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Phytochemical and Biological Evaluation of *Buddleja asiatica*

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ABSTRACT

The phytochemical investigation of *Buddleja asiatica* Lour (family Scrophulariaceae), resulted in the separation and identification of six compounds not isolated previously from *B. asiatica*, which have been identified as olian-12-en-3-one, taraxaster-12-en-3-one, 4-acetoxybenzaldehyde, 4-acetoxyacetophenone, (E) 3,4-dihydroxycinnamic acid and (E) 3,4-dihydroxy-5-methoxycinnamic acid, in addition to many compounds that were isolated previously from the same species included β-sitosterol, stigmasterol, oleanane glycosides (3-O-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→2)-β-D-fucopyranosyl]-olean-11,13(18)-dien-3β,23,28-triol, 3-O-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-β-D-fucopyranosyl]-olean-11,13(18)-dien-3β,23,28-triol and 3-O-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-β-D-xylopyranosyl-(1→2)-β-D-glucuronide]-olean-11,13 (18)-dien-3β,23,28-triol). The volatile constituents of essential oil, hexane and methylene chloride fractions were identified by GC/MS. Structures of separated compounds were elucidated by spectral analysis. The antimicrobial activities of methylene chloride and ethyl acetate extracts were comparable to ampicillin. The activities against *E. coli* were 83.3%; 91.7%, respectively, and against *S. aureus* were 86.4% for both extracts. Antioxidant activity was assessed by DPPH and ABTS methods. By the first one, it decreased in the following order: ascorbic acid > methylene chloride extract > ethyl acetate extract > butanol extract, and by the second one, it decreased in the following order: ascorbic acid > ethyl acetate > butanol > methylene chloride > essential oil Ba2 > hexane > essential oil Ba1. The cytotoxicity against HePG2 were found to be "strong" for ethyl acetate fraction, butanol fraction, methylene chloride fraction and essential oil Ba2, "moderate" for hexane fraction and essential oil Ba1. The cytotoxicity against MCF-7 was found to be "strong" for methylene chloride fraction, essential oil Ba2 and butanol fraction, "moderate" for ethyl acetate fraction, hexane fraction, and essential oil Ba1. The cytotoxicity against HCT-116 was found to be "strong" for ethyl acetate fraction, "moderate" for butanol fraction, and methylene chloride fraction, and "weak" for hexane fraction and essential oil Ba2, "non-cytotoxic" for essential oil Ba1. The cytotoxicity against PC3 was found to be "strong" for methylene chloride fraction, "moderate" for ethyl acetate fraction, hexane fraction, butanol fraction and essential oil Ba2, "weak" for essential oil Ba1.

Keywords: Scrophulariaceae; *Buddleja asiatica;* triterpenoidal glycosides; GC/MS; essential oil; antimicrobial; antioxidant; cytotoxicity.

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INTRODUCTION

The genus *Buddleja* comprises approximately 100 species, native to the tropics of Asia, USA and Africa; the more famous species are *B. asiatica, B. crispa, B. davidii* and *B. lindleyana* **[1, 2]**.

B. asiatica has been used in the treatment of cancer and as a cure of articular rheumatism in the Chinese traditional medicine. The whole plant is used as an abortifacient **[3, 4]**, as a medicine for skin disease, and for loss of weight **[5]**. A paste of its roots is used as a tonic when mixed with rice water **[6]**. A fusion of roots is used in the treatment of malaria **[7]**. It was reported that the leaves of *B. asiatica* have a hypertensive effect on cats and dogs, and that the leaves essential oil has in-vitro antifungal activities. The flowers are used in the treatment of cystitis and cold **[8]** and to treat edema **[9]**. *B. asiatica* is planted in gardens as an ornamental shrub **[10]**. The genus *Buddleja* has been reported to possess anti-inflammatory, antibacterial and cytotoxic properties **[11]**. It has been reported to contain terpenoids, flavonoids, phenylethanoids, neolignans and saponins **[12, 13]**.

RESULTS AND DISCUSSION

Phytochemical evaluation

Column chromatography for alcoholic extract of *B. asiatica* afforded eleven compounds: olian-12-en-3-one **1**, taraxaster-12-en-3-one **2**, β-sitosterol, stigmasterol, 4-acetoxybenzaldehyde, 4-acetoxyacetophenone, (E) 3,4-dihydroxycinnamic acid, (E) 3,4-dihydroxy-5-methoxycinnamic acid, 3-*O*-[*α*-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→3)-*β*-D-glucopyranosyl-(1→2)-*β*-D-fucopyranosyl]-olean-11,13(18)-dien-3*β*,23,28-triol **3,** 3-*O*-[*α*-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→3)-*β*-D-fucopyranosyl]-olean-11,13(18)-dien-3*β*,23, 28-triol **4** and 3-*O*-[*α*-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→2)-*β*-D-glucuronide]-olean-11,13(18)-dien-3*β*,23,28-triol **5** **[14, 15]**. The identity of the separated compounds were established by spectral means (^{1}H NMR and MS).

In addition, 80 compounds from acetogenins, terpenoids, steroids, shikimates and others were identified by the GC/MS from essential oil Ba1, essential oil Ba2, hexane Ba3, methylene chloride Ba4 and ethyl acetate extracts Ba5 by comparing the MS spectra with those in NIST library. A sample from essential oil Ba1 afforded 27 compounds, representing 55.02% from the sample, with dodecane (5.30%), decane (5.04%) and undecane (4.90%) being the major components. Essential oil Ba2 afforded 5 compounds, representing 33.93% from the sample, with heptadecane (2.95%), tetradecane (2.82%) and pentadecane (2.75%) being the major components. Hexane extract Ba3 afforded 37 compounds, representing 93.01 % from the sample, with fenchone (16.98%), anethole (14.28%) and phytol (11.09%) being the major components. Methylene chloride extract Ba4 afforded 7 compounds, representing 27.89% from the sample, with sulfolane (16.59%), 4-oxo-ß-ionol (2.33%), and loliolide (1.65%) being the major components. Ethyl acetate extract afforded 5 compounds, representing 26.18% from the sample, with 5,7-dimethoxy-2,2-dimethyl-2H-chromene (12.61%), 6-(3-Hydroxy-but-1-enyl)-1,5,5-trimethyl7oxabicyclo[4.1.0]heptan-2-ol (3.67%) and 4-isopropyl-5-methylphenol (3.18%) being the major components (Table 5).

Biological applications

Antimicrobial activity assessment

In our current study, the antimicrobial potentials of essential oil Ba1, essential oil Ba2, hexane Ba3, methylene chloride Ba4, ethyl acetate Ba5 and butanol Ba6 extracts of *B. asiatica* were examined by disc diffusion assay method, using pathogenic microbial species; *Staphylococcus aureus*, representing Gram positive bacteria, *Escherichia coli*, representing Gram negative bacteria, and *Candida albicans*, representing fungi (Table 1).

The activity index of ethyl acetate extract (Ba5) and methylene chloride extract (Ba4) against *E. coli* were comparable to *ampicillin* (91.7%, 83.3%, respectively). The activity index of ethyl acetate extract (Ba5) and methylene chloride extract (Ba4) against *S. aureus* were comparable to *ampicillin* (86.4% for both). Shivani et al., 2013 reported the antimicrobial inhibitory effect of leaves and flowers methanolic extracts against six strains **[16]**. Furthermore, the antibacterial and antifungal properties of crude extracts of *B. asiatica* aerial parts were evaluated and showed promising antimicrobial inhibitory effects **[17].** Also, our results are in agreement with the previous study done; which evaluated the antimicrobial activity of *B. asiatica* **[18].**

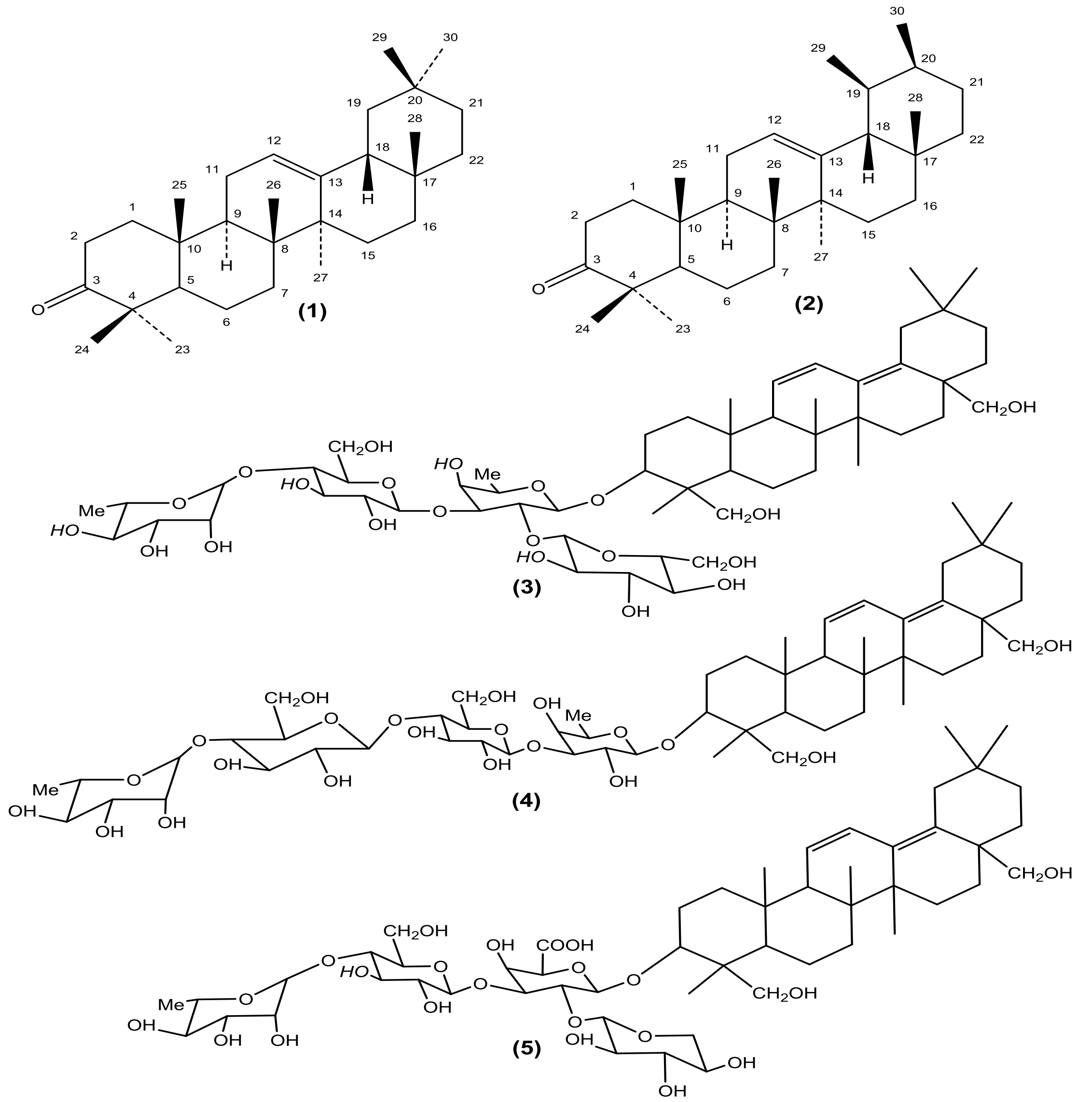

Fig. 1. Chemical structures of compounds 1-5

Table 1: The inhibition zone in mm and activity index% of extracts of *B. asiatica* compared to standard antibiotics

Microbial	C. Albicans (mg/ml)		*E. coli* (mg/ml)		*S. aureus* (mg/ml)	
Extract	Diameter of inhibition zone (mm)	% Activity index	Diameter of inhibition zone (mm)	% Activity index	Diameter of inhibition zone (mm)	% Activity index
essential oil (Ba1)	13	50.0	14	58.3	9	40.9
essential oil (Ba2)	10	38.5	15	62.5	12	54.5
Hexane (Ba3)	7	26.9	10	41.7	8	36.3
Methylene chloride (Ba4)	14	53.8	20	83.3	19	86.4
Ethyl acetate (Ba5)	20	76.9	22	91.7	19	86.4
Butanol (Ba6)	18	69.2	17	70.8	16	72.7
Colitrimazole	26	100	-	-	-	-
Ampicillin	-	-	24	100	22	100

Antioxidant activity assessment

There is a large number of *in vitro* methods that have been developed to evaluate the activity of natural antioxidants either in the form of pure compounds or as extracts **[19].** Furthermore, the researcher needs to use more than one method to evaluate antioxidant capacity due to the complex nature of phytochemicals. Therefore, the current study aimed to evaluate the antioxidant properties of *B. asiatica* via two different methods **[20]**.

Free radical scavenging method (DPPH)

Antioxidant activity of the extracts of *B. asiatica* were presented in Table 2, and Fig. 2. The free radicals of 1,1'-diphenyl-2-picrylhydrazyl (DPPH) is used for detection of the antioxidant activity of the plant extracts **[21, 22]**. Ethyl acetate extract has the highest scavenging activity. The scavenging effect of the extracts and standard on the DPPH radical decreased in the following order: ascorbic acid, ethyl acetate (Ba5), butanol (Ba6) (Table 2 and Fig. 2), respectively. Our results are in agreement with the previous study, which revealed that the methanol extract of the leaves of *B. asiatica* showed antioxidant activity toward the DPPH assay **[23].**

Table 2: Antioxidant activity of the extracts prepared from *B. asiatica* by DPPH

Extract	DPPH [IC_{50}] µg dry extract/ml
Ascorbic acid	**14.2**
Methylene chloride (Ba4)	**12.6**
Ethyl acetate (Ba5)	**135.8**
Butanol (Ba6)	**138.7**
Hexane (Ba3)	**-Ve**
methylene chloride essential oil (Ba2)	**-Ve**
Petroleum ether essential oil (Ba1)	**-Ve**

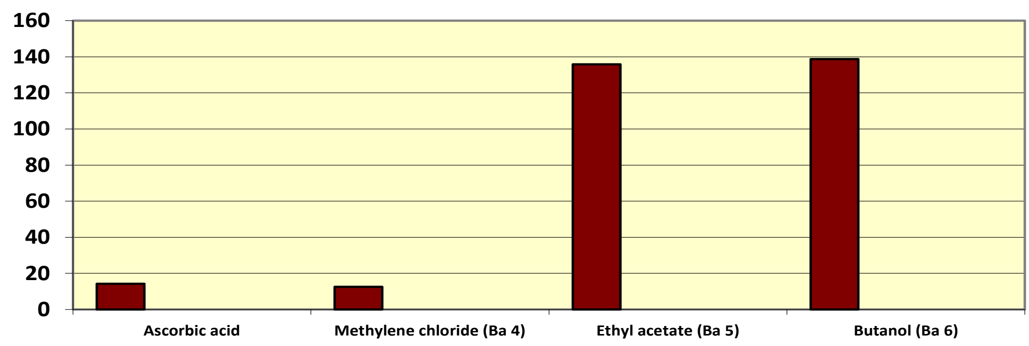

Fig. 2: The compare of antioxidant activity of the extracts prepared from *B. asiatica.*

Free radical scavenging method (ABTS).

The free radicals of 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) is used for detection of the antioxidant activity of the plant extracts **[24]. Ba5** has the highest scavenging activity. The scavenging effect of the extracts and standard on the ABTS radical decreased in the following order: ascorbic acid < **Ba5** < **Ba6** < **Ba4** < **Ba2** < **Ba3** < **Ba1**. (Table 3 and Fig. 3), respectively.

Table 3: The antioxidant activity of the extracts of *B. asiatica* by ABTS method.

Compound or extract	Absorbance of samples	%inhibition
Control of ABTS	0.510	0%
Ascorbic-acid	0.061	88.0%
Ba1	0.493	3.3%
Ba2	0.321	37.0%
Ba3	0.355	30.4%
Ba4	0.089	82.5%
Ba5	0.067	86.9%
Ba6	0.078	84.7%

Ba1= petroleum ether essential oil extract; **Ba2=** methylene chloride essential oil extract; **Ba3**= hexane extract; **Ba4=** methylene chloride extract; **Ba5=** ethyl acetate extract; **Ba6**= butanol extract.

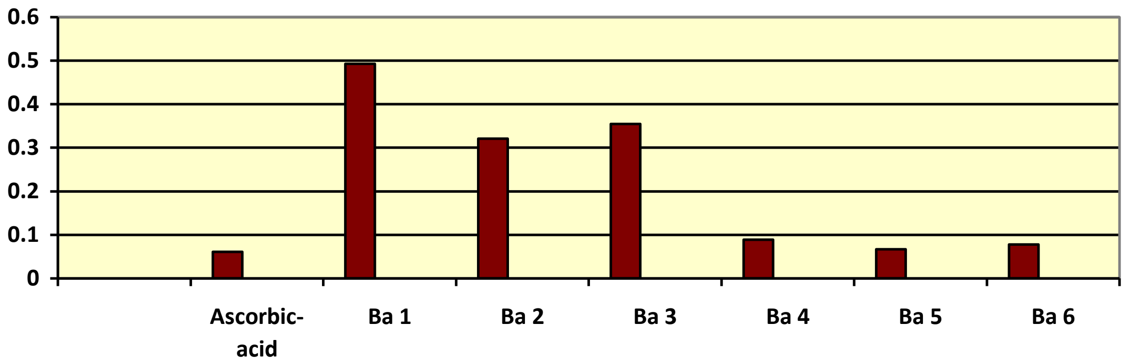

Fig. 3: the antioxidant activity of the extract of *B. asiatica* by ABTS Method.

Cytotoxic

In Egypt, liver cancer is the second cause of deaths from cancer after breast cancer and it is the third frequent occurring cancer after bladder and breast cancer and hepatocellular carcinoma (HCC) is a major health problem **[25].** The natural extracts have a vital role on cancer chemoprevention and chemotherapy **[26].**

The ethyl acetate, butanol, methylene chloride and essential oil Ba2, fractions were “strong”, which showed cytotoxic activity with IC_{50} ranged from 11.1±0.38 to 19.5±0.48 µg/ml. The hexane fraction and essential oil Ba1 fraction were “moderate” with IC_{50} 24.1±0.75 and 30.8±1.26 µg/ml, respectively. Mohamed *et al* reported that the methanol extract of *B. asiatica* showed significant cytotoxic activity against HepG2 cell line **[1]**.

The results (Table 4) indicated that the *in vitro* cytotoxicity against MCF-7, IC_{50} (µg/ml), of the methylene chloride fraction, butanol fractions and essential oil Ba2 fraction were “strong”, which showed cytotoxic activity with IC_{50} ranged from 11.9±0.72 to 20.5±1.04 µg/ml. The ethyl acetate fraction was “moderate” with IC_{50} 33.5±1.67 µg/ml. The hexane fraction and essential oil Ba1 fraction were “weak” with IC_{50} 51.9±2.83 and 69.9±3.41 µg/ml, respectively.

The results (Table 4) indicated that the *in vitro* cytotoxicity against HCT-116, IC_{50} (µg/ml), of the ethyl acetate fraction was “strong” with IC_{50} 19.8±0.68 µg/ml. On the other hand, the butanol, methylene chloride fractions were “moderate” with IC_{50} 28.8±1.66 and 41.4±1.90 µg/ml respectively, but the hexane and essential oil Ba2 fractions were “weak”. The essential oil Ba1 fraction was “non-cytotoxic”.

The results (Table 4) indicated that the *in vitro* cytotoxicity against PC3, IC_{50} (µg/ml), of the methylene chloride fraction was “strong” with IC_{50} 14.2±0.83 µg/ml, the butanol, essential oil Ba2, ethyl acetate and hexane fractions were "weak" with IC_{50} 23.0±0.85, 29.6±1.32, 39.9±2.65 and 50.3±3.11 µg/ml, respectively . The essential oil Ba1 fraction was "moderate" with IC_{50} 29.6±1.32 µg/ml.

Table 4: Cytotoxic activity assessment of *B. asiatica* extracts against human tumor cells HePG2, MCF-7, HCT-116 and PC3

Compounds	In vitro Cytotoxicity IC50 (µg/ml)•			
	HePG2	MCF-7	HCT-116	PC3
5-fluorouracil	6.6±0.24	4.7±0.11	8.4±0.20	9.6±0.27
essential oil Ba1	19.5±0.48	20.5±1.04	58.0±2.34	29.6±1.32
essential oil Ba2	30.8±1.26	69.9±3.41	>100	70.0±3.89
Hexane Ba3	24.1±0.75	51.9±2.83	83.7±4.21	50.3±3.11
methylene chloride Ba4	14.5±0.89	11.9±0.72	41.4±1.90	14.2±0.83
Ethyl acetate Ba5	11.1±0.38	33.5±1.67	19.8±0.68	39.9±2.65
Butanol Ba6	14.3±0.23	16.0±0.76	28.8±1.66	23.0±0.85

IC_{50} (µg/ml): 1 – 10 (very strong). 11 – 20 (strong). 21 – 50 (moderate). 51 – 100 (weak) and above 100 (non-cytotoxic).

Table 5: MS data of compounds identified by GC/MS analyses (m/z [identity] (rel.int. %))

Name	MS Data :m/z [identity](rel. abound.%)
decane	142 $[M]^+$ (6.66), 113 $[C_8H_{17}]^+$ (4.44), 99 $[C_7H_{15}]^+$ (7.77), 85 $[C_6H_{13}]^+$ (28.88), 71 $[C_5H_{11}]^+$ (42.22), 57 $[C_4H_9]^+$ (100).
undecane	156 $[M]^+$ (10), 127 $[C_9H_{19}]^+$ (3.33), 113 $[C_8H_{17}]^+$ (4.44), 99 $[C_7H_{15}]^+$ (10), 85 $[C_6H_{13}]^+$ (33.33), 71 $[C_5H_{11}]^+$ (55.55), 57 $[C_4H_9]^+$ (100).
dodecane	170 $[M]^+$ (6.66), 141 (2.22) $[C_{10}H_{21}]^+$, 127 $[C_9H_{19}]^+$ (5.55), 113 $[C_8H_{17}]^+$ (6.66), 99 $[C_7H_{15}]^+$ (10), 85 $[C_6H_{13}]^+$ (36.66), 71 $[C_5H_{11}]^+$ (62.22), 57 $[C_4H_9]^+$ (100).
tridecane	184 $[M]^+$ (5.55), 155 $[C_{11}H_{23}]^+$ (1.11), 141 $[C_{10}H_{21}]^+$ (7.77), 127 $[C_9H_{19}]^+$ (4.44), 113 $[C_8H_{17}]^+$ (5.55), 99 $[C_7H_{15}]^+$ (8.88), 85 $[C_6H_{13}]^+$ (41.11), 71 $[C_5H_{11}]^+$ (65.55), 57 $[C_4H_9]^+$ (100).
tetradecane	198 $[M]^+$ (5), 169 $[C_{12}H_{25}]^+$ (1.11), 155 $[C_{11}H_{23}]^+$ (2.22), 141 $[C_{10}H_{21}]^+$ (6.66), 127 $[C_9H_{19}]^+$ (5.55), 113 $[C_8H_{17}]^+$ (6.66), 99 $[C_7H_{15}]^+$ (11.11), 85 $[C_6H_{13}]^+$ (43.33), 71 $[C_5H_{11}]^+$ (71.11), 57 $[C_4H_9]^+$ (100).
pentadecane	212 $[M]^+$ (3.33), 169 $[C_{12}H_{25}]^+$ (2.22), 155 $[C_{11}H_{23}]^+$ (10), 141 $[C_{10}H_{21}]^+$ (4.44), 127 $[C_9H_{19}]^+$ (5), 113 $[C_8H_{17}]^+$ (6.66), 105 (23.33), 99 $[C_7H_{15}]^+$ (12.22), 85 $[C_6H_{13}]^+$ (45.55), 71 $[C_5H_{11}]^+$ (70), 57 $[C_4H_9]^+$ (100).
hexadecane	226 $[M]^+$ (12.22), 183 $[C_{13}H_{27}]^+$ (2.22), 169 $[C_{12}H_{25}]^+$ (4.39), 155 $[C_{11}H_{23}]^+$ (4.44), 141 $[C_{10}H_{21}]^+$ (5.49), 127 $[C_9H_{19}]^+$ (6.59), 113 $[C_8H_{17}]^+$ (10.98), 99 $[C_7H_{15}]^+$ (16.48), 85 $[C_6H_{13}]^+$ (57.14), 71 $[C_5H_{11}]^+$ (69.23), 57 $[C_4H_9]^+$ (100).
heptadecane	240 $[M]^+$ (3.33), 197 $[C_{14}H_{29}]^+$ (1.11), 183 $[C_{13}H_{27}]^+$ (2.22), 169 $[C_{12}H_{25}]^+$ (2.22), 155 $[C_{11}H_{23}]^+$ (3.33), 141 $[C_{10}H_{21}]^+$ (4.44), 127 $[C_9H_{19}]^+$ (5.55), 113 $[C_8H_{17}]^+$ (8.88), 105 (58.88), 99 $[C_7H_{15}]^+$ (15.55), 85 $[C_6H_{13}]^+$ (46.66), 71 $[C_5H_{11}]^+$ (68.88), 57 $[C_4H_9]^+$ (100).
octadecane	254 $[M]^+$ (3.33), 239 $[C_{17}H_{35}]^+$ (1.11), 255 $[C_{16}H_{33}]^+$ (1.11), 211 $[C_{15}H_{31}]^+$ (1.11), 197 $[C_{14}H_{29}]^+$ (1.11), 183 $[C_{13}H_{27}]^+$ (2.22), 169 $[C_{12}H_{25}]^+$ (3.33), 155 $[C_{11}H_{23}]^+$ (4.44), 141 $[C_{10}H_{21}]^+$ (5.55), 127 $[C_9H_{19}]^+$ (6.66), 113 $[C_8H_{17}]^+$ (11.11), 99 $[C_7H_{15}]^+$ (16.66), 85 $[C_6H_{13}]^+$ (48.88), 71 $[C_5H_{11}]^+$ (67.77), 57 $[C_4H_9]^+$ (100).
nonadecane	268 $[M]^+$ (3.33), 225 $[C_{16}H_{33}]^+$ (1.11), 197 $[C_{14}H_{29}]^+$ (3.33), 183 $[C_{13}H_{27}]^+$ (2.22), 169 $[C_{12}H_{25}]^+$ (3.33), 155 $[C_{11}H_{23}]^+$ (4.44), 141 $[C_{10}H_{21}]^+$ (5.55), 127 $[C_9H_{19}]^+$ (6.66), 113 $[C_8H_{17}]^+$ (9.99), 105 (60.66), 99 $[C_7H_{15}]^+$ (17), 85 $[C_6H_{13}]^+$ (49), 71 $[C_5H_{11}]^+$ (70), 57 $[C_4H_9]^+$ (100).
eicosane	282 $[M]^+$ (3.33), 239 $[C_{17}H_{35}]^+$ (5.55), 225 $[C_{16}H_{33}]^+$ (2.22), 197 $[C_{14}H_{29}]^+$ (2.22), 183 $[C_{13}H_{27}]^+$ (2.22), 169 $[C_{12}H_{25}]^+$ (3.33), 155 $[C_{11}H_{23}]^+$ (3.33), 141 $[C_{10}H_{21}]^+$ (5.55), 127 $[C_9H_{19}]^+$ (7.77), 113 $[C_8H_{17}]^+$ (10), 99 $[C_7H_{15}]^+$ (18), 85 $[C_6H_{13}]^+$ (51), 71 $[C_5H_{11}]^+$ (71), 57 $[C_4H_9]^+$ (100).
heneicosane	296 $[M]^+$ (2.22), 253 $[C_{18}H_{37}]^+$ (1), 239 $[C_{17}H_{35}]^+$ (1.11), 225 $[C_{16}H_{33}]^+$ (1.11), 197 $[C_{14}H_{29}]^+$ (1.66), 183 $[C_{13}H_{27}]^+$ (2.22), 169 $[C_{12}H_{25}]^+$ (3.33), 155 $[C_{11}H_{23}]^+$ (4.44), 141 $[C_{10}H_{21}]^+$ (5.55), 127 $[C_9H_{19}]^+$ (6.66), 113 $[C_8H_{17}]^+$ (11.11), 99 $[C_7H_{15}]^+$ (16.66), 85 $[C_6H_{13}]^+$ (48.88), 71 $[C_5H_{11}]^+$ (71.11), 57 $[C_4H_9]^+$ (100).
docosane	310 $[M]^+$ (2.22), 281 $[C_{20}H_{41}]^+$ (1), 255 $[C_{18}H_{37}]^+$ (1), 239 $[C_{17}H_{35}]^+$ (1),225 $[C_{16}H_{33}]^+$ (1.11), 197 $[C_{14}H_{29}]^+$ (1.33), 183 $[C_{13}H_{27}]^+$ (2.22), 169 $[C_{12}H_{25}]^+$ (3.33) 155 $[C_{11}H_{23}]^+$ (4.44), 141 $[C_{10}H_{21}]^+$ (5.55), 127 $[C_9H_{19}]^+$ (8.88), 113 $[C_8H_{17}]^+$ (11.11), 99 $[C_7H_{15}]^+$ (20), 85 $[C_6H_{13}]^+$ (56), 71 $[C_5H_{11}]^+$ (78.33), 57 $[C_4H_9]^+$ (100).
tricosane	324 $[M]^+$ (2.22), 281 $[C_{20}H_{41}]^+$ (1), 253 $[C_{18}H_{37}]^+$ (1), 239 $[C_{17}H_{35}]^+$ (1), 225 $[C_{16}H_{33}]^+$ (1.11), 197 $[C_{14}H_{29}]^+$ (1.33), 183 $[C_{13}H_{27}]^+$ (2.22), 169 $[C_{12}H_{25}]^+$ (3.33), 155 $[C_{11}H_{23}]^+$ (4.44), 141

	$[C_{10}H_{21}]^+$ (5.55), 127 $[C_9H_{19}]^+$ (6.66), 113 $[C_8H_{17}]^+$ (11.11), 99 $[C_7H_{15}]^+$ (16.66), 85 $[C_6H_{13}]^+$ (47.77), 71 $[C_5H_{11}]^+$ (72.22), 57 $[C_4H_9]^+$ (100).
tetracosane	338 $[M]^+$ (2.22), 281 $[C_{20}H_{41}]^+$ (1), 253 $[C_{18}H_{37}]^+$ (1), 239 $[C_{17}H_{35}]^+$ (1), 225 $[C_{16}H_{33}]^+$ (1.11), 197 $[C_{14}H_{29}]^+$ (1.33), 183 $[C_{13}H_{27}]^+$ (2.22), 169 $[C_{12}H_{25}]^+$ (3.33) 155 $[C_{11}H_{23}]^+$ (4.44), 141 $[C_{10}H_{21}]^+$ (5.55), 127 $[C_9H_{19}]^+$ (6.66), 113 $[C_8H_{17}]^+$ (11.11), 99 $[C_7H_{15}]^+$ (17.77), 85 $[C_6H_{13}]^+$ (50), 71 $[C_5H_{11}]^+$ (70), 57 $[C_4H_9]^+$ (100).
pentacosane	352 $[M]^+$ (2.22), 281 $[C_{20}H_{41}]^+$ (1), 253 $[C_{18}H_{37}]^+$ (1), 239 $[C_{17}H_{35}]^+$ (1), 225 $[C_{16}H_{33}]^+$ (1.11), 197 $[C_{14}H_{29}]^+$ (1.33), 183 $[C_{13}H_{27}]^+$ (2.22), 169 $[C_{12}H_{25}]^+$ (3.33), 155 $[C_{11}H_{23}]^+$ (4.44), 141 $[C_{10}H_{21}]^+$ (5.55), 127 $[C_9H_{19}]^+$ (6.66), 113 $[C_8H_{17}]^+$ (11.11), 99 $[C_7H_{15}]^+$ (17.77), 85 $[C_6H_{13}]^+$ (50), 71 $[C_5H_{11}]^+$ (72.22), 57 $[C_4H_9]^+$ (100).
hexacosane	366 $[M]^+$ (2.22), 309 $[C_{22}H_{45}]^+$ (1), 281 $[C_{20}H_{41}]^+$ (1), 253 $[C_{18}H_{37}]^+$ (1), 239 $[C_{17}H_{35}]^+$ (1), 225 $[C_{16}H_{33}]^+$ (1.11), 197 $[C_{14}H_{29}]^+$ (1.33), 183 $[C_{13}H_{27}]^+$ (2.22), 169 $[C_{12}H_{25}]^+$ (3.33), 155 $[C_{11}H_{23}]^+$ (4.44), 141 $[C_{10}H_{21}]^+$ (5.55), 127 $[C_9H_{19}]^+$ (6.66), 113 $[C_8H_{17}]^+$ (11.11), 99 $[C_7H_{15}]^+$ (16.66), 85 $[C_6H_{13}]^+$ (47.77), 71 $[C_5H_{11}]^+$ (73.33), 57 $[C_4H_9]^+$ (100).
heptacosane	380 $[M]^+$ (2.22), 323 $[C_{23}H_{47}]^+$ (1), 309 $[C_{22}H_{45}]^+$ (1), 281 (1) $[C_{20}H_{41}]^+$, 253 $[C_{18}H_{37}]^+$(1), 239 $[C_{17}H_{35}]^+$ (1), 225 $[C_{16}H_{33}]^+$ (1.11), 197 $[C_{14}H_{29}]^+$ (1.33), 183 $[C_{13}H_{27}]^+$ (2.22), 169 $[C_{12}H_{25}]^+$ (3.33), 155 $[C_{11}H_{23}]^+$ (4.44), 141 $[C_{10}H_{21}]^+$ (5.55), 127 $[C_9H_{19}]^+$ (6.66), 113 $[C_8H_{17}]^+$ (11.11), 99 $[C_7H_{15}]^+$ (16.66), 85 $[C_6H_{13}]^+$ (47.77), 71 $[C_5H_{11}]^+$ (73.33), 57 $[C_4H_9]^+$ (100).
octacosane	394 $[M]^+$ (2.22), 323 $[C_{23}H_{47}]^+$ (1), 309 $[C_{22}H_{45}]^+$ (1), 281 $[C_{20}H_{41}]^+$ (1), 253 $[C_{18}H_{37}]^+$ (1), 239 $[C_{17}H_{35}]^+$ (1), 225 $[C_{16}H_{33}]^+$ (1.11), 197 $[C_{14}H_{29}]^+$ (1.33), 183 $[C_{13}H_{27}]^+$ (2.22), 169 $[C_{12}H_{25}]^+$ (3.33), 155 $[C_{11}H_{23}]^+$ (4.44), 141 $[C_{10}H_{21}]^+$ (5.55), 127 $[C_9H_{19}]^+$ (6.66), 113 $[C_8H_{17}]^+$ (11.11), 99 $[C_7H_{15}]^+$ (16.66), 85 $[C_6H_{13}]^+$ (47.77), 71 $[C_5H_{11}]^+$ (73.33), 57 $[C_4H_9]^+$ (100).
nonacosane	408 $[M]^+$ (2.22), 323 $[C_{23}H_{47}]^+$ (1), 309 $[C_{22}H_{45}]^+$ (1), 281 $[C_{20}H_{41}]^+$ (1), 253 $[C_{18}H_{37}]^+$ (1), 239 $[C_{17}H_{35}]^+$ (1), 225 $[C_{16}H_{33}]^+$ (1.11), 197 $[C_{14}H_{29}]^+$ (1.33), 183 $[C_{13}H_{27}]^+$ (2.22), 169 $[C_{12}H_{25}]^+$ (3.33), 155 $[C_{11}H_{23}]^+$ (4.44), 141 $[C_{10}H_{21}]^+$ (5.55), 127 $[C_9H_{19}]^+$ (6.66), 113 $[C_8H_{17}]^+$ (11.11), 99 $[C_7H_{15}]^+$ (16.66), 85 $[C_6H_{13}]^+$ (47.77), 71 $[C_5H_{11}]^+$ (73.33), 57 $[C_4H_9]^+$ (100).
2-methylundecane	170 $[M]^+$ (1.11), 155 $[C_{11}H_{23}]^+$ (1.66), 148 (5.55), 141 $[C_{10}H_{21}]^+$ (1.66), 127 $[C_9H_{19}]^+$ (13.33), 119 (22.22), 113 $[C_8H_{17}]^+$ (4.44), 99 $[C_7H_{15}]^+$ (12.22), 85 $[C_6H_{13}]^+$ (48.88), 71 $[C_5H_{11}]^+$ (70), 57 $[C_4H_9]^+$ (100).
2-methyltridecane	198 $[M]^+$ (1.11), 183 $[C_{13}H_{27}]^+$ (1.11), 155 $[C_{11}H_{23}]^+$ (8.88), 141 $[C_{10}H_{21}]^+$ (2.22), 127 (5.55) $[C_9H_{19}]^+$, 113 $[C_8H_{17}]^+$ (10), 99 $[C_7H_{15}]^+$ (20), 85 $[C_6H_{13}]^+$ (47), 71 $[C_5H_{11}]^+$ (67), 57 $[C_4H_9]^+$ (100).
2-methyltetradecane	212 $[M]^+$ (3.33), 183 $[C_{13}H_{27}]^+$ (2.22), 169 $[C_{12}H_{25}]^+$ (2.22), 155 $[C_{11}H_{23}]^+$ (3.33), 141 $[C_{10}H_{21}]^+$ (6.66), 127 $[C_9H_{19}]^+$ (4.44), 113 $[C_8H_{17}]^+$ (5.55), 105 (23.33), 99 $[C_7H_{15}]^+$ ^{n}(13.33), 85 $[C_6H_{13}]^+$ (42.22), 71 $[C_5H_{11}]^+$ (68.88), 57 $[C_4H_9]^+$ (100).
2-methyloctadecane	268 $[M]^+$ (2.22), 225 $[C_{16}H_{33}]^+$ (2), 197 $[C_{14}H_{29}]^+$ (2.2), 183 $[C_{13}H_{27}]^+$ (2.22), 169 $[C_{12}H_{25}]^+$ (2.2), 155 $[C_{11}H_{23}]^+$ (4.4), 141 $[C_{10}H_{21}]^+$(5.55), 127 $[C_9H_{19}]^+$ (7.77), 119 (17.77), 113 $[C_8H_{17}]^+$ (9.99), 99 $[C_7H_{15}]^+$ (14.44), 85 $[C_6H_{13}]^+$ (46.66), 71 $[C_5H_{11}]^+$ (66.66), 57 $[C_4H_9]^+$ (100).
2-methylnonadecane	282 $[M]^+$ (2.22), 239 $[C_{17}H_{35}]^+$ (5.55), 225 $[C_{16}H_{33}]^+$ (2.22), 197 $[C_{14}H_{29}]^+$ (2.22), 183 $[C_{13}H_{27}]^+$ (3.33), 155 $[C_{11}H_{23}]^+$ (5.55), 127 $[C_9H_{19}]^+$ (6.66), 113 $[C_8H_{17}]^+$ (8.88), 99 $[C_7H_{15}]^+$ (16.66), 85 $[C_6H_{13}]^+$ (45), 71 $[C_5H_{11}]^+$ (63), 57 $[C_4H_9]^+$ (100).
cetene	224 $[M]^+$ (7.77), 195 $[C_{14}H_{27}]^+$ (4.44), 181 $[C_{13}H_{25}]^+$ (1.11), 167 $[C_{12}H_{23}]^+$ (2.22), 153 $[C_{11}H_{21}]^+$ (2.55), 139 $[C_{10}H_{19}]^+$ (5.55), 125 $[C_9H_{17}]^+$ (20) 111 $[C_8H_{15}]^+$ (48.88), 97 $[C_7H_{13}]^+$ (100), 83 $[C_6H_{11}]^+$ (97.77), 69 $[C_5H_9]^+$ (78.88) , 55 $[C_4H_7]^+$ (90).
1-octadecene	252 $[M]^+$ (8.79), 223 $[C_{16}H_{31}]^+$ (3.29), 195 $[C_{14}H_{27}]^+$ (1.09), 181 $[C_{13}H_{25}]^+$ (1.11), 153 $[C_{11}H_{21}]^+$ (3.44), 167 $[C_{12}H_{23}]^+$ (3.29), 139 $[C_{10}H_{19}]^+$ (8.79), 125 $[C_9H_{17}]^+$ (25.27), 111 $[C_8H_{15}]^+$ (57.14), 97 $[C_7H_{13}]^+$ (100), 83 $[C_6H_{11}]^+$ (96.70), 69 $[C_5H_9]^+$ (76.82) , 55 $[C_4H_7]^+$ (79.12).
1-nonadecene	266 $[M]^+$ (1.11), 207 $[C_{15}H_{27}]^+$ (4.44), 181 $[C_{13}H_{25}]^+$ (2.22), 195 $[C_{14}H_{27}]^+$ (2.09), 153 $[C_{11}H_{21}]^+$ (3.22), 167 $[C_{12}H_{23}]^+$ (3.33), 135 $[C_{10}H_{15}]^+$ (15.55) , 139 $[C_{10}H_{19}]^+$ (8.88) , 111 $[C_8H_{15}]^+$ (28.88), 97 $[C_7H_{13}]^+$ (100), 83 $[C_6H_{11}]^+$ (55.55), 69 $[C_5H_9]^+$ (64.44) , 55 $[C_4H_7]^+$ (73.33).
1-docosene	308 $[M]^+$ (7.14), 280 $[C_{20}H_{40}]^+$ (2.19), 195 $[C_{14}H_{27}]^+$ (2.19), 167 $[C_{12}H_{23}]^+$(4.39), 153 $[C_{11}H_{21}]^+$ (6.59), 139 $[C_{10}H_{19}]^+$ (10.98) ,125 $[C_9H_{17}]^+$ (26.37), 111 $[C_8H_{15}]^+$ (54.94), 97 $[C_7H_{13}]^+$ (100), 83 $[C_6H_{11}]^+$ (90.10), 69 $[C_5H_9]^+$ (65.93) , 57 $[C_5H_{11}]^+$ (79.12).
1-tetracosene	336 $[M]^+$ (4.39), 281 $[C_{20}H_{41}]^+$ (3.29), 223 $[C_{16}H_{31}]^+$ (3.29), 196 $[C_{14}H_{28}]^+$ (4.39), 167 $[C_{12}H_{23}]^+$ (7.69), 139 $[C_{10}H_{19}]^+$ (13.18), 125 $[C_9H_{17}]^+$ (26.37), 111 $[C_8H_{15}]^+$ (52.74), 97 $[C_7H_{13}]^+$ (100), 83 $[C_6H_{11}]^+$(70.32), 69 $[C_5H_9]^+$ (70.87) , 57 $[C_4H_9]^+$ (80.21).
2-methyl-1,11-dodecadiene	180 $[M]^+$ (2.22), 165 $[C_{12}H_{21}]^+$ (6.66), 139 $[C_{10}H_{19}]^+$ (11.11), 131 (21.11) 111 $[C_8H_{15}]^+$ (7.77), 99 $[C_7H_{15}]^+$ (12.22), 85 $[C_6H_{13}]^+$ (49.99), 71 $[C_5H_{11}]^+$ (69.99), 57 $[C_5H_9]^+$ (100).
1,12-tridecadiene	180 $[M]^+$ (2.22), 165 (5.55), 139 (2.22) $[C_{10}H_{19}]^+$, 125 $[C_9H_{17}]^+$ (3.33), 111 $[C_8H_{15}]^+$ (14.44), 99 $[C_7H_{15}]^+$ (12.22), 85 $[C_6H_{13}]^+$ (21.11), 71 $[C_5H_{11}]^+$ (78.88), 57 $[C_4H_9]^+$ (100).

1-eicosanol	298 $[M]^+$ (1.11), 280 $[C_{20}H_{40}]^+$ (8.79), 252 $[C_{18}H_{36}]^+$ (3.29), 209 $[C_{15}H_{29}]^+$ (2.22), 181 $[C_{13}H_{25}]^+$ (2.19), 153 $[C_{11}H_{21}]^+$ (6.59), 139 $[C_{10}H_{19}]^+$ (10.98), 125 $[C_9H_{17}]^+$ (27.47), 111 $[C_8H_{15}]^+$ (54.94), 97 $[C_7H_{13}]^+$ (100), 83 $[C_6H_{11}]^+$ (96.70), 69 $[C_5H_9]^+$ (74.72) , 57 $[C_5H_{11}]^+$ (87.91).
n-tetracosanol	354 $[M]^+$ (1.11), 336 $[C_{24}H_{48}]^+$ (7.69), 308 $[C_{22}H_{44}]^+$ (2.19), 237 $[C_{17}H_{33}]^+$ (1.09), 223 $[C_{16}H_{31}]^+$ (1.09), 209 $[C_{15}H_{29}]^+$ (1.64), 181 $[C_{13}H_{25}]^+$ (4.93), 167 $[C_{12}H_{23}]^+$ (6.59), 139 $[C_{10}H_{19}]^+$ (13.18), 125 $[C_9H_{17}]^+$ (28.57), 111 $[C_8H_{15}]^+$ (54.94), 97 $[C_7H_{13}]^+$ (100), 83 $[C_6H_{11}]^+$ (94.50), 69 $[C_5H_9]^+$ (70.32) , 57 $[C_5H_{11}]^+$ (91.20).
heptadecanoic acid	270 $[M]^+$ (11.53), 227 $[C_{14}H_{27}O_2]^+$ (6.59), 199 $[C_{12}H_{23}O_2]^+$ (6.59), 171 $[C_{10}H_{19}O_2]^+$ (9.89), 143 $[C_8H_{15}O_2]^+$ (21.97), 97 $[C_5H_5O_2]^+$ (27.47), 87 $[C_4H_7O_2]^+$ (71.42), 74 $[C_3H_6O_2]^+$ (100), 55 $[C_4H_7]^+$ (73.62).
oleic acid	282 $[M]^+$ (2.22), 264 $[C_{18}H_{32}O]^+$ (4.44), 235 $[C_{17}H_{31}]^+$ (1.11), 221 $[C_{16}H_{29}]^+$ (1.11), 207 $[C_{15}H_{27}]^+$ (4.44) , 153 $[C_{11}H_{21}]^+$ (6.66), 137 $[C_{10}H_{17}]^+$ (8.88), 111 $[C_8H_{15}]^+$ (18.88), 97 $[C_7H_{13}]^+$ (41.11) , 83 $[C_6H_{11}]^+$ (53.33), 69 $[C_5H_9]^+$ (64.44), 55 $[C_4H_7]^+$ (100).
linolic acid	280 $[M]^+$ (2.74), 207 $[C_{13}H_{19}O_2]^+$ (3.29), 167 $[C_{10}H_{15}O_2]^+$ (4.39), 139 $[C_8H_{11}O_2]^+$ (9.89), 111 $[C_8H_{15}]^+$ (50.54), 97 $[C_7H_{13}]^+$ (90.10), 83 $[C_5H_7O]^+$ (90.10), 69 $[C_5H_9]^+$ (84.61), 55 $[C_4H_7]^+$ (100).
methyl hexadecanoate	270 $[M]^+$ (5.55), 255 $[C_{16}H_{31}O_2]^+$ (1.68), 241 $[C_{15}H_{29}O_2]^+$ (2.38), 227 $[C_{14}H_{27}O_2]^+$ (5.55), 199 $[C_{12}H_{23}O_2]^+$ (3.33), 171 $[C_{10}H_{19}O_2]^+$ (4.44), 143 $[C_8H_{15}O_2]^+$ (14.44), 129 $[C_7H_{13}O_2]^+$ (6.66), 97 $[C_6H_9O]^+$ (12.22), 87 $[C_4H_7O_2]^+$ (60), 74 $[C_3H_6O_2]^+$ (100), 55 $[C_4H_7]^+$ (39).
octadecanoic acid methyl ester	**298** $[M]^+$ (35.16), 267 $[C_{18}H_{35}O]^+$ (8.79), 255 $[C_{16}H_{31}O_2]^+$ (16.48), 241 $[C_{15}H_{29}O_2]^+$ (4.38), 213 $[C_{13}H_{25}O_2]^+$ (5.49), 199 $[C_{12}H_{23}O_2]^+$ (13.18), 185 $[C_{11}H_{21}O_2]^+$ (4.39), 143 $[C_8H_{15}O_2]^+$ (24.17), 97 $[C_6H_9O]^+$ (10.11), 87 $[C_4H_7O_2]^+$ (68.13), 74 $[C_3H_6O_2]^+$ (100), 55 $[C_4H_7]^+$ (17.58).
(Z)9-octadecenoic acid methyl ester	296 $[M]^+$ (9.89), 264 $[C_{18}H_{32}O]^+$ (36.16), 236 $[C_{17}H_{34}]^+$ (6.59), 222 $[C_{16}H_{30}]^+$ (17.58), 207 $[C_{15}H_{27}]^+$ (3.29), 180 $[C_{13}H_{24}]^+$ (13.18), 166 $[C_{12}H_{22}]^+$ (8.79), 150 $[C_{10}H_{15}]^+$ (15.38), 111 $[C_8H_{15}]^+$ (32.96), 95 $[C_7H_{11}]^+$ (68.13), 85 $[C_6H_{13}]^+$ (100), 67 $[C_5H_7]^+$ (71.42), 55 $[C_4H_7]^+$ (93.40).
(Z,Z)-9,12-octadecadienoic acid methyl ester	294 $[M]^+$ (28.57), 263 $[C_{18}H_{31}O]^+$ (15.38), 220 $[C_{16}H_{28}]^+$ (5.49), 178 $[C_{13}H_{22}]^+$ (7.69), 163 $[C_{12}H_{19}]^+$ (8.79), 150 $[C_{11}H_{18}]^+$ (16.48), 123 $[C_9H_{15}]^+$ (19.78), 109 $[C_8H_{13}]^+$ (35.16), 95 $[C_7H_{11}]^+$ (72.52), 81 $[C_6H_9]^+$ (96.70), 67 $[C_5H_7]^+$ (100), 55 $[C_4H_7]^+$ (60.43).
Methyl 9,12,15-octadecatrienoate	292 $[M]^+$ (2.22), 263 $[C_{18}H_{31}O]^+$ (2.22), 163 $[C_{12}H_{19}]^+$ (3.33), 149 $[C_{11}H_{17}]^+$ (10), 134 $[C_{10}H_{15}]^+$ (11.11), 121 (16.66), 108 $[C_8H_{12}]^+$ (33.33), 95 $[C_7H_{11}]^+$ (57.77), 79 $[C_6H_7]^+$ (100), 67 $[C_5H_7]^+$ (67.77), 55 $[C_4H_7]^+$ (64.44).
6,9,12,15-docosatetraenoic acid methyl ester	346 $[M]^+$ (1.11), 281 $[C_{20}H_{25}O]^+$ (5.21), 207 $[C_{15}H_{27}]^+$ (21.21), 193 $[C_{14}H_{25}]^+$ (6.06), 165 $[C_{12}H_{21}]^+$ (6.66), 149 $[C_{11}H_{17}]^+$ (10.60), 111 $[C_8H_{15}]^+$ (15.15), 95 $[C_7H_{11}]^+$ (48.48), 71 $[C_5H_{11}]^+$ (96.96), 55 $[C_4H_7]^+$ (100).
(Z,Z,Z)-9,12,15-octadecatrienoic acid ethyl ester	306 $[M]^+$ (13.18), 277 $[C_{18}H_{29}O_2]^+$ (4.39), 264 $[C_{18}H_{32}O]^+$ (8.79), 222 $[C_{16}H_{30}]^+$ (6.59), 178 $[C_{13}H_{22}]^+$ (7.69), 163 $[C_{12}H_{19}]^+$ (8.79), 149 $[C_{11}H_{17}]^+$ (19.78), 135 $[C_{10}H_{15}]^+$ (23.07), 121 $[C_9H_{13}]^+$ (28.57), 108 $[C_8H_{12}]^+$ (50.54), 95 $[C_7H_{11}]^+$ (65.93), 79 $[C_6H_7]^+$ (100), 67 $[C_5H_7]^+$ (69.23).
Propyl-9.12.15-eicosatrien oate	348 $[M]^+$ (28.57), 319 $[C_{21}H_{35}O_2]^+$ (3.29), 261 $[C_{19}H_{33}]^+$ (7.69), 221 $[C_{16}H_{29}]^+$ (6.59), 191 $[C_{14}H_{23}]^+$ (5.49), 163 $[C_{12}H_{19}]^+$ (8.79), 149 $[C_{11}H_{17}]^+$ (17.58), 135 $[C_{10}H_{15}]^+$ (20.87), 121 $[C_9H_{13}]^+$ (25.27), 108 $[C_8H_{12}]^+$ (51.64), 95 $[C_7H_{11}]^+$ (59.34), 71 $[C_5H_{11}]^+$ (100), 67 $[C_5H_7]^+$ (53.84), 55 $[C_4H_7]^+$ (57.14).
(E)-10-heptadecen-8-ynoic acid methyl ester	278 $[M]^+$ (1.72), 247 $[C_{17}H_{27}O]^+$ (1.11), 205 $[C_{15}H_{25}]^+$ (8.62), 189 $[C_{14}H_{21}]^+$ (51.72), 175 $[C_{13}H_{19}]^+$ (6.89) , 161 $[C_{12}H_{17}]^+$ (60.34), 147 $[C_{11}H_{15}]^+$ (31.03), 133 $[C_{10}H_{13}]^+$ (74.13), 105 (81.03) ,91 (94.82). 69 $[C_5H_9]^+$ (60.34), 55 $[C_4H_7]^+$ (100).
α-Pinene	136 $[M]^+$ (8.88), 121 $[C_9H_{13}]^+$ (11.11), 105 $[C_8H_9]^+$ (12.22), 93 $[C_7H_9]^+$ (100), 77 $[C_6H_5]^+$ (35.55).
sabinene	136 $[M]^+$ (9), 121 $[C_9H_{13}]^+$ (10) ,105 $[C_8H_9]^+$ (14.44), 93 $[C_7H_9]^+$ (100), 77 $[C_6H_5]^+$ (25).
α-Terpinyl acetate	196 $[M]^+$ (1.11), 154 $[C_{10}H_{18}O]^+$ (10), 136 $[C_{10}H_{16}]^+$ (18.88), 119 $[C_9H_{11}]^+$ (21.11), 107 $[C_8H_{11}]^+$ (18.88) , 93 $[C_7H_9]^+$ (86.66), 79 $[C_6H_7]^+$ (43.33), 68 $[C_5H_8]^+$ (100).
fenchone	152 $[M]^+$ (11.11), 137 $[C_9H_{13}O]^+$ (2.22), 123 $[C_8H_{11}O]^+$ (1.11) ,109 $[C_7H_9O]^+$ (6.66) , 81 $[C_6H_9]^+$ (100), 68 $[C_5H_9]^+$ (47.77).
camphor	152 $[M]^+$ (23.33), 137 $[C_9H_{13}O]^+$ (3.33), 123 $[C_8H_{11}O]^+$ (3.33) ,108 $[C_7H_8O]^+$ (40), 95 $[C_6H_7O]^+$ (100), 83 $[C_6H_{11}]^+$ (93.33), 69 $[C_5H_9]^+$ (64.44).
thymoquinone	164 $[M]^+$ (63.73), 149 $[C_9H_9O_2]^+$ (100), 136 $[C_8H_8O_2]^+$ (30.76), 121 $[C_7H_5O_2]^+$ (87.91), 108 $[C_6H_4O_2]^+$ (16.48), 93 $[C_6H_5O]^+$ (42.85), 77 $[C_6H_5]^+$ (38.46).
Loliolide	196 $[M]^+$ (12.08), 178 $[C_{11}H_{14}O_2]^+$ (49.45), 163 $[C_{10}H_{11}O_2]^+$ (26.37), 135 $[C_8H_7O_2]^+$ (40.65), 111 $[C_7H_{11}O]^+$ (45.82), 95 $[C_7H_{11}]^+$ (37.36), 67 $[C_5H_7]^+$ (38.46), 57 $[C_4H_9]^+$ (43.95), 43 $[C_3H_7]^+$ (100).
farnesane	212 $[M]^+$ (1.11), 183 $[C_{13}H_{27}]^+$ (1.11), 165 $[C_{12}H_{25}]^+$ (5.55), 155 $[C_{11}H_{23}]^+$ (2.22), 141

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	$[C_{10}H_{21}]^+$ (2.22), 127 $[C_9H_{19}]^+$ (2.22), 113 $[C_8H_{17}]^+$ (20), 99 $[C_7H_{15}]^+$ (5.55), 85 $[C_6H_{13}]^+$ (14.44), 71 $[C_5H_{11}]^+$ (90), 57 $[C_4H_9]^+$ (100).
2,6,10-trimethyltridecane	226 $[M]^+$ (3.29), 197 $[C_{14}H_{29}]^+$ (1.11), 183 $[C_{13}H_{27}]^+$ (10.98), 155 $[C_{11}H_{23}]^+$ (5.49), 141 $[C_{10}H_{21}]^+$ (20.87), 127$[C_9H_{19}]^+$ (6.59), 113 $[C_8H_{17}]^+$ (19.78), 99 $[C_7H_{15}]^+$ (18.68), 85 $[C_6H_{13}]^+$ (54.94), 71 $[C_5H_{11}]^+$ (96.70), 57 $[C_4H_9]^+$ (100).
6,10,14-trimethyl2-pentadecanone	268 $[M]^+$ (2.19), 253 $[C_{17}H_{33}O]^+$ (2.33), 250 (15.38), 225 $[C_{15}H_{29}O]^+$ (2.33), 210 $[C_{14}H_{26}O]^+$ (9.89), 165 $[C_{11}H_{17}O]^+$ (13.18), 124 $[C_8H_{12}O]^+$ (24.17), 109 $[C_7H_9O]^+$ (39.56), 85 $[C_5H_9O]^+$ (49.45), 71 $[C_4H_7O]^+$ (76.60), 58 $[C_3H_6O]^+$ (100).
4,5-oxyacoran-3-one	236 $[M]^+$ (0.54), 207 $[C_{13}H_{19}O_2]^+$ (3.22), 170 (39.56), 149 $[C_9H_9O_2]^+$ (17.58), 137 $[C_8H_9O_2]^+$ (21.11), 124 $[C_7H_8O_2]^+$ (100), 109 $[C_6H_5O_2]^+$ (55.54), 95 $[C_6H_7O]^+$ (33.12), 69 $[C_5H_9]^+$ (51.84), 55 $[C_4H_7]^+$ (60.43).
α-copaene	204 $[M]^+$ (1.11), 189 $[C_{14}H_{21}]^+$ (2.77), 161 $[C_{12}H_{17}]^+$ (27.77), 147 $[C_{11}H_{15}]^+$ (3.33), 133 $[C_{10}H_{13}]^+$ (4.44) , 119 $[C_9H_{11}]^+$ (14.44), 105 $[C_8H_9]^+$ (33.33), 91 $[C_7H_7]^+$ (100), 77 $[C_6H_5]^+$ (7.77), 69 $[C_5H_9]^+$ (8.79), 55 $[C_4H_7]^+$ (15.38).
trans-phytol	296 $[M]^+$ (1.11), 278 $[C_{20}H_{38}]^+$ (1.11), 225 $[C_{16}H_{33}]^+$ (1.11), 196 $[C_{14}H_{28}]^+$ (1.66),123 $[C_9H_{15}]^+$ (25.55), 111 $[C_8H_{15}]^+$ (10), 99 $[C_7H_{15}]^+$ (16.66), 81 $[C_6H_9]^+$ (30), 71 $[C_5H_{11}]^+$ (100), 55 $[C_4H_7]^+$ (40).
phytol	296 $[M]^+$(1.11), 278$[C_{20}H_{38}]^+$(1.11), 225$[C_{16}H_{33}]^+$(1.11),196 $[C_{14}H_{28}]^+$(1.66), 123$[C_9H_{15}]^+$(23.33), 111$[C_8H_{15}]^+$(10), 99 $[C_7H_{15}]^+$(12.22), 83$[C_6H_{11}]^+$(17.77), 81$[C_6H_9]^+$(28.88), 71$[C_5H_{11}]^+$(100), 55 $[C_4H_7]^+$ (33.33).
olian-12-en-3-one	424 $[M]^+$ (14.28), 409 $[C_{29}H_{45}O]^+$ (6.59), 381 $[C_{27}H_{41}O]^+$ (0.54), 299 $[C_{21}H_{31}O]^+$ (1.44), 257 $[C_{18}H_{25}O]^+$ (1.09), 218 $[C_{15}H_{22}O]^+$ (100), 203 $[C_{14}H_{19}O]^+$ (49.45), 189 $[C_{13}H_{17}O]^+$ (17.58), 175 $[C_{12}H_{15}O]^+$ (6.59), 135 $[C_9H_{11}O]^+$ (8.79), 109 $[C_7H_9O]^+$ (42.85), 95 $[C_6H_7O]^+$ (15.38), 69 $[C_4H_5O]^+$ (10.98).
taraxaster-12-en-3-one	424 $[M]^+$ (35.16), 409 $[C_{29}H_{45}O]^+$ (13.18), 381 $[C_{27}H_{41}O]^+$ (1.44), 325 $[C_{23}H_{33}O]^+$ (1.44), 297 $[C_{21}H_{29}O]^+$ (1.44), 218 $[C_{15}H_{22}O]^+$ (12.08), 205 $[C_{14}H_{21}O]^+$ (100), 189 $[C_{13}H_{17}O]^+$ (21.97), 161 $[C_{11}H_{13}O]^+$ (5.49), 149 $[C_{10}H_{13}O]^+$ (10.98), 133 $[C_9H_9O]^+$ (10.98), 109 $[C_7H_9O]^+$ (42.85), 95 $[C_6H_7O]^+$ (32.96), 81 $[C_5H_5O]^+$ (14.28), 55 $[C_3H_3O]^+$ (12.08).
β-sitosterol	414 $[M]^+$ (38.46), 399 $[C_{28}H_{47}O]^+$ (13.18), 369 $[C_{26}H_{41}O]^+$ (2.11), 355 $[C_{25}H_{39}O]^+$ (16.49), 327 $[C_{23}H_{35}O]^+$ (3.29), 300 $[C_{21}H_{32}O]^+$ (15.38), 271 $[C_{20}H_{31}]^+$ (21.33), 255 $[C_{19}H_{27}]^+$ (30.96), 231$[C_{17}H_{27}]^+$ (15.33), 207 (42.33), 201 $[C_{15}H_{21}]^+$ (3.69), 173 $[C_{13}H_{17}]^+$ (10.89), 161 $[C_{12}H_{17}]^+$ (34.33), 147 $[C_{11}H_{15}]^+$ (26.37), 119 $[C_9H_{11}]^+$ (37.21), 107 $[C_8H_{11}]^+$ (28.11), 81 $[C_6H_9]^+$ (51.64), 69 $[C_5H_9]^+$ (72.16), 55 $[C_4H_7]^+$ (100).
stigmasterol	412 $[M]^+$ (33.45), 397 $[C_{28}H_{45}O]^+$ (11.77), 369 $[C_{26}H_{41}O]^+$ (10.98), 355 $[C_{25}H_{39}O]^+$ (5.49), 327 $[C_{23}H_{35}O]^+$ (3.29), 300 $[C_{21}H_{32}O]^+$ (15.38), 271 $[C_{20}H_{31}]^+$ (100), 255 $[C_{19}H_{27}]^+$ (32.96), 213 $[C_{16}H_{21}]^+$ (15.38), 201 $[C_{15}H_{21}]^+$ (7.69), 173 $[C_{13}H_{17}]^+$ (10.89), 161 $[C_{12}H_{17}]^+$ (16.48), 147 $[C_{11}H_{15}]^+$ (26.37), 119 $[C_9H_{11}]^+$ (21.97), 107 $[C_8H_{11}]^+$ (35.16), 81 $[C_6H_9]^+$ (51.64), 69 $[C_5H_9]^+$ (35.16), 55 $[C_4H_7]^+$ (50.54).
pregnane	288 $[M^+]$ (1.11),273 $[C_{20}H_{33}]^+$ (3.53), 248 $[C_{18}H_{32}]^+$ (6.59), 219 $[C_{16}H_{27}]^+$ (1.42), 205 $[C_{15}H_{25}]^+$ (5.49), 191 $[C_{14}H_{23}]^+$ (1.11), 177 $[C_{13}H_{21}]^+$ (4.39), 149 $[C_{11}H_{17}]^+$ (25.27), 125 $[C_9H_{17}]^+$ (40.65), 111 $[C_8H_{15}]^+$ (25.27), 97 $[C_7H_{13}]^+$ (59.34), 84 $[C_6H_{12}]^+$ (100), 71 $[C_5H_{11}]^+$ (70.32), 57 $[C_4H_9]^+$ (61.53).
2,6-dimethylundecane	184 $[M]^+$ (1.11), 169 $[C_{12}H_{25}]^+$ (1.11), 141 $[C_{10}H_{21}]^+$ (2.22), 127 $[C_9H_{19}]^+$ (1.11), 113 $[C_8H_{17}]^+$ (10), 98 $[C_7H_{14}]^+$ (15.55), 85 $[C_6H_{13}]^+$ (14.44), 71 $[C_5H_{11}]^+$ (52.22), 57 $[C_4H_9]^+$ (100).
4-oxo-ß-ionol	208 $[M]^+$ (1.09), 193 $[C_{12}H_{17}O_2]^+$ (1.09) 165 $[C_{11}H_{17}O]^+$ (1.09), 152 $[C_{10}H_{16}O]^+$ (15.38), 135 $[C_9H_{11}O]^+$ (6.59), 108 $[C_7H_8O]^+$ (100), 95 $[C_6H_7O]^+$ (12.08), 55 $[C_4H_7]^+$ (10.98).
3-hydroxy-5,6-oxymegastigm-7-en-9-one	224 $[M]^+$ (0.54), 182 $[C_{10}H_{14}O_3]^+$ (6.59), 151 $[C_9H_{11}O_2]^+$ (2.19), 123 $[C_8H_{11}O]^+$ (100), 95 $[C_6H_7O]^+$ (10.98), 79 $[C_6H_7]^+$ (7.69), 55 $[C_4H_7]^+$ (10.98).
4,5-oxymegastigm-7-en-9-ol	210 $[M]^+$ (10.98), 182 $[C_{11}H_{18}O_2]^+$ (39.56), 151 $[C_{10}H_{15}O]^+$ (17.58), 135 $[C_9H_{11}O]^+$ (48.35), 123 $[C_8H_{11}O]^+$ (65.93), 108 $[C_8H_{12}]^+$ (50.54), 83 $[C_6H_{11}]^+$ (50.54), 69 $[C_5H_9]^+$ (53.84), 55 $[C_4H_7]^+$ (60.43), 43 $[C_3H_7]^+$ (100).
6-hydroxymegastigm-4,7-dien-3,9-dione	222 $[M]^+$ (1.09), 196 $[C_{11}H_{16}O_3]^+$ (2.19), 166 $[C_9H_{10}O_3]^+$ (12.08), 149 $[C_9H_9O_2]^+$ (6.59), 124 $[C_8H_{12}O]^+$ (100), 95 $[C_6H_7O]^+$ (17.58), 81 $[C_6H_9]^+$ (10.98), 69 $[C_5H_9]^+$ (18.68), 55 $[C_4H_7]^+$ (21.97).
6-(3-Hydroxy-but-1-enyl)-1,5,5-trimethyl-7-oxabicyclo[4.1.0]heptan-2-ol	226 $[M]^+$ (1.09), 208 $[C_{13}H_{20}O_2]^+$ (82.41), 181 $[C_{11}H_{17}O_2]^+$ (5.49), 166 $[C_{10}H_{14}O_2]^+$ (16.48), 125 $[C_8H_{13}O]^+$ (69.23), 109 $[C_7H_9O]^+$ (79.12), 95 $[C_6H_7O]^+$ (28.57), 82 $[C_6H_{10}]^+$ (41.75), 71 $[C_5H_{11}]^+$ (36.26), 55 $[C_4H_7]^+$ (25.27), 43 $[C_3H_7]^+$ (100).
estragole	148 $[M]^+$ (100), 133$[C_9H_9O]^+$ (23.33), 117 $[C_9H_9]^+$ (57.77) ,105 $[C_8H_9]^+$ (44.44) , 91 $[C_7H_7]^+$(60), 77 $[C_6H_5]^+$ (58.88), 55 $[C_4H_7]^+$ (34.44).
cis-anethole	148 $[M]^+$ (100), 133 $[C_9H_9O]^+$ (30), 117 $[C_9H_9]^+$ (52.22) ,103 $[C_8H_7]^+$ (26.66) , 91 $[C_7H_7]^+$ (48.88), 77 $[C_6H_5]^+$ (57.77), 55 $[C_4H_7]^+$ (35.16).
trans-anethole	148 $[M]^+$ (100), 133 $[C_9H_9O]^+$ (24.44), 117 $[C_9H_9]^+$ (37.77) ,103 $[C_8H_7]^+$ (19.44) , 91 $[C_7H_7]^+$

	(26.66), 77 $[C_6H_5]^+$ (38.88), 55 $[C_4H_7]^+$ (16.66).
4-(benzyloxy)-3-hydroxybenzaldehyde	228 $[M]^+$ (17.58), 210 $[C_{14}H_{10}O_2]^+$ (3.29), 91 $[C_7H_7]^+$ (100), 77 $[C_6H_5]^+$ (6.88).
1-(3,4-dihydroxy-5-isopentylphenyl)ethan-1-one	222 $[M]^+$ (4.39), 166 $[C_{10}H_{14}O_2]^+$ (63.73), 151 $[C_9H_{11}O_2]^+$ (100), 123 $[C_7H_7O_2]^+$ (29.67), 91$[C_7H_7]^+$(3.21).
2,6-dimethoxy-3-(3'-methyl-2'-butenyl)-1,4-benzoquinone	236 $[M]^+$ (72.52), 221 $[C_{12}H_{13}O_4]^+$ (100), 193 $[C_{10}H_9O_4]^+$ (29.67), 179 $[C_9H_7O_4]^+$ (46.15), 151 $[C_9H_{11}O_2]^+$ (23.07), 109 $[C_7H_9O]^+$ (8.79), 69 $[C_5H_9]^+$ (17.58), 57 $[C_4H_9]^+$ (28.57).
5,7-dimethoxy-2,2-dimethyl-2H-chromene	220 $[M]^+$(26.37), 205$[C_{12}H_{13}O_3]^+$(100), 191$[C_{11}H_{11}O_3]^+$(3.22), 177$[C_{10}H_9O_3]^+$(10.98), 105$[C_8H_9]^+$(5.49), 81$[C_6H_9]^+$(6.04), 57$[C_4H_9]^+$(9.89).
luteolin	286 $[M]^+$ (7.69), 268 $[C_{15}H_8O_5]^+$ (3.44), 256 (14.28), 250 $[C_{15}H_6O_4]^+$ (2.22), 232 $[C_{15}H_4O_3]^+$ (4.38), 111 $[C_6H_7O]^+$ (48.35), 97 $[C_5H_5O]^+$ (83.51), 57 $[C_3H_5O]^+$ (100).
sulfolane	120 $[M]^+$ (48.38), 107 $[C_3H_7O_2S]^+$ (2.21), 93 $[C_2H_5O_2S]^+$ (1.61), 78 $[CH_3O_2S]^+$ (1.61), 64 $[HO_2S]^+$ (3.21), 55 $[C_4H_7]^+$ (100).

EXPERIMENTAL

^{1}H NMR: The NMR spectra were recorded in deuterated chloroform ($CDCl_3$) or dimethylsulphoxide (DMSO-d6) at either Faculty of Pharmacy, Benisweef University, on Bruker Avance III 400 MHz for ^{1}H and 100 MHz for ^{13}C (Bruker AG, Switzerland) with BBFO Smart Probe and Bruker 400 AEON Nitrogen-Free Magnet, and Topspin 3.1 software for data analysis, or at Faculty of Science, Cairo University, on Varian Mercury VX-300 NMR spectrometer, run at 300 MHz for ^{1}H and at 75.46 MHz for ^{13}C. Chemical shifts are quoted in δ and were related to that of the solvents.

GC/MS analysis: The GC/MS analysis was performed at National Research Center, Dokki, Cairo, using a Thermo Scientific, Trace GC Ultra / ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (30 m, 0.251 mm, 0.1 mm film thickness). For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used, Helium gas was used as the carrier gas at a constant flow rate of 1 mL/min. The injector and MS transfer line temperature was set at 280ºC. The oven temperature was programmed at an initial temperature 150ºC (hold 4 min) to 280ºC as a final temperature at an increasing rate of 5ºC/min (hold 4 min). The quantification of all the identified components was investigated using a percent relative peak area. A tentative identification of the compounds was performed based on the comparison of their relative retention time and mass spectra with those of the NIST, WILLY library data of the GC/MS system.

Material and reagents: For column chromatography (CC), silica gel for column and sephadex LH-20 were used. PTLC were performed on silica gel (Kieselgel 60, GF 254) of 0.25 mm thickness; petroleum ether (60-80), diethyl ether, hexane, methylene chloride, ethyl acetate, acetone, butanol and methanol were obtained from Adwic Company; The cell lines hepatocellular carcinoma HePG-2, mammary gland breast cancer MCF-7, Human prostate cancer PC3 and Colorectal carcinoma HCT-116 were obtained from ATCC via Holding company for biological products and vaccines (VACSERA), Cairo, Egypt; The reagents RPMI-1640 medium, MTT, DMSO and 5-fluorouracil were obtained from Sigma co., St. Louis, USA, and Fetal Bovine serum was obtained from GIBCO, UK.

Plant material: *B. asiatica* Lour was collected from Orman Botanical Garden, Giza, Egypt, in April 2014 and identified by Dr. Wafaa M. Amer, Department of Botany, Faculty of Science, Cairo University, Giza, Egypt. Voucher specimens (Reg. No.: B–1) were deposited in the herbarium of Medicinal Chemistry Department, Theodor Bilharz Research Institute, Giza, Egypt **[1].**

Processing of the plant material: Fresh aerial parts of *B. asiatica* (500 g) were percolated in petroleum ether for 2 days, subjected to hydro-steam distillation then the distillate was separated into two layers by a separatory funnel. The petroleum ether layer was dried over Na_2SO_4 and evaporated to give the petroleum ether extract, Ba1 (7.81 g). The aqueous layer was extracted by methylene chloride to give and methylene chloride extract, Ba2 (1.22 g). The residual plant matter after distillation was soaked in MeOH at room temperature for 48 hour, and filtrated. The filtrate was extract by hexane, methylene chloride, ethyl acetate and butanol to give hexane extract, Ba3 (1.14 g), methylene chloride extract, Ba4 (0.93 g), ethyl acetate extract, Ba5 (1.42 g) and butanol extract, Ba6 (1.73 g), respectivelly.

A sample from essential oil fraction Ba1 gave by GC/MS: decane (R_t 11.45 min, 5.04%), undecane (R_t 15.23 min, 4.90%), 2-methylundecane (R_t 17.47 min, 0.83%), dodecane (R_t 18.84min, 5.30%), 2,6-dimethylundecane (R_t 19.24 min,1.07%), 2-Methyl-1,11-dodecadiene (R_t 20.92 min, 0.82%), 1,12-tridecadiene (R_t 21.23 min, 1.04%), Tridecane (R_t 22.25 min, 2.39%), 2-methyltridecane (R_t 24.20 min, 0.47%), Tetradecane (R_t 25.47 min, 2.98%), 2-methyltetradecane (R_t 28.51 min, 1.52%), pentadecane (R_t 28.70 min, 4.84%), 2-methyloctadecane (R_t 39.53 min, 0.38%), nonadecane (R_t 39.77 min, 1.16%), methyl palmitate (R_t 40.22 min, 0.91%), 2-methylnonadecane (R_t 40.93 min, 0.39%), eicosane (R_t 41.89 min, 3.37%), heneicosane (R_t 44.08 min, 3.10%), trans-phytol (R_t 44.44 min, 1.85%), docosane (R_t 46.23 min, 3.20%), tricosane (R_t 48.27 min, 2.52%), tetracosane (R_t 50.26 min, 2.06%), pentacosane (R_t 52.16 min, 1.63%), hexacosane (R_t 53.99 min, 1.16%), heptacosane (R_t 55.77 min, 0.80%), octacosane (R_t 57.48 min, 0.50%) and nonacosane (R_t 59.15min, 0.32%).

A sample from essential oil fraction Ba2 gave by GC/MS: undecane (R_t 15.10 min, 1.22%), Dodecane (R_t 18.70 min, 1.91%), Sulfolane (R_t 19.55 min, 1.83%), farnesane (R_t 21.15 min, 0.56%), tridecane (R_t 22.11 min, 2.24%), tetradecane (R_t 25.35 min, 2.82%), pentadecane (R_t 28.43 min, 2.75%), heptadecane (R_t 34.15 min, 2.95%), octadecane (R_t 36.77 min, 2.52%), nonadecane (R_t 39.25 min, 2.51%), methyl hexadecanoate (R_t 39.90 min, 0.59%), eicosane (R_t 41.60 min, 2.33%), heneicosane (R_t 43.89 min, 2.16%), methyl 9,12,15-octadecatrienoate (R_t 43.99 min, 0.85%), trans-phytol (R_t 44.27 min, 0.95%), docosane (R_t 46.05 min, 1.89%), tricosane (R_t 48.14 min, 1.49%), tetracosane (R_t 50.15 min, 1.13%), pentacosane (R_t 52.07 min, 0.77%) and hexacosane (R_t 53.94 min, 0.46%).

A sample from hexane extract Ba3 gave by GC/MS: α-pinene (R_t 8.89 min, 2.67%), sabinene (R_t 9.22 min, 0.37%), α-terpinyl acetate (R_t 12.49 min, 1.30%), fenchone (R_t 14.57 min, 16.98%), camphor (R_t 16.61 min, 0.64%), thymoquinone (R_t 18.58 min, 4.30%), estragole (R_t 19.83 min, 0.40%), cis-anethole (R_t 19.98 min, 0.59%), anethole (R_t 22.61 min, 14.28%), 2,6,10-trimethyltridecane (R_t27.13min, 0.32%), 2,6-dimethoxy-3-(3'-methyl-2'-butenyl)-1,4-benzoquinone (R_t 30.70 min, 0.89%), cetene (R_t 31.01 min, 0.74%), hexadecane (R_t 31.21 min, 0.65%), 1-(3,4-dihydroxy-5-isopentylphenyl)ethan-1-one (R_t 31.76 min, 1.51%), 10-heptadecen-8-ynoic acid methyl ester (R_t 31.95 min, 0.31%), a-copaene (R_t 32.19 min, 0.40%), 1-octadecene (R_t 36.52 min, 3.10%), 1-nonadecene (R_t 36.67 min, 0.91%), hexahydrofarnesyl acetone (R_t 37.73 min, 2.82%), 2-pentadecanone,6,10,14trimethyl (R_t 37.88 min, 1.77%), 4-(benzyloxy)-3-hydroxybenzaldehyde (R_t 38.86 min, 1.27%), Pregnane (R_t 39.69 min, 1.42%), methyl hexadecanoate (Rt 40.01 min, 0.89%), 1-eicosanol (R_t 41.44 min, 2.00%), linoleic acid methyl ester (R_t 43.76 min, 1.29%), oleic acid methyl ester (R_t 43. 98min, 2.56%), oleic acid (R_t 44.07min, 1.63%), phytol (R_t 44.37min, 11.09%), methyl stearate (R_t 44.49 min, 1.60%), linolenic acid ethyl ester (R_t 45.38 min, 1.23%),1-docosene (R_t 45.89 min, 1.62%), n-tetracosanol (R_t 49.99 min, 0.59%), propyl 9.12.15-eicosatrienoate (R_t 50.58 min, 1.45%), 6,9,12,15-docosatetraenoic acid methyl ester (Rt 50.71 min, 0.59%), taraxaster-12-en-3-one (R_t 66.32 min, 1.33%), stigmasterol (R_t 66.34 min, 3.05%), β-sitosterol (R_t 66.36 min, 2.69%), and Olian-12-en-3-one (R_t 66.48 min, 1.16%).

A sample from methylene chloride extract Ba4 gave by GC/MS: sulfolane (R_t 20.13 min, 16.59%), 4-oxo-ß-ionol (R_t 32.84 min, 2.33%),3-hydroxy-5,6-oxymegastigm-7-en-9-one (R_t 33.99 min, 1.45%),4,5 -oxymegastigm-7-en-9-ol (R_t 34.42 min, 0.85%), (-)-loliolide (R_t 36.55 min, 1.65%),4,5-oxyacoran-3-one (Rt 37.50 min, 0.47%) 6-hydroxymegastigm-4,7-dien-3,9-dione (R_t 37.04 min, 1.15%),4,5-oxyacoran-3-one (R_t 37.50 min, 0.47%), heptadecanoic acid (R_t 39.76 min, 0.30%), methyl hexadecanoate (R_t 40.25 min, 0.57%), linolic acid (R_t 41.29 min, 0.80%), (E)-10-heptadecen-8-ynoic acid methyl ester (R_t 41.43 min, 0.30%) and oleic acid (R_t 44.10 min, 1.43%).

A sample from ethyl acetate Ba5 gave by GC/MS: camphor (R_t 16.48 min, 2.28%), 5,7-dimethoxy-2,2-dimethyl-2H-chromene (R_t 28.71 min, 12.61%), 6-(3-Hydroxy-but-1-enyl)-1,5,5-trimethyl-7-oxabicyclo [4.1.0]heptan-2-ol (R_t 31.36 min, 3.67%), 1-docosene (R_t 45.85 min, 3.03%), tetracosene (R_t 49.98 min, 0.63%), luteolin (R_t 53.81 min, 0.78%).

Hexane extract (Ba3, 1.00g) was subjected to silica gel CC using hexane/ethyl acetate as an eluent with gradient increasing polarity. The eluted fractions were monitored and collected based on their TLC patterns. The fraction eluted by hexane/ethyl acetate 47:3 (90 mg) gave by TLC (silica gel, hexane/benzene/ethyl acetate 6:3:1) a mixture of **1** and **2** (2:3, R_f 0.81, 12 mg). The fraction eluted by hexane/ethyl acetate 13:7 (82 mg) gave by TLC (silica gel, hexane/benzene/ethyl acetate 4:3:3) a mixture of β-sitosterol and stigmasterol (1:4, R_f 0.42, 10 mg).

Methylene chloride extract (Ba4, 0.80g) was subjected to silica gel CC using hexane/ethyl acetate as an eluent with gradient increasing polarity. The eluted fractions were monitored and collected based on their TLC patterns. The fraction eluted by hexane/ethyl acetate 17:3 (161 mg) gave by TLC (silica gel, hexane/ ethyl acetate 7:3) 4-acetoxybenzaldehyde (R_f 0.85, 27 mg).

The ethyl acetate extract (Ba5, 1.20 g) was subjected to using hexane/ethyl and ethyl acetate/methanol as an eluent with gradient increasing polarity. The eluted fractions were monitored and collected based on their TLC patterns. The fraction eluted by hexane/ethyl acetate 4:1 (80 mg) gave by TLC (silica gel, hexane/ethyl acetate 9:1) 4-acetoxyacetophenone (R_f 0.81, 11mg). The fraction eluted by methylene chloride/methanol 17:3 (78 mg) gave by TLC (silica gel, hexane/benzene/ ethyl acetate / 2:2.1) (E) 3,4-dihydroxycinnamic acid (R_f 0.77, 18 mg). The fraction eluted by methylene chloride/methanol 4:1 (92 g) gave by TLC (silica gel, hexane/benzene/ ethyl acetate / 2:1.2) (E) 3,4-dihydroxy-5-methoxycinnamic acid (R_f 0.73, 21 mg).

The Butanol extract (Ba6, 1.60 g) was subjected to silica gel CC using ethyl acetate/methanol as an eluent with gradient increasing polarity. The eluted fractions were monitored and collected based on their TLC patterns. The fraction eluted by ethyl acetate /methanol 7:3 (0.51 g) was reseparated on a sephadex LH-20 CC (methylene chloride/methanol, 1:9), followed by C8 RP TLC plates (methanol/ H_2O 19:5) to give **3** and **4** in a mixture at R_f 0.74 (5:6 97 mg) as well as **5** at R_f 0.65 (87 mg).

Antimicrobial activity assessment

Chemical compounds were individually tested against a panel of Gram positive *Staphylococcus aureus*, Gram negative *Escherichia coli* bacterial and fungal *Candida albicans*. Each of the compounds was dissolved in DMSO and solution of the concentration 1 mg /ml were prepared separately paper discs of Whatman filter paper were prepared with standard size (5mm) were cut and sterilized in an autoclave. The paper discs soaked in the desired concentration of the complex solution were places aseptically in the petridishes containing nutrient agar media (agar 20g + beef extract 3 g + peptone 5 g) seeded with *Staphylococcus aureus*, *E. coli* and *Candida albicans*. The petridishes were incubated at 36 c and the inhibition zones were recorded after 24h of incubation. Each treatment was replicated three times. The antibacterial activity of a common standard antibiotic Ampicillin and Antifungal Colitrimazole was also recorded using the same procedure as above at the same concentration and solvents. The % activity index for the complex was calculated by the formula as under:

$$\text{\% Activity Index} = \frac{\text{[Zone of inhibition by test extract (diameter)]}}{\text{[Zone of inhibition by standard (diameter)]}} \times 100$$

Antioxidant activity assessment

Free radical scavenging method (DPPH)

The antioxidant activity of extract was determined at the Regional Center for Mycology and Biotechnology (RCMB) at Al-Azhar University *by* the DPPH free radical scavenging assay in triplicate and average values were considered.

Freshly prepared (0.004%w/v) methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was prepared and stored at 10C in the dark. A methanol solution of the test compound was prepared. A 40 uL aliquot of the methanol solution was added to 3ml of DPPH solution. Absorbance measurements were recorded immediately with a UV-visible spectrophotometer (Milton Roy, Spectronic 1201). The decrease in absorbance at 515 nm was determined continuously, with data being recorded at 1 min intervals until the absorbance stabilized (16 min). The absorbance of the DPPH radical without antioxidant (control) and the reference compound ascorbic acid were also measured. All the determinations were performed in three replicates and averaged. The percentage inhibition (PI) of the DPPH radical was calculated according to the formula:

$$PI = [\{(AC- AT)/ AC\} \times 100]$$

Where AC = Absorbance of the control at t = 0 min and AT = absorbance of the sample+ DPPH at t = 16 min **[27].**

Free radical scavenging method (ABTS)

For each of the investigated compounds (2 mL) of ABTS solution (60 μM) was added to 3 mL MnO2 solution (25mg/mL), all prepared in (5 mL) aqueous phosphate buffer solution (pH 7, 0.1 M). The mixture was shaken, centrifuged, filtered and the absorbance of the resulting green blue solution (ABTS radical solution) at 734 nm was adjusted to approx. ca. 0.5. Then, 50 μl of (2 mM) solution of the tested compound in spectroscopic grade MeOH/phosphate buffer (1:1) was added. The absorbance was measured and the reduction in color intensity was expressed as inhibition percentage. L –ascorbic acid was used as standard antioxidant (Positive control). Blank sample was run without ABTS and using MeOH/phosphate buffer (1:1) instead of tested compounds. Negative control was run with ABTS and MeOH/phosphate buffer (1:1) only.

Cytotoxicity assay

The cell lines HePG-2, MCF-7, PC3 and HCT-116 were used to determine the inhibitory effects of extracts on cell growth using the MTT assay. This colorimetric assay is based on the conversion of the yellow tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. HepG2 was cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/ml penicillin and 100μg/ml streptomycin at 37°C in a 5% CO_2 incubator. The cell line was seeded in a 96-well plate at a density of 1.0×10^4 cells/well **[28],** at 37°C for 48 h under 5% CO_2. After incubation the cells were treated with different concentration of compounds and incubated for 24 h. After 24 h of drug treatment, 20 μl of MTT solution at 5 mg/ml was added and incubated for 4 h. Dimethyl sulfoxide (DMSO) in volume of 100μl is added into each well to dissolve the purple formazan formed. The colorimetric assay is measured and recorded at absorbance of 570 nm using a plate reader (EXL 800). The relative cell viability in percentage was calculated as:

(A570 of treated samples/A570 of untreated sample) X 100.

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