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GC-MS Analysis of Volatile Oil and Fatty Acids Composition of *Abutilon hirtum* (Lam.) Sweet Leaves

Alshymaa A. Gomaa[1], Mamdouh N. Samy[1,*], Samar Y. Desoukey[1], Mohamed S. Kamel[1,2]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Minia University, Minia 61519, Egypt.

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

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Abstract

The Volatile oil of *Abutilon hirtum* (Lam.) Sweet leaves extracted by a Clevenger approach and the fatty acid content of this species as a result of petroleum ether extraction were investigated using gas chromatography-mass spectrometry (GC-MS) technique. GC-MS analysis of the volatile oil revealed the presence of 63 compounds, with (2*E*,6*E*)-farnesol (6.56%), *E*-caryophyllene (4.68%), caryophyllene oxide (4.37%) and β-copaen-4-α-ol (3.51%) as the major components. On the other hand, 8 fatty acids of which three were identified as linolenic acid methyl ester, which was the major identified fatty acid methyl ester (38.74%), followed by palmitic acid methyl ester (29.09%) and linoleic acid methyl ester (18.14%). The identification of the compounds depends on the retention time and mass spectrum.

Key words

Malvaceae, *Abutilon hirtum*, volatile oil, fatty acids, GC-MS

1. Introduction

Abutilon Miller is a large genus belonging to family Malvaceae comprises about 150 annual or perennial herbs, shrubs or even small trees native to the tropical and subtropical countries of America, Africa, Asia and Australia [1, 2]. *Abutilon hirtum* (Lam.) Sweet [syn. *A. graveolens* (Roxb. Ex Hornem.) Wight & Arn. and *Sida hirta* Lam.], commonly known as Florida Keys Indian Mallow. It is a perennial herb or shrub, 0.5-2.5 m height, widely distributed in tropical Africa, Asia and South America. The leaves are simple, alternate with long petioles and hairy surfaces. The flowers are hermaphrodite and solitary with orange –yellow petals appearing in April until June, with schizocarpic fruit containing reniform black seeds [2-4]. Traditionally, many *Abutilon* species are used in treatment of inflammation, piles, gonorrhea, bronchitis, diarrhea, cleaning wounds and ulcers [5]. In Malaysia, *Abutilon hirtum* is used as a poultice to ease the pain of kidney gravel and often mixed with glutinous rice and applied to ulcers. In Thailand, the roots are used against cough and toothache and as an antipyretic. The leaves or flowers are applied to abscesses. In Kenya the fruits are eaten raw, while the leaves are browsed by goats and camels. Water extract of the bark is given to ease childbirth in Kenya and Uganda. In India, traditionally the leaves are used as demulcent, diuretic and to treat diarrhea. The decoction of the leaves is used as mouth wash and to cure bladder inflammations, wounds and ulcers, since alkaloids are reported from the roots of the plant [2,6,7]. Previous phytochemical investigation of *A. hirtum* led to isolation of flavonoids and phenolic acids [4]. The aim of the present study was to investigate the chemical properties of *A. hirtum* leaves collected from Egypt concerning the chemical combination of the volatile oil and composition of fatty acids.

2. Experimental

2.1. Plant material

The leaves of *A. hirtum* were collected in November 2013 from El-Zohria botanical garden, Cairo, Egypt and identified by Prof. Dr. Mahmoud Abdelhady Hassan Professor of Horticulture, Faculty of Agriculture, Minia University. A voucher sample (Mn-ph-Cog-016) was kept in the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Minia University, Minia, Egypt.

2.2. Preparation of volatile oil

Fresh leaves of the plant (107g) were subjected to water distillation using Clevenger apparatus (for volatile oils lighter than water) to give 0.1ml yellowish oil with concentration 0.09% V/W. Heating was continuous for 6 hours and the isolated oil was dissolved in 30 ml of diethyl ether. The solvent was removed at room temperature and the oil was kept for further investigation [8].

2.3. Preparation of fatty acids

About 2.3 g of the dried petroleum ether fraction of the air-dried powder of the leaves of *A. hirtum* was subjected to alkaline hydrolysis for saponification. The extract was refluxed with 50 ml of N/2 alcoholic potassium hydroxide for about 8 hours 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 with water, extracted with several portions of chloroform until exhaustion. The combined chloroform extracts

**** Correspondence:** **Mamdouh Nabil Samy***

***Tel.**: 01212030906**; Fax:** +2086-236-90-75.*

***Email Address:** mamdouhnabil.2006@yahoo.com*

were washed with sodium hydroxide (10%) then with distilled water until the washing were free from any alkalinity. The chloroform extracts were dehydrated over anhydrous sodium sulphate and then the chloroform was distilled off. The residue (1g) obtained (represent the unsaponifiable matter) was orange red in colour. It was reserved for further investigation. 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 small portions of chloroform. The combined chloroform extracts were washed with distilled water, till the washing was neutral to litmus paper. The chloroform was distilled off and the residue of the total fatty acids was dried over calcium chloride. It was semisolid and brown in colour [9-11].

2.4. Preparation of fatty acid methyl esters

The fatty acids were converted to their methyl esters by refluxing with 50 ml methanol and 1.5 ml conc. sulphuric acid for 2 hours. The major part of alcohol was distilled off, and the residue was diluted with distilled water and then extracted with several portions of chloroform. The combined chloroform extracts were washed with distilled water, till washing was free from any acidity. The chloroform extract was concentrated, and the residue (300 mg) was dried over calcium chloride overnight, and then kept for further investigation [8-10].

2.5. GC-MS analysis

GC-MS analysis was performed on a QP-2010 Ultra gas chromatography (Shimadzu, Kyoto, Japan) equipped with head space AOC-5000 auto injector using a fused-silica capillary column (30 m length × 0.25 mm ID × 0.25 μm film thichness), packed with Rtx-5MS (5% diphenyl/ 95% dimethyl polysiloxane, Fisher Scientific). The carrier gas was helium with a flow rate of 0.8 ml/min. The injected volume was 1 μl and the injector and detector temperature was 230 ºC. The analysis was carried at programmed temperature using flame ionization detector (FID).

For the volatile oil, the initial temperature was 40 ºC, then increased at a rate of 5 ºC/min and final temperature 210 ºC, and the total program time was 54 mi. For fatty acid methyl esters, the initial temperature was 70 ºC, then increased at a rate of 3 ºC/min and final temperature 220 ºC and the total run time was 26 min. The spectrometer was operated in electron impact (EI) mode of ionization; with mass range of *m/z* 40-500; the ionization energy was 70 eV and the scan rate was 0.20 s per scan.

3. Results

Sixty-three compounds were identified in volatile oil of *A. hirtum* leaves, representing 64.3% of the total oil constituents (Table 1 and Figure 1). The major components were identified as (2*E*, 6*E*)-farnesol (6.56%), *E*-caryophyllene (4.68%), caryophyllene oxide (4.37%) and β-copaen-4-α-ol (3.51%). The oil has yellow colour with faint odour and was found to be rich in oxygenated compounds (39.8%), while non-oxygenated compounds were 24.5%. The oil contains monoterpenes hydrocarbons (3.1%), oxygenated monoterpenes (3%), sesquiterpenes hydrocarbons (16.3%), oxygenated sesquiterpenes (13.3%), diterpenes hydrocarbons (2%), oxygenated diterpenes (2.1%) and other compounds (24.5%) (Oxygenated compounds (21.4%) and aliphatic compounds (3.1%)).

Table 1. Identified compounds of the volatile oil of *A. hirtum* leaves.

No.	Compounds	Molecular Formula	Molecular Weight	R_t (min)	Relative Area (%)
1	1,2-Propanediol	$C_3H_8O_2$	76	4.56	0.31
2	Phenol	C_6H_6O	94	10.89	0.48
3	*p*-Cymene	$C_{10}H_{14}$	134	12.16	0.34
4	Limonene	$C_{10}H_{16}$	136	12.29	0.47
5	Terpinolene	$C_{10}H_{16}$	136	14.20	0.43
6	Camphor	$C_{10}H_{16}O$	152	16.03	0.97
7	Borneol	$C_{10}H_{18}O$	154	16.70	0.29
8	β-Cyclocitral	$C_{10}H_{16}O$	152	17.57	0.42
9	2,3-Dihydro-benzofuran	C_8H_8O	120	18.25	0.61
10	1,4- Benzenediol	$C_6H_6O_2$	110	19.88	1.54
11	2-(Acetylmethyl)-(+)-3-carene	$C_{13}H_{20}O$	192	21.63	0.40
12	α-Cubebene	$C_{15}H_{24}$	204	22.09	0.46
13	α –Copaene	$C_{15}H_{24}$	204	22.87	0.36
14	β-Damascenone	$C_{13}H1_8O$	190	23.08	2.37
15	β-Copaen-4α-ol	$C_{15}H_{24}O$	220	23.17	3.51
16	β –Elemene	$C_{15}H_{24}$	204	23.28	1.00
17	2,3-Dehydro-4-oxo-beta-ionol	$C_{13}H1_8O_2$	206	23.48	0.73
18	6-Methyl-5-(1-methylethylidene)-6,8-nonadien-2-one	$C_{13}H_{20}O$	192	23.78	1.55
19	4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)butan-2-one	$C_{13}H_{20}O$	192	23.90	2.64
20	*E*- Caryophyllene	$C_{15}H_{24}$	204	24.11	4.68
21	7,8-Dihydro-β-ionone	$C_{13}H_{22}O$	194	24.34	0.74
22	γ-Elemene	$C_{15}H_{24}$	204	24.40	0.57
23	Dihydrocurcumene	$C_{15}H_{24}$	204	24.59	1.00
24	Geranyl acetone	$C_{13}H_{22}O$	194	24.81	0.53
25	α-Humulene	$C_{15}H_{24}$	204	25.02	1.21
26	4-Hydroxy- β -ionone	$C_{13}H_{20}O_2$	208	25.29	2.65
27	β-Panasinsene	$C_{15}H_{24}$	204	25.57	0.42
28	Germacrene-D	$C_{15}H_{24}$	204	25.74	1.32
29	β –Ionone	$C_{13}H_{20}O$	192	25.79	0.43
30	Torreyol	$C_{15}H_{26}O$	222	26.07	0.61

No.	Compounds	Molecular Formula	Molecular Weight	R_t (min)	Relative Area (%)
31	Farnesene	$C_{15}H_{24}$	204	26.24	0.30
32	1,5,5,8-Tetramethyl-3,7-cycloundecadien-1-ol	$C_{15}H_{26}O$	222	26.61	0.85
33	δ-Cadinene	$C_{15}H_{24}$	204	26.77	2.43
34	Nerolidol	$C_{15}H_{26}O$	222	27.15	0.43
35	Germacrene B	$C_{15}H_{24}$	204	27.72	1.22
36	Dehydroaromadendrene	$C_{15}H_{22}$	202	27.82	0.47
37	(2*E*,6*E*)-Farnesol	$C_{15}H_{26}O$	222	28.02	6.56
38	Caryophyllene oxide	$C_{15}H_{24}O$	220	28.39	4.37
39	Viridiflorol	$C_{15}H_{26}O$	222	28.59	0.30
40	*E* –longipinocarveol	$C_{15}H_{24}O$	220	29.21	0.55
41	Di-epi- α –cedrene (α-Funebrene)	$C_{15}H_{24}$	204	29.43	1.45
42	*E*-10-Pentadecenol	$C_{15}H_{30}O$	226	29.61	0.35
43	Carotol	$C_{15}H_{26}O$	222	29.76	2.19
44	4-Hexadecen-6-yne	$C_{16}H_{28}$	220	30.02	3.19
45	1-Hexadecanol	$C_{16}H_{34}O$	242	30.32	0.37
46	α-Calacorene	$C_{15}H_{20}$	200	30.36	0.32
47	4-Methylene-2,8,8-trimethyl-2-vinyl-Bicyclo[5.2.0]nonane	$C_{15}H_{24}$	204	30.50	0.66
48	Ledol	$C_{15}H_{26}O$	222	30.63	0.29
49	Octadecane	$C_{18}H_{38}$	254	30.80	0.49
50	*Z*-7-Hexadecenal	$C_{16}H_{30}O$	238	31.06	0.38
51	Tetradecanal	$C_{14}H_{28}O$	212	31.19	0.50
52	(2*Z*,6*E*)-Farnesol	$C_{15}H_{26}O$	222	31.440	0.66
53	Benzyl benzoate	$C_{14}H_{12}O_2$	212	32.548	0.70
54	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	$C_{15}H_{24}O$	220	32.675	0.43
55	Farnesyl acetate	$C_{17}H_{28}O_2$	264	34.03	1.57
56	3-Heptadecen-5-yne	$C_{17}H_{30}$	234	34.27	0.71
57	*E*-Phytol	$C_{20}H_{40}O$	296	34.82	2.02
58	9,12-Octadecadien-1-ol	$C_{18}H_{34}O$	266	35.03	0.35
59	2-Heptadecanone	$C_{17}H_{34}O$	254	35.28	0.47
60	Linoleoyl chloride	$C_{18}H_{31}ClO$	298	35.73	1.01
61	(*E*,*E*,*E*)-3,7,11,15-Tetramethylhexadeca-1,3,6,10,14-pentaene	$C_{20}H_{32}$	272	36.03	0.77
62	Phytane (2,6,10,14-Tetramethylhexadecane)	$C_{20}H_{42}$	282	37.29	0.77
63	Geranyl linalool	$C_{20}H_{34}O$	290	38.17	2.24

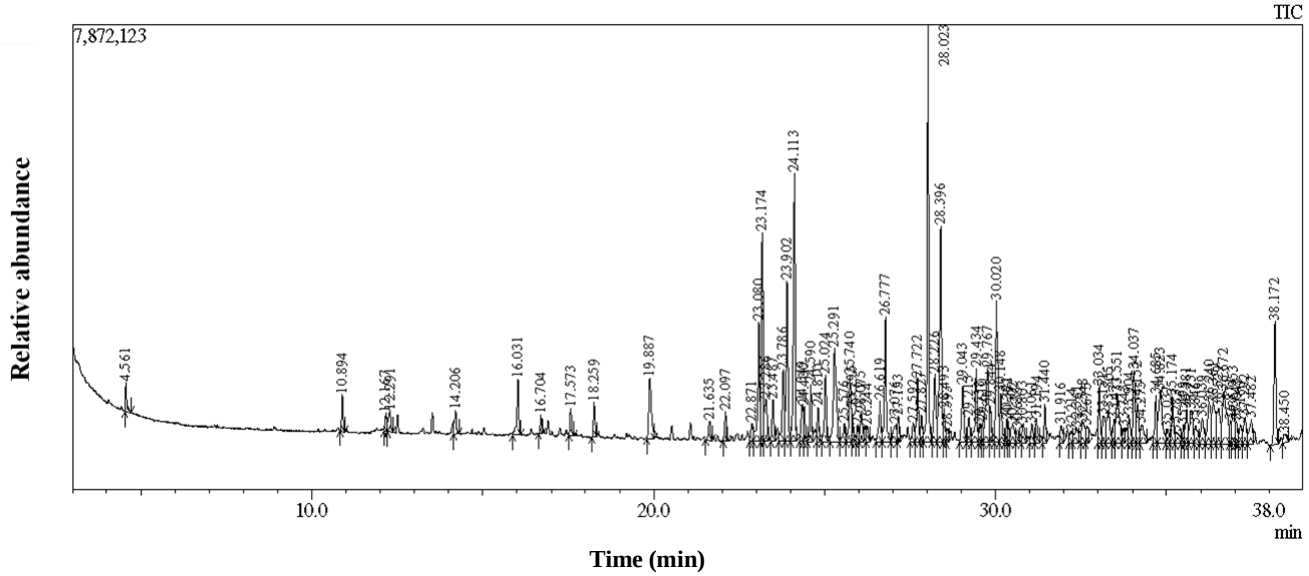

Figure 1. GLC chromatogram of volatile oil of *A. hirtum* leaves.

On the other hand, the results of the GC-MS analysis of fatty acid methyl esters of *A. hirtum* leaves (Table 2 and Figure 2**)** revealed the presence eight fatty acids of which seven (87.5%) were identified. The unsaturated fatty acids formed 37.5% of the fatty acid fraction, while the saturated fatty acids consisted of 50% of the fatty acid fraction. The major identified fatty acid methyl ester was linolenic acid methyl ester was (38.74%), followed by palmitic acid methyl ester (29.09%) and linoleic acid methyl ester (18.14%).

The comparison of GC-MS fatty acids methyl esters analysis of A. hirtum leaves with previously reported GC fatty acids methyl esters analysis [13], showed that the major fatty acid in this study was linolenic acid (38.74%), while in previous one was palmitic acid (32.9%). This variation was probably due to different conditions of analysis.

Table 2. Fatty acids identified as methyl esters of *A. hirtum* leaves.

Peak No.	Compounds	Molecular Formula	Molecular Weight	R_t (min)	Relative Area (%)
1	Myristic acid methyl ester	$C_{15}H_{30}O_2$	242	16.15	2.65
2	Palmitoleic acid methyl ester	$C_{17}H_{32}O_2$	268	21.43	2.85
3	Palmitic acid methyl ester	$C_{17}H_{34}O_2$	270	21.50	29.09
4	Arachidic acid methyl ester	$C_{21}H_{42}O_2$	326	21.77	1.46
5	Linoleic acid methyl ester	$C_{19}H_{34}O_2$	294	24.07	18.14
6	Linolenic acid methyl ester	$C_{19}H_{32}O_2$	292	24.20	38.74
7	Stearic acid methyl ester	$C_{19}H_{38}O_2$	298	24.61	3.25

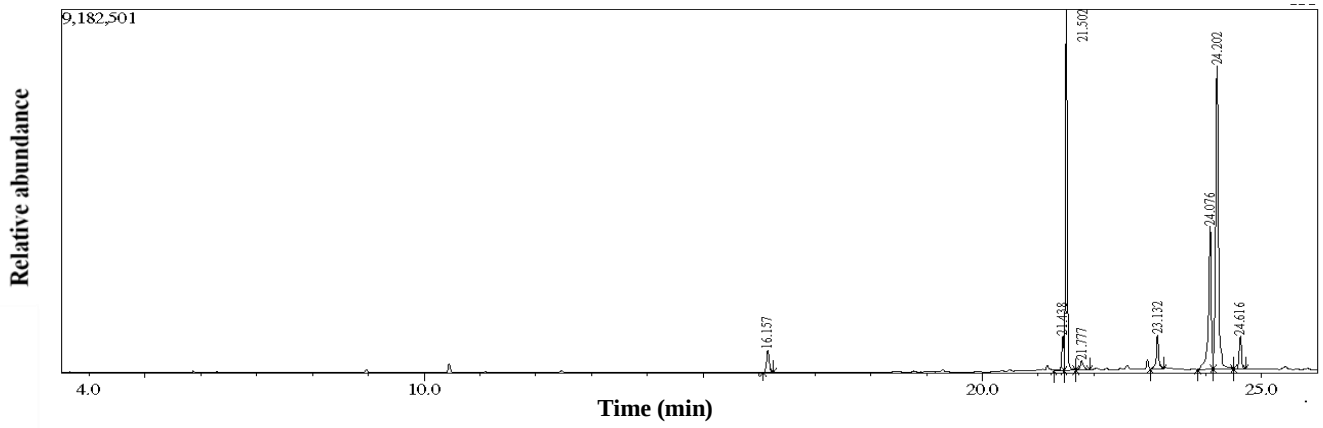

Figure 2. GLC chromatogram of fatty acid methyl esters of *A. hirtum* leaves.

Identification of the compounds was confirmed by comparing their fragmentation pattern with those of the reference compounds reported in literature [11], in addition to matching with the database of National Institute Standard and Technology (NIST library).

4. Discussion

The essential oil profile of *A. hirtum* leaves was different with that of A. indicum flowering tops [12], revealing the presence of limonene, terpinolene, camphor, β-cyclocitral, α-cubebene, β-damascenone, β-copaen-4-α-ol, germacrene-D, torreyol, δ-cadinene, carotol, farnesyl acetate and phytol only in *A. hirtum*. Furthermore, α-pinene, α-cineole, geraniol, geranyl acetate and eudesmol were found only in A. indicum. Caryophyllene oxide, β–elemene, γ-elemene, (2E,6E)-farnesol and (2Z,6E)-farnesol were detected in high amount in *A. hirtum* leaves. On the other hand, caryophyllene, geranyl acetate and borneol were found in high amount in A. indicum flowering tops.

Stearic acid methyl ester was detected in high amount in this study. Moreover, myristic acid methyl ester, arachidic acid methyl ester and linoleic acid methyl ester were found in high amount in previous study. Capric acid, lauric acid, oleic acid and Cis,11-eicosenoic acid were identified in previous study and were not detected even in trace amount in this study.

5. Conclusion

The present study includes isolation and identification of volatile components and saponifiable matter of *A. hirtum* leaves using gas chromatography-mass spectroscopy (GC-MS) technique. The volatile oil of the plant leaves contains a high percentage of oxygenated compounds 39.8% that may exhibit a potent antioxidant and antibacterial activities of the oil that need more investigations, and this is the first time for the GC-MS analysis of *A. hirtum* leaves essential oil. Furthermore, the leaves contain a significant percentage of linolenic acid (38.74%) and may be used a source of it.

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References:

[1] Gomaa AA-R, Samy MN, Desoukey SY, Kamel MS. Anti-inflammatory, analgesic, antipyretic and antidiabetic activities of *Abutilon hirtum* (Lam.) Sweet. Clinical Phytoscience 2018;4:11.
[2] Gomaa AA-R, Samy MN, Desoukey SY, Kamel MS. Phytochemistry and pharmacological activities of genus *Abutilon*: a review (1972-2015). Journal of Advanced Biomedical and Pharmaceutical Sciences 2018;1:56.
[3] Gomaa AA, Samy MN, Desoukey SY, Kamel MS. Pharmacognostical studies of leaf, stem, root and flower of *Abutilon hirtum* (Lam.) Sweet. International Journal of Pharmacognosy and Phytochemical Research 2016;8:199-216.
[4] Kassem HA. Study of polyphenolic components and macro- and micromorphological characters of *Abutilon hirtum* (Lam.) Sweet. Bulletin of Faculty of Pharmacy, Cairo University 2007;45:173-183.
[5] Khadabadi SS, Bhajipale NS. A review on some important medicinal plants of *Abutilon spp*. Research Journal of Pharmaceutical, Biological and Chemical Sciences 2010**;**1:718-729.
[6] Brink, M, Dako EG. Plant Resources of Tropical Africa 16. Fibres. Prota Foundation, Wageningen, Netherlands 2012; 25.
[7] Wesley SP, Devi CB, Moin S, Shibu SB. *In vitro* phytochemical screening, free radical scavenging activity and anticancer activity of *Abutilon hirtum* (Lam.) Sweet (Malvaceae). International Journal of PharmTech Research 2013;5:155-161.
[8] Mahmoud BK, Hamed ANE, Samy MN, Wanas AS, Kamel MS. Antimicrobial and GC/MS studies for saponifiable and volatile oil of *Markhamia platycalyx* leaves. European Journal of Pharmaceutical and Medicinal Research 2015;2:57-63
[9] Refaat J, Kamel MS, Ramadan MA, Ali AA. GC/MS studies of *Crinum asiaticum* L. leaves and flowers. Research Journal of Pharmacognosy and Phytochmistry 2011; 3:232-235.
[10] Abdelhafez OH, Fahim JR, Abdelmohsen UR, Desoukey SY. GC–MS analysis of fatty acids of *Malvaviscus arboreus* leaves. Journal of Advanced Biomedical and Pharmaceutical Sciences 2020;4:12-15
[11] Adams RP. Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy, 4th edition, Allured books, Illinois, USA 2009.
[12] Kaushik P, Kaushik D, Khokra S, Chaudhary B. *Abutilon indicum* (Atibala): ethno-botany, phytochemistry and pharmacology- A review. International Journal of Pharmaceutical and Clinical Research 2009;1:4-9
[13] Kassem HA. Investigation of lipids, mucilage and cytotoxic activity of *Abutilon hirtum* (Lam.) Sweet grown in Egypt. Bulletin of Faculty of Pharmacy, Cairo University 2001;39:156-159.