sulphate, and evaporated to dryness under reduced pressure to give the three residues of unsaponifiable matter (USM) 2.4, 1.7, and 1.2 g for *A. pannosum*, *A. hybridum* and *A. hirtum*, respectively.

The remaining saponifiable aqueous layer left after extraction with ether was acidified with 2 N hydrochloric acid and the liberated fatty acids were extracted exhaustively with ether. The extracts collected were separately washed with distilled water till neutralization, dried over anhydrous sodium sulfate, followed by evaporation of ether to give 1.6, 1.2, and 0.9 g, respectively, residues of total fatty acids (TFA).

Preparation of fatty acid methyl esters[16-18]

The three fatty acids dried residues of *A. pannosum*, *A. hybridum* and *A. hirtum* were separately methylated by dissolving in anhydrous methanol, followed by the addition of an ethereal solution of

Table 1. GLC analysis of USM of the aerial parts of *A. pannosum*, *A. hybridum*, and *A. hirtum*

No.	Identified HC/sterol	Molecular formula	*A. Pannosum*			*A. hybridum*			*A. hirtum*		
			RT	PA%	RRT	RT	PA%	RRT	RT	PA%	RRT
A) Hydrocarbons											
1	*n*-Octane	C_8H_{18}	2.76	0.61	0.15	-	-	-	2.26	0.71	0.13
2	*n*-Nonane	C_9H_{20}	3.48	0.62	0.19	-	-	-	3.44	0.69	0.19
3	*n*-Decane	$C_{10}H_{22}$	4.34	0.32	0.24	-	-	-	-	-	-
4	*n*-Hendecane	$C_{11}H_{24}$	4.92	0.43	0.27	-	-	-	-	-	-
5	*n*-Tetradecane	$C_{14}H_{30}$	-	-	-	-	-	-	9.08	2.97	0.51
6	*n*-Pentadecane	$C_{15}H_{32}$	10.25	1.24	0.57	10.13	0.49	0.57	10.79	1.49	0.61
7	*n*-Hexadecane	$C_{16}H_{34}$	11.72	4.08	0.65	11.55	2.57	0.65	11.52	1.30	0.65
8	*n*-Heptadecane	$C_{17}H_{36}$	12.12	1.61	0.67	12.50	4.67	0.71	12.79	5.02	0.72
9	*n*-Octadecane	$C_{18}H_{38}$	13.17	7.84	0.73	13.88	5.23	0.78	13.84	1.90	0.78
10	*n*-Nonadecane	$C_{19}H_{40}$	14.47	7.02	0.80	15.19	2.59	0.86	15.17	4.19	0.86
11	*n*-Eicosane	$C_{20}H_{42}$	16.39	2.84	0.91	16.25	5.66	0.92	16.24	2.67	0.92
12	*n*-Heneicosane	$C_{21}H_{44}$	17.97	8.31	1.00	17.70	5.38	1:00	17.70	8.65	1.00
13	*n*-Docosane	$C_{22}H_{46}$	19.56	3.74	1.09	19.60	5.63	1.11	19.34	7.83	1.09
14	*n*-Tricosane	$C_{23}H_{48}$	20.49	2.61	1.14	20.65	2.71	1.17	20.31	11.88	1.15
15	*n*-Tetracosane	$C_{24}H_{50}$	21.93	3.12	1.22	21.66	4.03	1.22	21.28	7.93	1.20
16	*n*-Pentacosane	$C_{25}H_{52}$	22.85	4.62	1.27	22.59	2.19	1.28	22.20	6.49	1.25
17	*n*-Hexacosane	$C_{26}H_{54}$	23.75	3.81	1.32	23.51	1.89	1.33	23.12	5.01	1.31
18	*n*-Heptacosane	$C_{27}H_{56}$	24.83	3.20	1.38	24.64	1.39	1.39	24.66	3.79	1.39
19	*n*-Octacosane	$C_{28}H_{58}$	25.48	2.94	1.42	25.25	2.04	1.43	25.47	2.95	1.44
B) Sterols and triterpenes											
20	Cholesterol	$C_{27}H_{46}O$	26.29	2.45	1.46	26.07	1.28	1.47	26.28	1.51	1.48
21	Campesterol	$C_{28}H_{48}O$	27.18	2.26	1.51	26.98	3.23	1.52	27.11	3.84	1.53
22	Stigmasterol	$C_{29}H_{48}O$	28.67	2.31	1.59	28.32	1.83	1.60	28.31	2.45	1.60
23	*β*-Sitosterol	$C_{29}H_{50}O$	30.39	6.69	1.69	29.02	11.37	1.64	28.93	4.68	1.63
24	*α*-Amyrin	$C_{30}H_{50}O$	32.24	0.24	1.79	30.92	2.16	1.75	30.87	0.75	1.74
Total hydrocarbons				**58.96 %**			**46.47 %**			**75.47 %**	
Total sterols and triterpenes				**13.95 %**			**19.87 %**			**13.23 %**	
Unidentified compounds				**27.09%**			**33.66 %**			**11.3 %**	

RT: Retention time; HC: Hydrocarbon; PA: peak area; RRT: relative retention time relative to n-Heneicosane with RT: 17.97, 17.7, and 17.7 min, respectively.

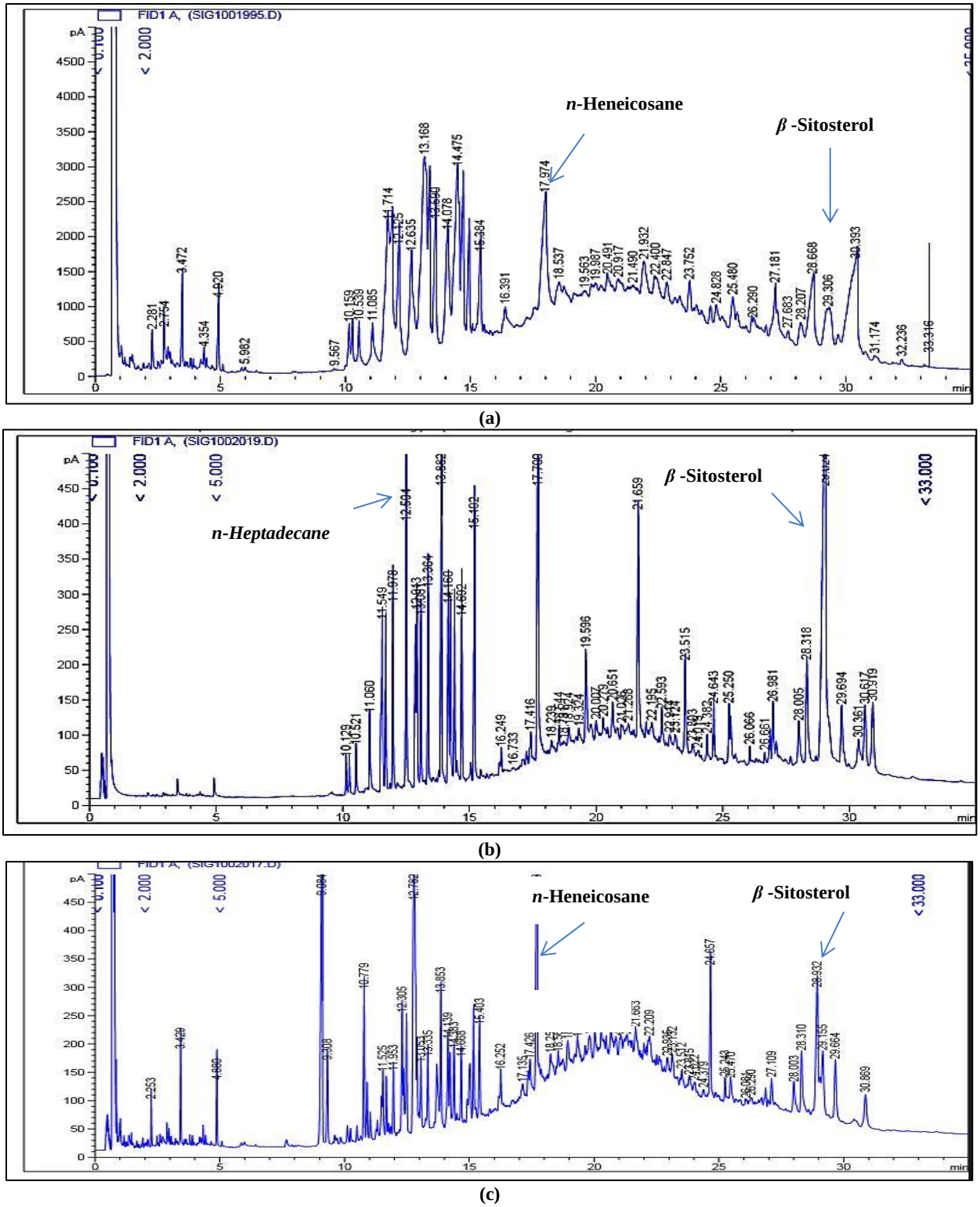


Figure 1. GLC-chromatogram of unsaponifiable matter of aerial parts of: (a) *A. pannosum,* (b) *A.hybridum* and (c) *A. hirtum.*

diazomethane portion wise until gas evolution ceased. The mixture was then left for 10 min at room temperature, evaporated under a nitrogen stream at room temperature. The fatty acids methyl esters (FAME) were dissolved by adding two drops of re-distilled chloroform solution and kept in sealed vials for GLC analysis.

GLC analysis of the unsaponifiable matter

The unsaponifiable matter was analyzed by the GLC technique by comparison with the available authentic hydrocarbons and sterols. The analysis was carried at the principal laboratory, NRC, Cairo, Egypt, on Pye UNIC GLC, series 304 equipped with a flame ionization detector (FID). A coiled glass column (UNICAM Pro GC, Portugal, 2.8 m × 4 mm) packed with Diatomite (100 - 120 mesh) and coated with 3% Ohio Valley (OV-17) was used for the chromatographic separation. The oven temperature was programmed at 10ºC/min from 70ºC, then 270ºC for 25 min. The nitrogen flow rate was adjusted to 30 ml/min. The detector, injector temperatures, and hydrogen, air flow rates were generally adjusted to 300ºC, 280ºC, and 33 ml/min, 330 ml/min, respectively.

GLC analysis of the FAME

GLC analysis of FAME was done at the principal laboratory, NRC, Cairo, Egypt using almost the same parameters as in unsaponifiable investigation except for the usage of a coiled glass column (UNICAM Pro GC, Portugal, 1.5 m × 4 mm) packed with diatomite (100 - 120 mesh) and coated with 10% polyethylene glycol adipate (PEGA). The column oven temperature was programmed at 8 ºC/min from 70ºC to 190ºC, then isothermally at 190ºC for 25 min with nitrogen flow rate at 30 ml/min.

Identification of USM and TFA

The peaks of the pure available authentic reference standards were used to confirm the identity of the hydrocarbons, sterols, and fatty acids by comparing them with the relative retention times of the recorded peaks. Quantitative estimation was done by peak area measurement using computing integration software (PU 4810, Philips Egypt).

RESULTS

The results of the GLC analysis of the unsaponifiable matter of the three *Abutilon* species *pannosum*, *hybridum*, and *hirtum* aerial parts revealed tentative identification of 23 compounds in the unsaponifiable matter (USM) of *A. pannosum* accounting for 58.96% as hydrocarbons and 13.95% sterols and triterpenes, 19 compounds in *A.hybridum* accounting for 46.47% as hydrocarbons and 19.87% sterols and triterpenes and 22 compounds in *A. hirtum* accounting for 75.47% as hydrocarbons and 13.23% sterols and triterpenes. The most abundant hydrocarbon identified in the aerial parts of *A. pannosum* was *n*-heneicosane (21 straight-chain hydrocarbons) representing 8.31% of the USM, *n*-eicosane in *A. hybridum* 5.66%, and *n*-tricosane in *A. hirtum*11.88%. Four sterols; cholesterol, campesterol, stigmasterol, and *β*-sitosterol were identified in the three USM of *A. pannosum*, *hybridum*, and *hirtum Abutilon* species. *β*-sitosterol was the major sterol identified in the three *Abutilon* species representing 6.69, 11.37, and 4.68 %, respectively. Besides, *α*-amyrin was the only identified *triterpene* in the three *Abutilon* species, *pannosum*, *hybridum*, and *hirtum*, representing 0.24, 2.16, and 0.75%, respectively. The results are shown in **Figure 1 (a, b, and c)**, **Figure 2** and summarized in **Table 1**.

Results of GLC analysis of the FAME (saponifiable matters) of the three *Abutilon* species *pannosum*, *hybridum*, and *hirtum* aerial parts are shown in **Figure 3 (a, b, and c),** represented in **Figure 4** and summarized in **Table 2**. A total of 14 compounds were specified in the saponifiable fraction of *A. pannosum*, 18 for *A. hybridum*, and 19 compounds for *A. hirtum*. The saturated fatty acids in *A. pannosum* (48.35%) represent a higher percentage than that of unsaturated ones (35.25%), while in *A.hybridum* and *A.hirtum* the unsaturated fatty acids (51.8% and 48.14%, respectively) represent a higher percentage than that of saturated ones (39.09% and 40.54%, respectively). The major identified saturated fatty acid in the three *Abutilon* species is palmitic acid accounting for 23.18% for *A. pannosum*, 23.97% for *A. hybridum*, and 19.23% for *A. hirtum*, while oleic acid represents the major unsaturated fatty acid in three *Abutilon* species *pannosum*, *hybridum*, and *hirtum* (17.78, 34.44 and 22.13%, respectively).

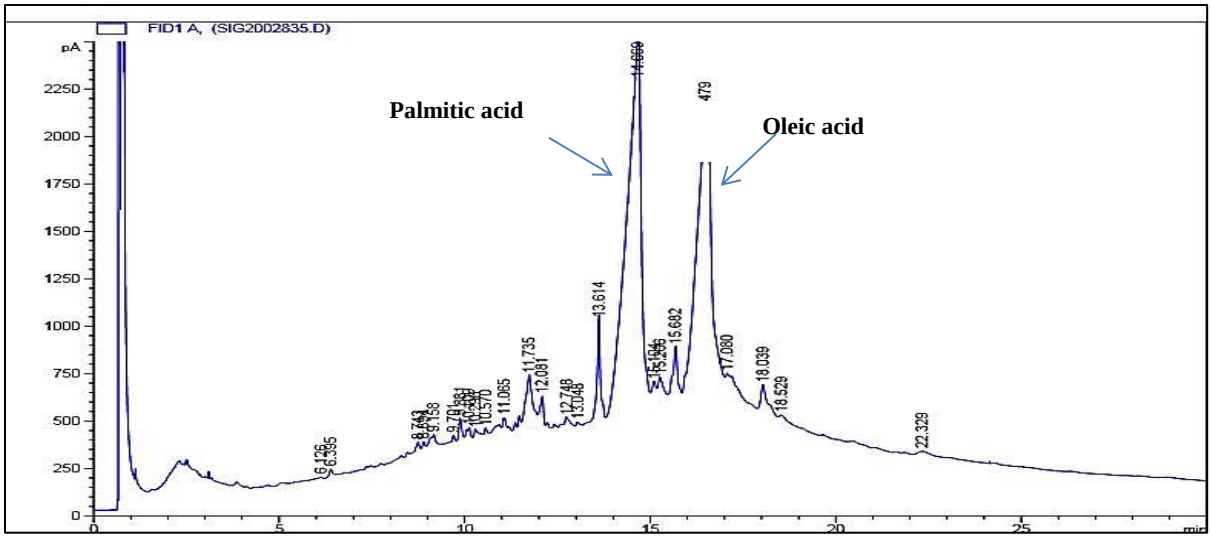
DISCUSSION

The qualitative identification of the lipoidal matter of the petroleum ether extracts of *A. pannosum*, *A. hybridum*, and *A. hirtum* using GLC-analysis revealed the presence of the hydrocarbons, sterols, unsaturated and saturated fatty acids by comparing the relative retention times of the identified peaks with those of the authentic available reference standards, while relative peak area integration method was used for the quantitative estimation. The results showed that the most abundant hydrocarbon identified in USM of the aerial parts of *A. pannosum* was *n*-heneicosane which was found to have antibacterial and larva growth inhibition effects[19], *n*-eicosane in *A. hybridum* was reported as antifungal compound and can be extracted and purified for obtaining novel antibiotic compounds to treat human pathogenic bacterial infection[20-21], while the *n*-tricosane (the most abundant hydrocarbon identified in the *A. hirtum* USM) was found to have antibacterial effect[22]. Steroids identified in the three *Abutilon* species as

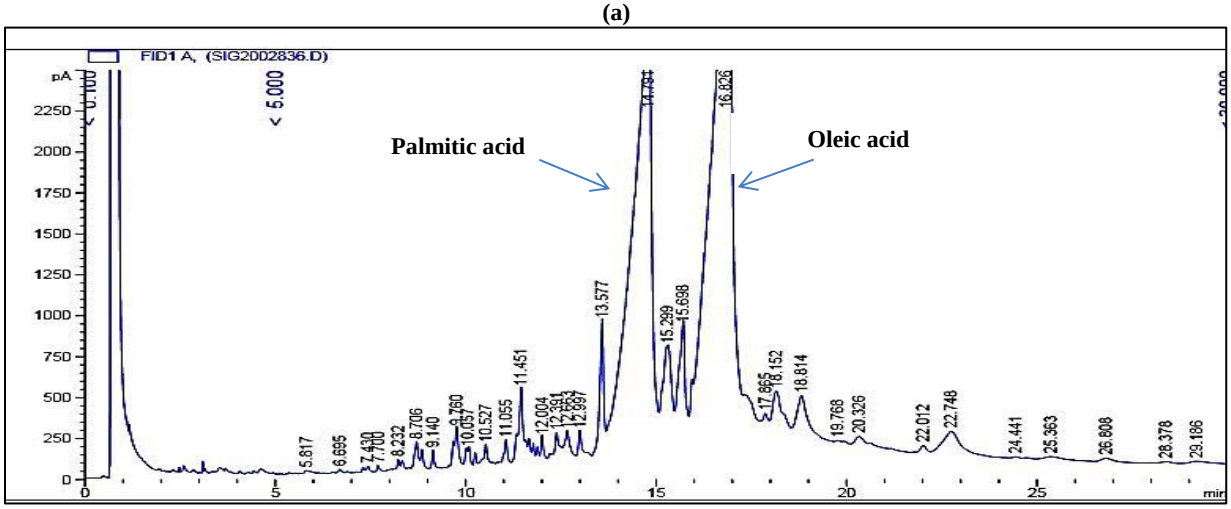
Table 2. GLC-analysis of the saponifiable fraction (FAME) of the aerial parts of *A. pannosum*, *A. hybridum*, and *A. hirtum*

No.	Fatty acids	C. No.: No. of d.b	*A. pannosum*			*A. hybridum*			*A. hirtum*		
			RT	PA%	RRT	RT	PA%	RRT	RT	PA%	RRT
1	Caprylic	8:0	-	-	-	5.82	0.10	0.34	-	-	-
2	Capric	10:0	-	-	-	7.43	0.20	0.44	-	-	-
3	Undecylic	11:0	8.74	3.84	0.53	8.70	0.62	0.52	8.74	1.49	0.53
4	Lauric	12:0	9.70	6.02	0.59	9.76	2.10	0.58	9.71	3.28	0.59
5	Tridecylic	13:0	11.06	2.63	0.67	11.05	0.56	0.66	11.08	1.26	0.67
6	Myristic	14:0	11.73	3.85	0.71	11.45	1.83	0.68	11.47	2.76	0.69
7	Myristoleic	14:1	12.08	1.86	0.73	12.00	0.57	0.71	-	-	-
8	Pentadecylic	15:0	12.75	3.78	0.77	12.66	2.50	0.75	12.44	5.23	0.75
9	Palmitic	16:0	14.67	23.18	0.89	14.79	23.97	0.88	14.44	19.23	0.87
10	Palmitoleic	16:1	15.27	3.38	0.93	15.30	3.25	0.90	15.11	2.40	0.91
11	Stearic	18:0	15.68	3.06	0.95	15.70	2.96	0.93	15.64	3.40	0.94
12	Oleic	18:1	16.48	17.78	1.00	16.83	34.44	1.00	16.56	22.13	1.00
13	Linoleic	18:2	18.04	8.29	1.09	18.15	3.02	1.08	18.02	7.40	1.09
14	Linolenic	18:3	19.65	2.52	1.19	19.77	5.50	1.17	19.20	8.82	1.16
15	Arachidic	20:0	20.46	1.99	1.24	20.33	3.60	1.20	20.50	2.15	1.24
16	Paullinic	20:1	21.04	1.42	1.28	-	-	-	21.11	2.32	1.27
17	Eicosadienoic	20:2	-	-	-	22.75	3.86	1.35	22.75	1.24	1.37
18	Arachidonic	20:4	-	-	-	-	-	-	23.32	1.93	1.41
19	Eicosapentaenoic	20:5	-	-	-	-	-	-	24.58	1.29	1.48
20	Behenic	22:0	-	-	-	-	-	-	25.64	0.94	1.55
21	Lignoceric	24:0	-	-	-	28.38	0.65	1.69	28.13	0.80	1.70
22	Nervonic	24:1	-	-	-	29.19	1.16	1.73	29.16	0.61	1.76
	Saturated fatty acid			**48.35%**			**39.09%**			**40.54%**	
	Unsaturated fatty acid			**35.25%**			**51.8%**			**48.14%**	
	Unidentified compounds			**16.4%**			**9.11 %**			**11.32**	

RT: Retention time; HC: Hydrocarbon; PA: peak area; C. No.: number of carbon; No. of d.b: number of double bonds; RRT: relative retention time relative to oleic acid with RT: 16.48, 16.83, and 16.56 min, respectively.



(a)



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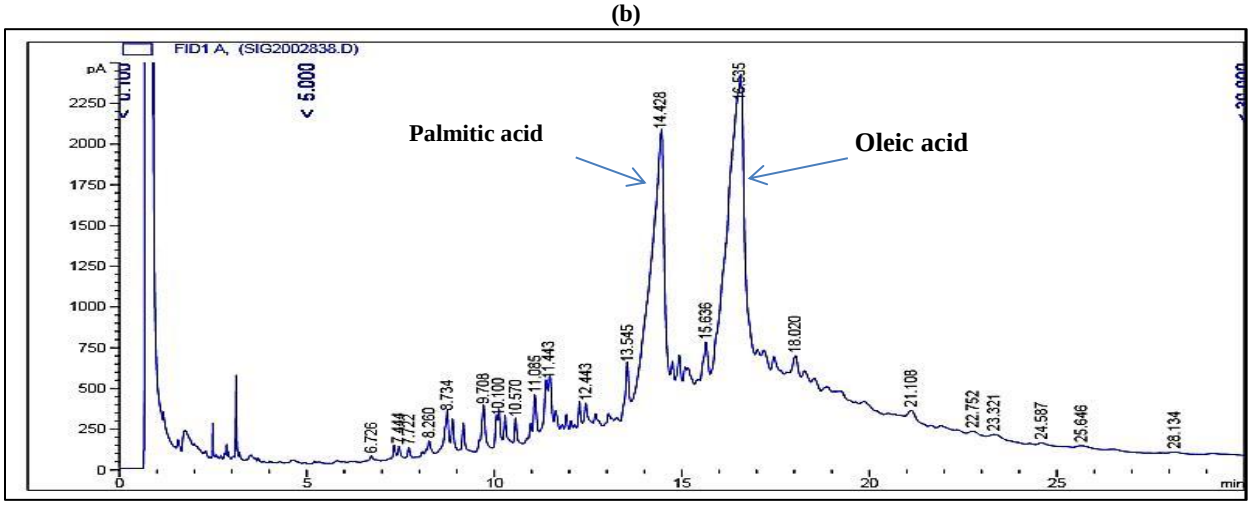

(c)

Figure 3 (a, b and c): GLC-chromatogram of the saponifiable fraction of aerial parts of: (a) *A. pannosum,* (b) *A.hybridum* and (c) *A. hirtum.*

stigmasterol, *β*-sitosterol, and campesterol are considered to have miscellaneous biological activities including anti-inflammatory, anti-oxidant, anti-carcinogenic activities, and also have cholesterol-lowering effect[23-24]. The major sterol identified in the three *Abutilon* species, *β*-sitosterol exhibited significantly antidiabetic activity, and this activity was measured on the basis of the glucose uptake in yeast cells. The results obtained revealed that *β*-sitosterol exhibited significantly antidiabetic activity at all glucose concentrations[25]. Additionally, previous studies on the pentacyclic *triterpene* found in the three *Abutilon* species, *α*-amyrin showed significant anti-inflammatory activity through a reduction of COX-2 expression[26].

The most abundant saturated fatty acid present in the three *Abutilon* species, palmitic acid was reported to have antibacterial, antifungal, antioxidant anti-inflammatory, hypocholesterolemic effects[27-29], while the oleic acid which represents the major unsaturated fatty acid could help in preventing the progression of metastasis in several human cancers and reduce the risk of developing certain types of cancer, especially colorectal, breast and prostate cancer. Oleic acid was found to be able to attenuate palmitic acid hepatotoxicity, also plays an important role in the treatment of heart disease[30-32].

From the previous data it can be shown that the *A. pannosum* aerial parts (collected from Saudi Arabia) represents a higher percentage of saturated fatty acids than that of unsaturated ones while in *A.hybridum* and *A.hirtum* (which were collected from Egypt) the unsaturated fatty acids represent a higher percentage of unsaturated fatty acids than that of saturated ones. There is a well-established consensus that replacing saturated fats in the diet with oleic acid or other unsaturated fats contributes to the maintenance of normal blood cholesterol levels[32].

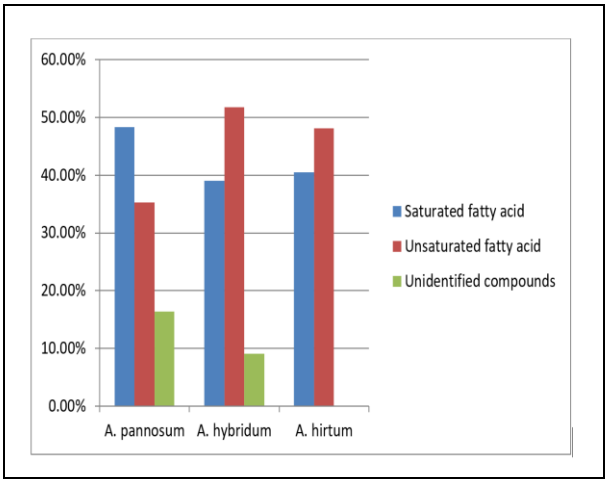

Figure 4. FAME percentage of the aerial parts of the three *Abutilon* species.

CONCLUSION

Lipoidal matter investigation by Gas-liquid chromatography coupled with flame ionization detector (GLC/FID) was used for the tentative identification of different bioactive lipoidal constituents as hydrocarbons, sterols, unsaturated and saturated fatty acids from *A. pannosum*, *A. hybridum*, and *A. hirtum* which suggest that the three *Abutilon* species are worthy candidates for further pharmacological and phytochemical studies. The present study is the first report on the petroleum ether extracts of *A. hybridum*, *A. hirtum* plants and the first report on the aerial parts of *A. pannosum.*

Conflict of interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

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