ejpmr, 2015,2(7), 57-63

SJIF Impact Factor 2.026

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article
ISSN 3294-3211
EJPMR

ANTIMICROBIAL AND GC/MS STUDIES FOR SAPONIFIABLE MATTER AND VOLATILE OIL OF *MARKHAMIA PLATYCALYX* LEAVES

Basma Khalaf Mahmoud, Ashraf Nageeb El-Sayed Hamed*, Mamdouh Nabil Samy, Amira Samir Wanas, Mohamed Salah Kamel

Pharmacognosy Department, Faculty of Pharmacy, Minia University, 61519 Minia, Egypt.

***Correspondence for Author: Dr. Ashraf Nageeb El-Sayed Hamed**
Pharmacognosy Department, Faculty of Pharmacy, Minia University, 61519 Minia, Egypt.

Article Received on 29/09/2015 **Article Revised on 22/10/2015** **Article Accepted on 15/11/2015**

ABSTRACT

Bignoniaceae Juss. (Bignonia family) includes many genera of high economic and medicinal values. It comprises 104 genera and 860 species**.** *Markhamia* genus is traditionally used in the treatment of several diseases. Literature survey showed few chemical works on the *Markhamia platycalyx* and nothing could be found about the antimicrobial study. This provoked us to carry out an extensive study of this plant including GC/MS for saponifiable matter and volatile oil. The main recognized fatty acids were linolenic (44.66%), followed by palmitic (30.63%) of the saponifiable matter composition. While, *E*-phytol was the major identified compound of the volatile oil composition (12.95%). Moreover, this study included the antimicrobial activity of the volatile oil. It exhibited a potent antibacterial effect against *E. coli* with MIC 7.48 µg/ml compared to the standard antibiotics MICs, amikacin (28.96 µg/ml) and gentamycin (26.44 µg/ml).

KEYWORDS: *Markhamia platycalyx*; Bignoniaceae; antimicrobial; volatile oil; saponifiable matter; GC/MS.

INTRODUCTION

Bignoniaceae Juss. (Bignonia family) is rich in secondary metabolites viz., iridoids, lignans, flavonoids, triterpenes in addition to other phytochemical constituents (Abdel-Wahab et al, 2014). It includes many genera of high economic and medicinal values (Abdel-Wahab et al, 2014). It varies from trees, to shrubs or lianas and rarely herbs (Ugbabe et al, 2008). It comprises 104 genera and 860 species, according to the last taxonomic revision (Fischer et al, 2004). *Markhamia platycalyx* Sprague, synonym*; Dolichandrone platycalyx* belongs to family Bignoniaceae . Many species of *Markhamia* are traditionally used in the treatment of several diseases viz., *M. zanzibarica* used in toothache, headache and back pains (Khan and Mlungwana, 1999). Moreover, *M. tomentosa* bark used in treatment of dysentery, worm infestation and migraine (Nia et al, 1997). Furthermore, *M. lutea* root alleviate symptoms of watery, bloodless diarrhea (Kernan et al, 1998), while the foliage of *M. platycalyx* are important food of the red colobus monkeys (Baranga, 1983), indicating the safety margin of this plant. Literature survey showed few chemical works on the *M. platycalyx*, in which three quinones, lapachol, *β*-lapachone and dehydro-iso-*α*-lapachone, two lignans *d*-sesamin and paulownin, in addition to stigmasterol from the hexane extract of the *M. platycalyx* heartwood (Joshi et al, 1985). Nothing could be found about the antimicrobial study. This provoked us to carry out an extensive studies of this plant including characterization of the saponifiable matter of the plant. Moreover, investigation of volatile oil constituents of the leaves. Finally, evaluation of the antibacterial and antifungal activities of the volatile oil.

MATERIALS AND METHODS

Plant material

The plant material used in this work consisted of *M. platycalyx* Sprague leaves, cultivated in El-Zohria botanical garden, Giza, Egypt. It was collected in May 2012. The plant was identified by Dr. Mamdouh Shokry, Director of El- Zohria Botanical Garden, Giza, Egypt. A voucher specimen (Mn-Ph-Cog-015) was kept in the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Minia University.

Preparation of the saponifiable matter

1-Preparation of the unsaponifiable matter

The dried petroleum ether extract (2.0 g) of the air-dried powdered leaves of *M. platycalyx* was subjected to alkali hydrolysis by refluxing with 50 ml of N/2 alcoholic potassium hydroxide for eight hrs on a boiling water bath. The major part of the alcohol was distilled off and the liquid left was diluted with twice its volume of distilled water, extracted with several portions of dichloromethane (DCM) until exhaustion. The combined DCM extracts were washed with sodium hydroxide solution (5%), then with distilled water until the washings were free from any alkalinity. The DCM residue was dehydrated over anhydrous sodium sulphate and then the DCM was distilled off. The obtained residue

(0.6 g) was brown in color (Refaat et al, 2011; Johnson and Davenport, 1971).

2-Preparation of the fatty acids

The alkaline aqueous solution (soap), remained after removal of the unsaponifiable matter was acidified with sulphuric acid (10%). The liberated fatty acids were extracted with successive portions of DCM. The combined DCM extracts were washed with distilled water till the washing was neutral to litmus paper. The DCM was distilled off and the residue of the total fatty acids (saponifiable matter) was dried. It was semisolid and brown in color (El-Said and Amer, 1965).

3-Preparation of the fatty acids methyl esters

The fatty acids were converted to their methyl esters by refluxing with 50 ml of methanol in presence of 1.5 ml of sulphuric acid for 2 hrs on boiling water bath. The major part of the alcohol was distilled off and the liquid remained was diluted with twice its volume of distilled water, extracted with several portions of DCM until exhaustion. The collective DCM extracts were washed with distilled water till the washing became neutral to litmus paper. The DCM was distilled off and the remaining residue represented the fatty acids methyl esters, was dried over anhydrous sodium sulphate. It was semisolid and brownish yellow in color. This residue was reserved for further investigation (Jonson and Davenport, 1971).

Preparation of the volatile oil

M. platycalyx leaves volatile oil (V.O.) was prepared according to the method mentioned in the Egyptian pharmacopeia. The fresh cutted leaves (100 g) were subjected to water distillation, using Clevenger apparatus for V.O. lighter than water and the heating was continued for seven hrs till exhaustion. The obtained product was an emulsion of oil droplets in the water and then the oil was extracted with diethyl ether and dehydrated over anhydrous sodium sulphate. The solvent was evaporated at room temperature and the oil was kept in cooled, dry, amber glass bottles for GC analysis. (yield: 0.15%, v/w).

GC/MS analysis

1-Fatty acid methyl esters

The used column was 0.25 mm in internal diameter, 30 m length, packed with Rtx-MS and 0.25 µm film thickness. The injected volume was 1 µl, using helium as carrier gas at flow rate 40 ml/min. The analysis was carried out at a programmed temperature; the initial temperature was 70 °C then increased at a rate 30-50 °C to the final temperature 220 °C (kept for 5 min). Injector and detector had the same temperature 240 °C. The total run time was 26 min and split ratio 1:50.

2-Volatile oils

The analysis was performed with the same conditions for fatty acid methyl esters, except the injector and detector temperature was 230 °C. The initial temperature was 40 °C (kept for 2 min) then increased to the final temperature (210 °C) (kept for 5 min). The total run time was 26 min with splitless ratio.

3-Mass analysis

Total ion chromatograms and mass spectra were recorded in the electron impact ionization mode at 70 eV, using ACQ Mode of scan from 35 to 500 m/z in 0.3 s.

4-Equipments

Shimadzu GC/MS with Head Space system provided by FID (Flame Ionization Detector), connected to the Mass Spectrometer Model: QP2010 Ultra, Rotary evaporator (BÜCHI R-144, Switzerland), (HEIDOLPH 4000, Germany), Water distiller (BHANU Basic/PH4 MK-I, India) and Water bath (BÜCHI B466, Switzerland).

Microbiological study of the volatile oil

Microbial strains

Test organisms used in this study include:

1-Bacterial strains

Staphylococcus aureus (*S. aureus*) [Gram positive, Facultative anaerobic bacteria]. *Escherichia coli* (*E. coli*) and *Klebsiella pneumonia* (*K. pneumonia*) [Gram negative, Facultative anaerobic bacteria].

2-Fungal strains

Candida krusei (C. krusei), Candida glabrata (C. glabrata) and Candida albicans (C. albicans). All the bacterial and fungal strains used in the study were clinical isolates obtained from Microbiology Department, Faculty of Pharmacy, Minia University. Bacterial strains were cultured on Mueller Hinton agar and fungal strains were cultured on Sabouraud agar.

Screening of the antimicrobial activity

The cultures were adjusted to 0.5 ml of 1x 106 CFU/m1 (0.5 Mcfarland turbidity). The sterile, molten and cooled media (15 ml) of either Mueller Hinton agar or Sabouraud agar was added to the petri dishes then the plates were rotated slowly and allowed to solidify on flat surface. The media are then inoculated with the microorganisms using a sterile swab to evenly distribute bacterial or fungal culture over the appropriate medium. The plates were allowed to dry for 15 min before use then four equidistant and circular wells of 10 mm diameter were carefully punched into the agar medium using a sterile cork borer (Gemeda et al, 2008; Delahaye et al, 2009). The wells were then filled with 100 µl of 7 mg/ml of volatile oil in addition to solvent blank then the plates were allowed to stand for one hr to allow the prediffusion then they were incubated overnight at 37 °C after that the antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism (Ogbulie et al, 2007; El-Kashef, 2014).

RESULTS

GC/MS analysis of fatty acid methyl esters

Identification of fatty acids methyl esters was carried out by direct comparison of retention time and fragmentation

pattern of each of the separated compounds with archive mass spectra lipid library (Archive of Mass Spectra). The quantitation was based on peak area integration. The results are illustrated in Fig. 1 and listed in Table 1.

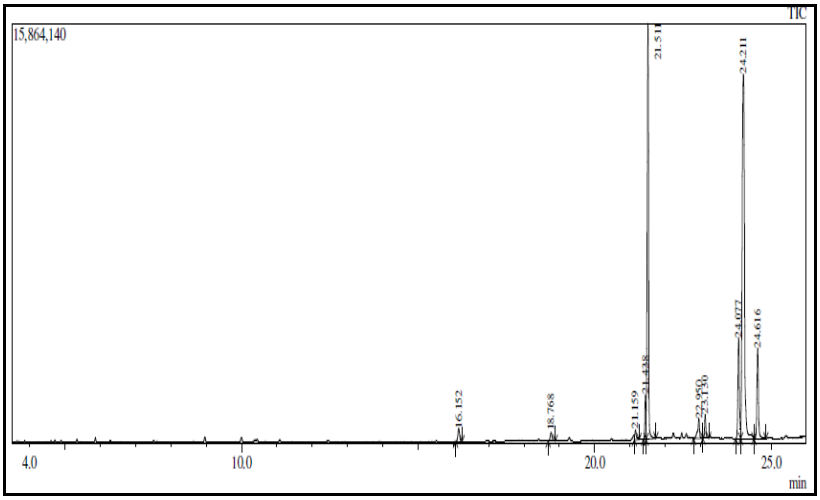

Figure 1: GC chromatogram of saponifiable matter.

Table 1: Identification of fatty acids as methyl esters.

Peak no.	Compounds	Molecular formula	Molecular weight	R_t (min)	Relative area (%)
1	Myristic acid	$C_{15}H_{30}O_2$	242	16.150	1.21
2	5-Octadecenoic acid	$C_{19}H_{36}O_2$	296	18.767	0.84
3	Unknown	----	----	21.158	0.73
4	Palmitoleic acid	$C_{17}H_{32}O_2$	268	21.438	2.73
5	Palmitic acid	$C_{17}H_{34}O_2$	270	21.508	30.63
6	Heptadecanoic acid	$C_{18}H_{36}O_2$	284	22.950	1.98
7	Unknown	----	----	23.133	1.66
8	Linoleic acid	$C_{19}H_{34}O_2$	294	24.075	8.17
9	Linolenic acid	$C_{19}H_{32}O_2$	292	24.208	44.66
10	Stearic acid	$C_{19}H_{38}O_2$	298	24.617	7.39
Percentage of identified saturated long chain fatty acids methyl esters					**40.0%**
Percentage of identified unsaturated long chain fatty acids methyl esters					**57.61%**
Percentage of unidentified compounds					**2.39%**

R_t: Retention time.

GC/MS of volatile oil

Identification of V.O. constituents was carried out by direct comparison fragmentation pattern of each of the separated compounds with those of the reference (Adams, 2009). The results are demonstrated in Fig. 2 and presented in Table 2.

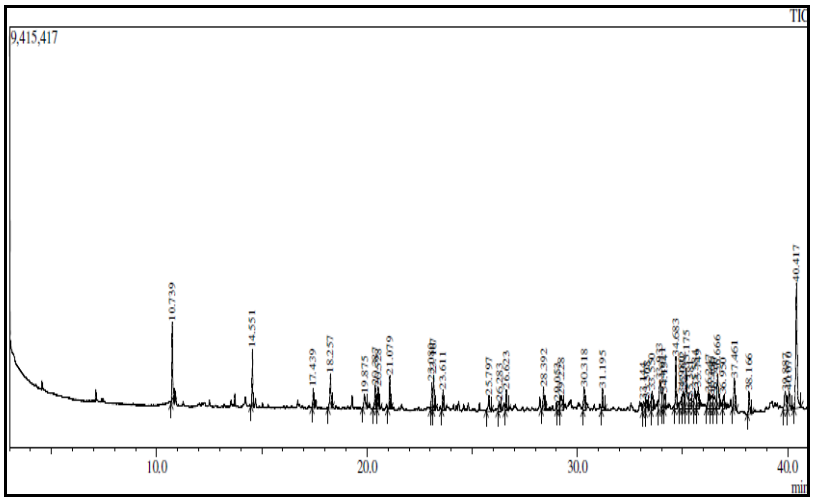

Figure 2: GC chromatogram of V.O.

Table 2: GC/MS data of V.O. constituents.

No.	Name	Molecular formula	R_t	Area %	Molecular weight	Base peak	Characteristic peaks
1	1-Octen-3-ol	$C_8H_{16}O$	10.739	5.00	128	57	43(30%), 72(20%)
2	*L*-Linalool	$C_{10}H_{18}O$	14.551	3.23	154	71	93(70%), 41(60%), 55(55%)
3	*P*-Menth-1-en-8-ol	$C_{10}H_{18}O$	17.439	1.12	154	59	93(60%), 121(50%), 136(40%)
4	2,3-Dihydrobenzofuran	C_8H_8O	18.257	2.70	120	120	91(50%), 65(20%), 39(10%)
5	2,5-Cyclohexadiene-1,4-dione	$C_{12}H_{10}O4$	19.875	1.71	218	110	81(25%), 53(20%), 39(15%)
6	Dihydroedulan Π	$C_{13}H_{22}O$	20.387	1.78	194	179	69(65%), 107(40%), 43(35%)
7	Indole	C_8H_7N	20.528	1.57	117	117	90(45%), 63(10%)
8	2-Methoxy-4-vinyl phenol	$C_9H_{10}O_2$	21.079	2.19	150	150	135(90%), 107(42%), 77(35%)
9	*β*-Demascenone	$C_{13}H_{18}O$	23.080	1.79	190	69	121(70%), 105(30%) 41(25%)
10	*β*-Copaen-4-α-ol	$C_{15}H_{24}O$	23.167	2.38	220	159	91(70%), 105(65%), 119(60%)
11	Unidentified	----	23.611	1.40	----	----	----
12	*E*-*β*-Ionone	$C_{13}H_{22}O$	25.797	1.54	192	177	43(55%), 123(45%), 91(20%)
13	2-Isopropyl-5-methyl-6-oxabicyclo[3.1.0]hexane-1-carboxaldehyde	$C_{10}H_{16}O_2$	26.283	0.97	168	125	43(45%), 153(20%)
14	3-Nonen-5-one	$C_9H_{16}O$	26.623	1.45	140	83	111(45%), 55(45%)
15	(-) Caryophyllene oxide	$C_{15}H_{24}O$	28.392	1.82	220	79	93(90%), 41(80%), 69(65%)
16	Unidentified	----	29.053	1.20	-----	71	----
17	14-Chloro-1-tetradecanol	$C_{14}H_{29}ClO$	29.228	2.31	248	55	69(70%), 95(50%)
18	8-Hydroxylinalool	$C_{10}H_{18}O_2$	30.318	2.13	170	43	67(95%), 93(30%)
19	Tetradecanal	$C_{14}H_{28}O$	31.195	1.65	212	57	82(85%), 96(55%)
20	Unidentified	----	33.144	1.06	----	----	----
21	Unidentified	----	33.308	1.36	----	----	----
22	Unidentified	----	33.550	1.73	----	----	----
23	4,9,13,17-Tetramethyl-4,8,12,16-octadecatetraen-1-ol	$C_{22}H_{38}O$	33.933	3.99	318	81	67(60%), 95(50%), 107(25%)
24	6,10,14-Trimethyl-2-pentadecanone	$C_{18}H_{36}O$	34.071	2.21	268	58	43(95%), 71(90%), 109(35%)
25	Unidentified	----	34.154	1.21	----	----	----
26	Unidentified	----	34.683	3.80	----	----	----
27	Heneicosane	$C_{21}H_{44}$	34.980	1.68	296	57	71(75%), 85(65%), 99(30%)
28	9,17-Octadecadienal	$C_{18}H_{32}O$	35.032	1.49	264	67	81(80%), 55(65%), 95(55%)
29	11,14,17-Eicosatrienoic acid, methyl ester	$C_{21}H_{36}O_2$	35.175	4.30	320	79	67(85%), 55(70%), 93(55%)
30	Unidentified	-----	35.381	1.45	----	----	----
31	Unidentified	-----	35.584	2.41	----	----	----
32	Methyl palmitate	$C_{17}H_{34}O_2$	35.749	2.28	270	74	87(70%), 55(45%)
33	Unidentified	----	36.247	1.83	----	----	----
34	2-Pentyl-2-nonenal	$C_{14}H_{26}O$	36.366	2.20	210	55	41(80%), 81(65%), 69(60%)
35	Unidentified	----	36.530	1.91	----	----	----
36	Unidentified	----	36.666	3.80	----	----	----
37	Unidentified	----	36.950	1.15	----	----	----
38	Unidentified	----	37.461	2.72	----	----	----
39	Farnesyl acetate	$C_{17}H_{28}O_2$	38.166	1.76	264	69	81(55%), 93(50%), 107(40%)
40	*n*-Pentacosane	$C_{25}H_{52}$	39.887	2.71	352	57	71(80%), 85(60%), 43(50%)
41	9,12,15-Octadecatrienoic acid, methyl ester (Methyl linolenate)	$C_{19}H_{32}O_2$	40.07	2.08	292	79	67(65%), 93(55%), 108(40%)
42	*E*-Phytol	$C_{20}H_{40}O$	40.417	12.95	296	71	57(55%), 123(40%), 95(35%)

Percentage of unidentified compounds	**27.01%**		
Percentage of total identified compounds	**72.99%**		
Percentage of total identified non-oxygenated compounds	**5.96%**		
Percentage of total identified oxygenated compounds	**67.03%**	Alcoholics	33.11%
		Aldehydics	5.34%
		Ketonics	9.67%

		Esters	10.42%
		Oxides	6.30%
		Phenolics	2.19%

R_t: Retention time.

Determination of the minimum inhibitory concentration (MIC)

Two-fold serial dilutions were performed on tested V.O. and the antimicrobial agents. The initial concentration was 7 mg/ml and the antimicrobial agents were applied separately to each well using a micropipette (Esimone et al, 1998).

All plates were incubated overnight at 37 °C then the plates were collected and the developed inhibition zones were measured. The MICs was calculated by plotting the natural logarithm of the concentration of volatile oil against the square of inhibition zones. A regression line was drawn trough the points. The antilogarithm of the intercept on the logarithm of concentration axis gave the MIC values (Esimone et al, 1998). The results are shown in Table 3.

Table 3: Antimicrobial activity of the volatile oils of *M. platycalyx* leaves.

	S. aureus	***E. coli***	***K. pnuemoniae***	***C. krusei***	***C. glabrata***	***C. albicans***
Volatile oil	59.80	7.48	NT	53.70	7.80	10.52
Amikacin	1.73 S	28.96 S	9.59 S	----	----	----
Gentamycin	16.45 R	26.44 R	37.60 R	----	----	----
Ketoconazole	----	----	----	1.04	1.58	2.60

S=Susceptible, R=Resistant according to CLSI (Clinical and laboratory Standards Institute, 2011), NT means not active.

DISCUSSION

GC/MS of the saponifiable matter

The results of GC/MS of the saponifiable matter of *M. platycalyx* leaves, revealed the presence of ten fatty acids, of which eight have been identified. The unsaturated fatty acids have been assumed to be protective against inflammatory disorders. The drugs containing unsaturated fatty acids are now developed and provided (Mutoh and Ueda, 2013). The unsaturated fatty acids represented 57.61% of the identified fatty acids, while identified saturated fatty acids were 40.0%; refer Table 1. The major identified fatty acids was linolenic acid (44.66%), n-3 fatty acid and reported to be essential nutrient for retinal and brain function, especially during fetal and postnatal development (Connor et al, 1992), followed by palmitic acid (30.63%).

Myristic, 5-octadecenoic and palmitoleic acids are mono unsaturated fatty acids, which have been shown to reduce metabolic syndrome risk factors. They do this by promoting insulin sensitivity, increasing glycaemic control and regulating HDL and LDL levels (Salas-Salvadó et al, 2008; Misra et al, 2010).

By reviewing the literatures, it showed that myristic acid, 5-octadecenoic acid, palmitoleic acid, palmitic acid, heptadecanoic acid, linoleic acid and linolenic acid were reported for the first time in *Markhamia* genus, While stearic acid was previously reported in *M. acuminata* (Gormann et al, 2004), but firstly reported in *M. platycalyx*.

GC/MS of the volatile oil

The GC chromatogram and mass fragmentation of detected compounds revealed the presence of 42 compounds, of which 28 compounds were identified represented 72.99% of the V.O. composition; refer Fig. 2 and Table 2. The oxygenated compounds were about 67.03% of the identified compounds, including alcoholic compounds (33.11%), aldehydes (5.34%), ketones (9.67%), esters (10.42%), oxides (6.30%) and phenolic compounds (2.19%). *E*-Phytol (12.95%) followed by 1-octen-3-ol (5.00%) were the major identified oxygenated compounds; refer Table 2. The relative areas of the non-oxygenated identified compounds were about 5.96% of the total identified compounds.

Antimicrobial activity of the volatile oil

The high content of the oxygenated compounds in the V.O. of *M. platycalyx* leaves suggested that it may has an antimicrobial activity. The main oxygenated compound was *E*-Phytol. It is a diterpene alcohol and reported to exhibit an anti-tubercular activity against *Mycobacterium tuberculosis* H37Rv strain at 100 µg/ml and used as precursor for different semisynthetic compounds with lower MIC (15.6-50 µg/ml) against the same organism (Saikia et al, 2010). It exhibited an anti-inflammatory activity, proposed by inhibiting neutrophil migration that is partly caused by reduction in IL-1β and TNF-α levels and oxidative stress tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) levels (Silva et al, 2014).

The antibacterial activity of V.O. of *M. platycalyx* leaves was tested against two gram negative bacteria viz., *E. coli* and *K. pneumonia*, in addition to one gram positive bacteria viz., *S. aureus*. This study showed a potent antibacterial activity against *E. coli* with MIC 7.48 µg/ml compared to the standard antibiotics MICs, amikacin (28.96 µg/ml) and gentamycin (26.44 µg/ml). It

displayed also a weak activity against *S. aureus* 59.8 µg/ml and no activity against *K. pneumonia*. While, the antifungal activity of the V.O. was tested against *C. krusei*, *C. glabrata* and *C. albicans*, showing weak or mild activity against yeasts compared to the standard antifungal agent (ketoconazole); refer Table 3.

CONCLUSION

This study demonstrates that eight and twenty eight chemical constituents have been identified from the saponifiable matter and the V.O. of *M. platycalyx* Sprague leaves by GC/MS, respectively. In addition to, the V.O. showed a potent antibacterial activity against *E. coli*. Therefore, further research on this plant in order to develop new antibacterial agents with less drug resistance is recommended.

ACKNOWLEDGEMENT

We would like to thank Dr. Rehab M. Abd-Elbaky, Department of Microbiology, Faculty of Pharmacy, Minia University for her contribution in the antimicrobial study.

REFERENCES

1. Abdel-Wahab NM, Hamed ANE, Khalil HE, Samy MN, Wanas AS, Fouad MA, Kamel MS. Phenolic acid glycosides from *Parmentiera cereifera* Seem. (Candle tree). Phytochem Lett, 2014; 9: 74-77.
2. Adams RP. Identification of essential oil components by Gas chromatography/mass spectrometry. Illinois, USA, Allured books, 4th Ed. 1989.
3. Archive of Mass Spectra: http://lipidlibrary.aocs.org/ms/arch_me/index.htm (Retrieved 15th Feb., 2015).
4. Baranga D. Changes in chemical composition of food parts in the diet of Colobus Monkeys. Ecology, 1983; 64: 668-673.
5. Connor WE, Neuringer M, Reisbick S. Essential fatty acids: The importance of n-3 fatty acids in the retina and brain. Nutr Rev, 1992; 50 (4): 21-29.
6. Delahaye C, Rainford L, Nicholson A, Mitchell S, Lindo J, Ahmad M. Antibacterial and antifungal analysis of crude extracts from the leaves of *Callistemon viminalis*. J Med Biol Sci, 2009; 3 (1): 1-7.
7. El-Kashef DFAH. A pharmacognostical study of *Pachypodium lamerei* drake, family Apocyanaceae, cultivated in Egypt. M.Sc. Thesis, Pharmacognosy Department, Minia university, Minia, Egypt, 2014.
8. El-Said FM, Amer MM. Oils, fats, waxes and surfactants. CairoAnglo-Egyptian Bookshop, 1965, 130.
9. Esimone CO, Adiukwu MU, Okonta JM. Preliminary antimicrobial screening of the ethanolic extract from the lichen *Usnea subfloridans* (L). J Pharm Res Dev, 1998: 3(2): 99-102.
10. Fischer E, Theisen I, Lohmann LG, Kadereit JW. Bignoniaceae flowering plants dicotyledons. The Families and Genera of Vascular Plants. K. Kubitzki, Springer Berlin Heidelberg. 2004; 7: 9-13.
11. Gemeda N, Urga K, Tadele A, Lemma H, Melaku D, Mudie K. Antimicrobial activity of topical formulation containing *Eugenia caryophyllata* L. (Krunfud) and *Myritus communis* L. (Ades) essential oils on selected skin disease causing microorganisms. Ethiop J Health Sci, 2008; 18(3): 101-107.
12. Gormann R, Schreiber L, Kolodziej H. Cuticular wax profiles of leaves of some traditionally used african Bignoniaceae. Z Naturforsch, 2004; 59c: 631-635.
13. Johnson AR, Davenport JB. Biochemistry and methodology of lipids. New York: John Wiley and sons, Inc., 1971, 31.
14. Joshi KC, Singh P, Sharma MS. Quinones and other constituents of *Markhamia platycalyx* and *Bignonia unguiscati*. J Nat Prod, 1985; 48: 145.
15. Kernan MR, Amarquaye A, Chen JL, Chan J, Sesin DF, Parkinson N, Ye Z, Barrett M, Bales C, Stoddart CA, Sloan B, Blanc P, Limbach C, Mrisho S, Rozhon EJ. Antiviral phenylpropanoid glycosides from the medicinal plant *Markhamia lutea*. J Nat Prod, 1998; 61(5): 564-570.
16. Khan MR, Mlungwana SM. γ-Sitosterol, a cytotoxic sterol from *Markhamia zanzibarica* and *Kigelia africana*. Fitoterapia, 1999; 70: 96-97.
17. Misra A, Singhal N, Khurana L. Obesity, the metabolic syndrome, and type 2 diabetes in developing countries: role of dietary fats and oils. J Am Coll Nutr, 2010; 29(3): 289S–301S.
18. Mutoh A, Ueda S. Peroxidized unsaturated fatty acids stimulate Toll-like receptor 4 signaling in endothelial cells. Life Sci, 2013; 92: 984-992.
19. Nia R, Adesanya SA. Palustrine from *Markhamia tomentosa*. Niger J Nat Pro Med, 1997; 1: 39-40.
20. Ogbulie JN, Ogueke CC, Okoli IC, Anyanwu BN. Antibacterial activities and toxicological potentials of crude ethanolic extracts of *Euphoria hirta*. Afr J Biotechnol, 2007; 6(13): 1544-1548.
21. Refaat J, Kamel MS, Ramadan MA, Ali AA. GC-MS studies of *Crimm asiaticum* L. leaves and flowers. Res J Pharmacogn Phytochhem, 2011; 3(5): 232-235.
22. Saikia D, Parihar S, Chanda D, Ojha S, Kumar JK, Chanotiya CS, Shanker K, Negi AS. Antitubercular potential of some semisynthetic analogues of phytol. Bioorg Med Chem Lett, 2010; 20: 508–512.
23. Salas-Salvadó J, Fernández-Ballart J, Ros E, Martínez-González MA, Fitó M. Effect of a Mediterranean diet supplemented with nuts on metabolic syndrome status: one year results of the predimed randomized trial. Arch Intern Med, 2008; 168(22): 2449-58.
24. Silva RO, Sousa FBM, Damasceno SRB, Carvalho NS, Silva VG, Oliveira FRMA, Sousa DP, Aragãod KS, Barbosa ALR, Freitas RM, Medeirosa JVR. Phytol, a diterpene alcohol, inhibits the inflammatory response by reducing cytokine

production and oxidative stress. Fundam Clin Pharmacol, 2014; 28(4): 455-464.
25. Ugbabe GE, Ayodele AE. Foliar epidermal studies in the family Bignoniaceae JUSS. in Nigeria. Afr J Agric Res, 2008; 3(2): 154-166.