ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Phytochemical and Biological Evaluation of *Plantago arenaria*.

El Sayeda Ramadan[1], Manal G Elfedawy[1], Maha M ElShamy[2], and Mamdouh Abdel-Mogib[1,*]

[1]Chemistry Department, [2]Botany Department, Faculty of Science, Mansoura University, Mansoura-35516, Mansoura, Egypt

ABSTRACT

This article presents the phytochemical and biological evaluation of *Plantago arenaria*. The phytochemical investigation resulted in the separation and identification of the triterpenoid β-oleanolic acid from methylene chloride fraction, the iridoidal glycoside plantarenaloside and the phenylpropanoidal glycoside verbascoside from ethyl acetate fraction, in addition to the identification of the volatile constituents of petroleum ether and methylene chloride fractions by GC/MS analysis. Structures of separated compounds were elucidated by spectral analyses. Additionally, the antimicrobial, antioxidant activities and cytotoxicity of different fractions of *Plantago arenaria* were evaluated. The antimicrobial activity index of ethyl acetate extract fraction (Pa 3) decreased in the following order: against *Escherichia coli* (58.3 **%**) > against *Staphylococcus aureus* (50.0**%**) > against *Candida albicans* (34.6**%**). The activity index of butanol fraction (Pa 4), ethyl acetate fraction (Pa3), methylene chloride fraction (Pa 2) and petroleum ether extract (Pa 1) against *Staphylococcus aureus* were found to be 59.1**%,** 50.0**%,** 50.0 **%** and 22.7**%**, respectively. Against *Candida albicans*, their activity indexes were 50.0**%,** 34.6**%,** 19.2**%** and 0%, respectively. The activity index of (Pa 4), (Pa 2), (Pa 1) against *Escherichia coli* were 41.7**%,** 29.2**%** and 12.5**%**, respectively. The radical scavenging activity of (Pa 4) had the highest antioxidant activity. The antioxidant activity of the extracts and standard decreased in the following order: ascorbic acid > (Pa 4) > (Pa 3) > (Pa 2) > (Pa 1). The IC_{50} values against MCF-7 indicated that the cytotoxicity of extracts decreased in the order: (Pa 3) was “very strong”, (Pa 4) was “strong”, (Pa2) and (Pa1) were “moderate”. The cytotoxicity against PC-3 of (pa 1) was “moderate”, (pa 2) was “strong”, (Pa 4) and (Pa 3) were “very strong”. The cytotoxicity against Hela of (Pa 1) was “week”, (Pa 2) was moderate, (Pa 3) was “very strong” and (Pa 4) were “strong”.

Keywords: *Plantago arenaria*, oleanolic acid, verbascoside, plantarenaloside, antimicrobial, antioxidant, cytotoxicity.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Plantago is a genus of about 200 species of small, inconspicuous plants commonly called plantains or fleaworts. They share this name with the very dissimilar plantain, a kind of banana. Most *Plantago* species are herbaceous plants, though a few are sub shrubs growing to 60 cm (24 inch) tall. *Plantago arenaria* (Branched plantain) is annual species, distributed from South Europe to South-west Asia and naturalized in Britain [1**]**.

Plantago can be proposed as a potential sources of natural antioxidants and bioactive phyto-pharmaceuticals [2**]**. Flavonoids derived from *Plantago* species may be effective in the treatment of inflammatory and microbial disease but further studies are still needed **[**3**]**. In this article, we present the results of the phytochemical reinvestigation, as well as the antimicrobial, antioxidant and antitumor activities of *Plantago arenaria.*

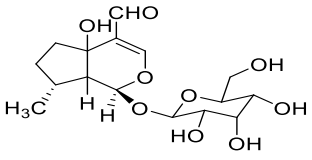
RESULTS AND DISCUSSION

Phytochemical evaluation

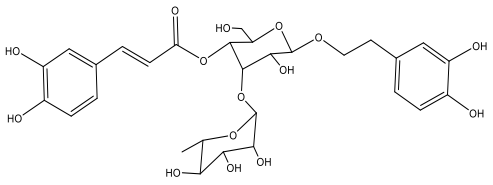
The separation of extracts of *Plantago arenaria* afforded β-oleanolic acid [4]**,** plantarenaloside **1** [5], and verbascoside **2** [6], in addition to the GC/MS identification of the volatile constituents of petroleum ether fraction, Pa1, and methylene chloride fraction, Pa2.

The ^{1}H NMR spectrum of **1** showed an iridoid glocoside signal pattern with the signal of the anomeric proton, H-1' present at δ 4.63 as a doublet with J of 8.0 Hz, indicating the β-D-glucopyranoside. A doublet at δ 5.88 with J of 1.6 Hz was assigned to the H_{acetal}, H-1. An aldehydic singlet at δ 9.28 was assigned to H-11. A singlet at δ 7.42 was assigned to the olefinic proton of the enol ether of the heterocyclic ring, H-3. A methyl group doublet at δ 0.97 with J of 6.8 Hz was assigned to H-10, indicating the presence of the 5-membered homocarbon ring. Comparing the NMR data with corresponding literature values indicated that **1** is plantarenaloside, isolated recently by Rana *et al*. from *Incarvillea emodi* [7] and by Venditti *et al*. from *Pedicularis kerneri* [8].

The ^{1}H NMR spectrum of **2** showed two 2H-triplets with J of 6.4 Hz at δ 2.80, 3.60, in addition to a broad singlet at δ 6.73, a doublet at δ 6.71 with J = 8 Hz, and a broad doublet with the same J value (8 Hz) at δ 6.58, indicating the presence of the phenylethanoid hydroxytyrosol [9]. The spectrum also contained the signals of caffeoyl ester (two 1-H doublets with J of 16 Hz at δ 7.62, 6.30, a broad singlet at δ 7.09, a doublet at δ 6.81 and a broad doublet at δ 6.97 sharing J value of 8 Hz) and the sugar α-L-rhamnopyranosyl-(1->3)-β-D-glucopyranose, with the two anomeric proton signals at δ 5.22 (br s H-1 of α-L-rhamnopyranos) and 4.41 (d, 7.6 Hz, of H-1 of β-D-glucopyranose) and the methyl group of rhamnose as a doublet of 3-Hs with J of 6.0 Hz at δ 1.11. Comparing the NMR data with corresponding literature values indicated that **2** is verbascoside isolated recently by Venditti *et al*. from *Pedicularis kerneri* [8].



plantarenaloside **1**



verbascoside **2**

Antimicrobial activity assessment

The results indicated that the activity index of ethyl acetate extract decreased in the following order: *Escherichia coli* (58.3 **%**) > *Staphylococcus aureus* (50.0**%**) > *Candida albicans* (34.6 **%**). The activity index of butanol (Pa 4), ethyl acetate extract (Pa 3), methylene chloride (Pa 2) and petroleum ether extract (Pa 1) against *Staphylococcus aureus* were found to be 59.1**%,** 50.0**%,** 50.0**%** and 22.7**%**, respectively, and against *Candida albicans*, their activity indexes were 50.0**%,** 34.6**%,** 19.2**%**, and no activity with petroleum ether extract (Pa 1), while the activity index of (Pa 4), (Pa 2) and (Pa 1) against *Escherichia coli* were 41.7**%,** 29.2**%** and 12.5**%**, respectively (Table 1).

ISSN: 0975-8585

Table 1: the inhibition zone in mm of extracts of *Plantago arenaria* compared to standard antibiotics

Compound	*E. coli* (mg/ml)		*S. aureus* (mg/ml)		*C. Albicans* (mg/ml)	
	Diameter of inhibition zone (mm)	% Activity index	Diameter of inhibition zone (mm)	% Activity index	Diameter of inhibition zone (mm)	% Activity index
Petrolium ether /Pa1	3	12.5	5	22.7	NA	----
Methylene chloride / Pa2	7	29.2	6	27.3	5	19.2
Ethyl acetate / Pa3	14	58.3	11	50.0	9	34.6
Butanol /Pa4	10	41.7	13	59.1	13	50.0
Ampicillin	24	100	22	100	NA	----
Colitrimazole	NA	----	NA	----	26	100

Pa 1= petroleum ether extract; *Pa* 2= methylene chloride extract; *Pa* 3= ethyl acetate extract; *Pa* 4= butanol extract; NA = No activity

Free radical scavenging activity assessment

The antioxidant activity data of *Plantago arenaria* were presented in table 2. The free radicals of ABTS were used for detection of the antioxidant activity of the extracts [10]. Butanol extract (Pa 4) had the highest antioxidant activity. The antioxidant activity of the extracts and standard decreased in the following order: ascorbic acid > butanol extract (Pa 4) > ethyl acetate extract (Pa 3) > methylene chloride extract (Pa 2) > petroleum ether extract (Pa 1).

Antioxidant activity = **Abs(control) - Abs(test) / Abs(control) X 100**

Table 2: Antioxidant activity of the extracts of Plantago arenaria by ABTS method

No.	Fraction or compound	Absorbance of samples	Inhibition
	Control of ABTS	0.495	0%
	Ascorbic-acid	0.055	88.9%
1	Methylene chloride fraction	0.356	28.1%
2	Ethyl acetate fraction	0.083	83.2%
3	Petroleum ether fraction	0.475	4.0%
4	Butanol fraction	0.068	86.3%

Cytotoxic activity assessment

The IC_{50} values against MCF-7 (Table 3) indicated that the cytotoxicity of extracts decreased in the order: ethyl acetate fraction (Pa 3) was “very strong”, butanol fraction (Pa 4) was “strong”, methylene chloride fraction (Pa 2) and petroleum ether fraction (Pa 1) were “moderate”. The cytotoxicity against PC-3 of the petroleum ether fraction (pa 1) was “moderate”, methylene chloride fraction (Pa 2) was “strong”, butanol fraction (Pa 4) and ethyl acetate fraction (Pa 3) were “very strong”, The cytotoxicity against Hela of petroleum ether fraction (Pa 1) was “week”, methylene chloride fraction (Pa 2) was “moderate”, ethyl acetate fraction (Pa 3) was “very strong” and butanol fraction (Pa 4) was “strong”.

Table 3: Cytotoxic activity assessment of *Plantago arenaria* extracts against human tumor cells MCF-7, PC-3 and Hela

Fraction or compound	In vitro Cytotoxicity IC50 (µg/ml)*		
	MCF-7	PC-3	Hela
5-FU*	**5.4±0.34**	**8.3±0.44**	**7.8±0.32**
Petrolium ether fraction (Pa 1)	**40.4±3.06**	**50.9±4.32**	**62.8±4.53**
Methylene chloride fraction (Pa 2)	**35.3±2.81**	**29.3±1.87**	**26.0±1.76**
Ethyl acetate fraction (Pa 3)	**9.1±0.48**	**5.2±0.35**	**8.7±0.71**
Butanol fraction (Pa 4)	**14.3±1.10**	**9.6±0.59**	**16.6±1.15**

•IC_{50} (µg/ml): 1 – 10 (very strong). 11 – 20 (strong). 21 – 50 (moderate). 51 – 100 (weak) and above 100 (non-cytotoxic). 5-FU* = 5-Flurouracil

ISSN: 0975-8585

EXPERIMENTAL

^{1}H-NMR

The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer. ^{1}H spectra were run at 300 MHz and ^{13}C spectra were run at 75.46 MHz in deuterated chloroform (CDCl3) or dimethylsulphoxide (DMSO-d_6). Chemical shifts are quoted in δ and were related to that of the solvents.

GC/MS

GC/MS analysis was performed at the Central Laboratory of the Ministry of Agriculture, Al Bhooth Str., Cairo, on Agilent 6890 gas chromatograph equipped with an Agilent mass spectrometric column HP-5 ms (30 m x 0.32 mm x0.25 um film thickness). Samples were injected under the following condition: Helium was used as carrier gas at approximately 1 ml /min, pulsed splitless mode. The solvent delay was 3 min and the injection size was 1.0 µl. The mass spectrophotometric detector was operated in electron impact ionization mode an ionizing energy of 70 eV, scanning from m/z 50 to 500. The ion source temperature was 230°C and the quadrupole temperature was 150°C. The electron multiplier voltage (EM voltage) was maintained at 1250 v above auto tune. The instrument was manually tuned using perfluorotributyl amine (PFTBA). The GC temperature program was started at 60°C then elevated to 280°C at rate of 8°C/min. and 10 min .hold at 280°C the detector and injector temperature were set at 280°C and 250°C, respectively. Wiley and Nist 05 mass spectral database was used in the identification of the separated peaks.

Materials and reagents

PTLC were performed on silica gel (Kieselgel 60, GF 254) of 0.25 mm thickness; CC was performed on silica gel (60- 120 MESH); petroleum ether (60-80), diethyl ether, hexane, methylene chloride, ethyl acetate, acetone, butanol and methanol were obtained from Adwic company;

Plant material

Plantago arenaria (Del.) Link was collected from Marine, Alkharga Oasis, Alwady Algaded on March, 2014. The plant species was identified by Dr. Maha Elshamy, Botany Department, Faculty of Science, Mansoura University.

Processing of the plant material

The whole plant material (root, stem, leaves) was dried in air at 24°C for 12 days and been grinded to a powdered material (344 g), which was extracted by a soxhlet extractor using different solvents, petroleum ether, methylene chloride, ethyl acetate and methanol, to give four fractions; petroleum ether fraction (Pa1, 12.03 g, 3.49% w/w), methylene chloride fraction (Pa2, 4.08 g, 1.18% w/w), ethyl acetate fraction (Pa3, 1.65 g, 0.47% w/w) and methanol fraction (12.0 g, 3,48% $_w/_w$). The methanol fraction was distributed in a separatory funnel between water and butanol to give a butanol fraction (12.0 g).

Two samples of the petroleum ether fraction and methylene chloride fraction were analyzed by GC/MS. The methylene chloride fraction (4.08 g) was separated by silica gel CC eluted by hexane /ethyl acetate with increasing polarity. The fraction eluted by hexane /ethyl acetate (8.2 : 1.6) gave oleanolic acid (1.6 mg). The ethyl aceatate fraction (1.65 g) was separated by silica gel CC, eluted by methylene chloride/methanol with increasing polarity to give oleanolic acid (1.9 mg). The butanol fraction (12.0 g) was separated by sephadex CC, eluted by ethyl acetate /methanol with increasing polarities to give verbascoside **2** at eluent ratio 7:3 (1.93 mg). The fraction eluted by ethyl acetate /methanol at eluent ratio 8:2 (1.96 mg) was purified by TLC (ethy acetate, methanol, water 3.8 : 4.0 : 6.0) to give plantarenaloside **1.**

GC/MS analysis of petroleum ether fraction (Pa 1) afforded Nonane, (R_t 5.09 min., 0.51%), decane, (R_t 7.18 min., 0.58%), dodecane, (R_t 11.28 min., 2.94%), sulfolane, (R_t 11.78 min., 2.10%), 2-methyl dodecane (R_t 12.59 min., 5.02%), tridecane, (R_t 13.14 min., 4.48%), tetradecane, (R_t 14.94 min., 4.14%), pentadecane, (R_t 16.53 min., 1.62%), hexadecane, (R_t 18.07 min., 2.95%), 7-ethyl-1,4-dimethyl -azulene, (R_t 19.16 min., 0.87%), heptadecane, (R_t 19.58 min., 3.20%), octadecane, (R_t 20.90 min., 3.40%), phytol, (R_t 21.37 min., 4.49%),

nonadecane, (R_t 22.19 min., 3.59%), eicosane, (R_t 32.28 min., 5.46%), heneicosane , (R_t 24.56 min., 2.49%), 3-hydroxy-14 βH pregnan-20-one, (R_t 27.33 min., 1.27%), tetracosane, (R_t 27.74 min., 2.33%), pentacosane, (R_t 28.69 min., 2.66%), hexacosane, (R_t 29.95 min., 1.50%), heptacosane, (R_t 30.47 min., 2.02%), octacosane, (R_t 31.36 min., 1.35%), squalene, (R_t 31.70 min., 0.62%), and docosane, (R_t 33.64 min., 1.07%), octadecanol (R_t 34.24 min., 0.38%), henteriacontane, (R_t 35.20 min., 2.32%), d1-alpha-tocopherol, (R_t 35.95 min., 0.73%), campestrol, (R_t 37.91 min., 1.44%), stigmasta-5,22-dien-3-ol, (R_t 38.60 min., 1.41%), -sitosterol, (R_t 40.04 min., 2.71%), tetradecyloxirane (R_t 40.78 min., 0.39%), stigmasta-4-en-3-one, (R_t 43.57 min., 0.32%), 1,19-eicosaidiene, (R_t 47.63 min., 0.17%).

GC/MS analysis of methylene chloride fraction (Pa 2) gave dodecane (R_t 11.19 min., 0.74%), 4,6-dimethyl dodecan (R_t 12.51 min., 0.35%), tridecane (R_t 13.01 min., 1.37%), tetradecane (R_t 14.70 min., 1.,96%), pentadecane (R_t 16.28 min., 2.31%), hexadecane (R_t 17.78 min., 2.92%), heptadecane (R_t 19.20 min., 2.31%), octadecane (R_t 12 .55 min., 2.94%), eicosane (R_t 26.10 min., 0.11%), henicosane (R_t 24.21 min., 2,48%), gamma-sitosterol (R_t 39.81 min., 1.10%), tetracosane (R_t 27.14 min., 0.55%), pentacosane (R_t 28.43 min., 2.50%), docosane (R_t 25.34 min., 2.50%), alpha-tocopherol (R_t 35.88 min., 0.39%), henicosane (R_t 24.56 min., 2.49%), nonadecane (R_t 21.83 min., 2.16%), benzoic acid, (R_t 11.01 min., 0.42%), (-) myrental, (R_t 14.55 min., 0.66%), p-methylcinnamic acid, (R_t 15.13 min., 1.41%), 6-methoxy-3-methyl-1Hindole, (R_t 15.61 min., 3.82%), bicyclo[2.2.1]heptan-2-ol, (R_t 15.82 min., 0.94%), 4-tetradecyne, (R_t 15.97 min., 0.17%), pentadecane,2,6,10-trimethyl, (Rt 18.47 min.,093%), tetradecanoic acid, (R_t 20.11 min., 1.32%), 9-methyl anthracen- (R_t 22.00 min., 0.93%), , hexadecanoic acid, (R_t 22.70., 5.69%), octadecanoic acid, (R_t 24.99min., 0.73%), tricosane (R_t 26.41 min., 2.41), 1-heneicosyl formate, (R_t 23.17 min., 0.91%), 9,12,15-octadecattrien-1-ol, (R_t 24.80 min., 6.60%), campestrol, ergost-5-en-3-ol, (R_t 37.76 min., 0.44%), stigmasta-5,22-dien-3-ol, (R_t 38.45 min., 0.50%).

Table 3: The MS data of compounds identified by the GC/MS

Compound	MS data: m/z [identity] (rel. abound.%)
nonane	128 $[M^+]$ (6.66), 106 (23.33), 99 $[C_7H_{15}]^+$ (9.66), 85 $[C_6H_{13}]^+$ (43).71 $[C_5H_{11}]^+$ (30).64 (3.33).57 $[C_4H_6]^+$ (100).
decane	142 $[M^+]$ (7.66), 133 (0.83), 120 (9.33), 105 (20.00), 95 (6), 85 $[C_6H_{13}]^+$ (31.33), 71 $[C_5H_{11}]^+$ (43.33), 57 $[C_4H_6]^+$ (100).
dodecane	170 $[M^+]$ (8.66), 162 (0.83), 155 $[C_{11}H_{23}]^+$ (0. 83), 148 (1.66), 141 $[C_{10}H_{21}]^+$ (1.33), 133 (1.66),126 $[C_9H_{18}]^+$ (3.33),119 (2.66), 112 $[C_8H_{16}]^+$ (6.22), 105 (1.66), 98 $[C_7H_{14}]^+$ (10),85 $[C_6H_{13}]^+$ (46.66), 78 (0.83), 71 $[C_5H_{11}]^+$ (70), 64 (0.83), 57 $[C_4H_6]^+$ (100).
tridecane	184 $[M^+]$ (10.73), 176 (0.83), 169 $[C_{12}H_{26}]^+$ (0. 83), 160 (1.66), 151 (0.83), 142 $[C_{10}H_{22}]^+$ (72.83), 135 (1.66), 127 $[C_9H_{19}]^+$ (6.63), 115 (21.33), 107 (3.33), 99 $[C_7H_{15}]^+$ (13.29), 92 (3.33), 85 $[C_6H_{13}]^+$ (56.56), 78 (1.66), 71 $[C_5H_{11}]^+$ (78.46), 64 (3.33), 57 $[C_4H_6]^+$ (100).
tetradecane	198 $[M^+]$ (9.93), 174 (1.66), 156 $[C_{11}H_{24}]^+$ (8.53), 141 $[C_{11}H_{21}]^+$ (18.26), 127 $[C_9H_{19}]^+$ (7.46), 113 $[C_8H_{17}]^+$ (9.43), 99 $[C_7H_{15}]^+$ (16.26), 85 $[C_6H_{13}]^+$ (62.56), 71 $[C_5H_{11}]^+$ (78.26), 57 $[C_4H_6]^+$ (100).
pentadecane	212 $[M^+]$ (8.66), 204 (0.83), 196 $[C_{14}H_{28}]^+$ (8.53), 188 (1.66), 176 (0.83), 168 $[C_8H_{24}]^+$ (7.83), 155 $[C_{11}H_{23}]^+$ (8.43), 141 $[C_{10}H_{21}]^+$ (9.16), 127 $[C_9H_{19}]^+$ (7.93), 113 $[C_8H_{17}]^+$ (10.36), 99 $[C_7H_{15}]^+$ (18.53), 85 $[C_6H_{13}]^+$ (64.66), 71 $[C_5H_{11}]^+$ (78.26), 57 $[C_4H_6]^+$ (100).
hexadecaene	226 $[M^+]$ (9.26), 214 (0.83), 202 (1.66), 191 (2.96), 182 $[C_{13}H_{26}]^+$ (12.96), 170 $[C_{12}H_{26}]^+$ (13.33), 155 $[C_{11}H_{23}]^+$ (30.63), 141 $[C_{10}H_{21}]^+$ (10.66), 127 $[C_9H_{19}]^+$ (9.73), 113 $[C_8H_{17}]^+$ (3.33), 99 $[C_7H_{15}]^+$ (21.73), 85 $[C_6H_{13}]^+$ (68.33), 71 $[C_5H_{11}]^+$ (83), 57 $[C_4H_6]^+$ (100).
heptadecane	226 $[M^+]$ (6.06), 230 (3.33), 220 (0.83), 210 $[C_{15}H_{30}]^+$ (10.66), 196 $[C_{14}H_{28}]^+$ (3.03), 183 $[C_{13}H_{27}]^+$ (10), 169 $[C_{12}H_{25}]^+$ (10.33), 155 $[C_{11}H_{23}]^+$ (7.46), 141 $[C_{10}H_{21}]^+$ (8.33), 127 $[C_9H_{19}]^+$ (11.26), 113 $[C_8H_{17}]^+$ (17.36), 99 $[C_7H_{15}]^+$ (21.56), 85 $[C_6H_{13}]^+$ (62), 71 $[C_5H_{11}]^+$ (83.33), 57 $[C_4H_6]^+$ (100).
octadecane	254 $[M^+]$ (6.66), 242 (0.83), 225 $[C_{16}H_{33}]^+$ (1.66), 211 $[C_{15}H_{31}]^+$ (3.33), 197 $[C_{14}H_{29}]^+$ (6.22), 183 $[C_{13}H_{27}]^+$ (7.43), 169 $[C_{12}H_{25}]^+$ (7.43), 155 $[C_{11}H_{23}]^+$ (9.43), 141 $[C_{10}H_{21}]^+$ (12.53), 127 $[C_9H_{19}]^+$ (12.53), 113 $[C_8H_{17}]^+$ (16.53), 99 $[C_7H_{15}]^+$ (24.13), 85 $[C_6H_{13}]^+$ (70), 71 $[C_5H_{11}]^+$ (83.33), 57 $[C_4H_6]^+$ (100).
nonadecane	254 $[M^+]$ (7.43), 256 (0.83), 239 $[C_{17}H_{35}]^+$ (1.11), 225 $[C_{16}H_{33}]^+$ (1.66), 211 $[C_{15}H_{30}]^+$ (7.43), 197 $[C_{14}H_{29}]^+$ (6.63), 183 $[C_{13}H_{27}]^+$ (7.43), 169 $[C_{12}H_{25}]^+$ (7.13), 155 $[C_{11}H_{23}]^+$ (9.16), 141 $[C_{10}H_{21}]^+$ (11.43), 127 $[C_9H_{19}]^+$ (12.73), 113 $[C_8H_{17}]^+$ (16.66), 99 $[C_7H_{15}]^+$(23.33), 85$[C_6H_{13}]^+$ (71.76), 71 $C_5H_{11}]^+$ (81.33), 57 $[C_4H_6]^+$(100).
eicosane	282 $[M^+]$ (5.83), 270 (3.33), 256 (36.66), 239 $[C_{17}H_{35}]^+$ (6.66), 227 (10.76), 212 $[C_{15}H_{32}]^+$(46.43), 197 $[C_{14}H_{29}]^+$ (17.33), 185 (21.06), 171 (21.66), 157 (21.66), 141 $[C_{10}H_{21}]^+$ (14.03), 129 (37.73), 111 (21.03), 97 (33.66), 85 $[C_6H_{13}]^+$ (71.63), 71 $[C_5H_{11}]^+$ (83.33), 57 $[C_4H_6]^+$ (100).
heneicosane	296 $[M^+]$ (7.33), 274 (5.13), 253 $[C_{18}H_{37}]^+$ (3.33), 238 $[C_{17}H_{34}]^+$ (6.23), 225 $[C_{16}H_{33}]^+$ (6.56), 211 $[C_{15}H_{31}]^+$ (8.23), 197 $[C_{14}H_{29}]^+$ (6.66), 183 $[C_{13}H_{27}]^+$ (7.46), 169 $[C_{12}H_{25}]^+$ (9.26), 155 $[C_{11}H_{23}]^+$ (10.76), 141 $[C_{10}H_{21}]^+$ (13.23), 127 $[C_9H_{19}]^+$ (15.06), 113 $[C_8H_{17}]^+$ (18.33), 99 $[C_7H_{15}]^+$(26.66), 85 $[C_6H_{13}]^+$

	(67.33), 71 $[C_5H_{11}]^+$ (83.33), 57 $[C_4H_6]^+$ (100).
docosane	310 $[M^+]$ (1,66), 281 $[C_{20}H_{41}]^+$ (2,11), 253 $[C_{18}H_{37}]^+$ (2,33), 225 $[C_{16}H_{33}]^+$ (2,33), 197 $[C_{14}H_{29}]^+$ (5.21), 169 $[C_{12}H_{25}]^+$ (6.63), 141 $[C_{10}H_{21}]^+$ (9.96), 113 $[C_8H_{17}]^+$ (16.63), 85 $[C_6H_{13}]^+$ (66.63), 57 $[C_4H_6]^+$ (100).
tricosane	324 $[M^+]$ (1.66), 309 $[C_{22}H_{45}]^+$ (0.83), 295 $[C_{21}H_{43}]^+$ (0.83), 281 $[C_{20}H_{41}]^+$ (0.83), 267 $[C_{19}H_{39}]^+$ (0.40), 253 $[C_{18}H_{37}]^+$ (1.66), 239 $[C_{17}H_{35}]^+$ (2.11), 225 $[C_{16}H_{33}]^+$ (2.22), 211$[C_{15}H_{31}]^+$ (2.33), 197 $[C_{14}H_{29}]^+$ (2.51), 183 $[C_{13}H_{27}]^+$ (2.66), 169 $[C_{12}H_{25}]^+$ (4.16), 155 $[C_{11}H_{23}]^+$ (5.33), 141 $[C_{10}H_{21}]^+$ (8.33), 127 $[C_9H_{19}]^+$ (15.66), 113 $[C_8H_{17}]^+$ (23.33), 99 $[C_7H_{15}]^+$(25,13), 85 $[C_6H_{13}]^+$ (66.66), 71 $[C_5H_{11}]^+$ (83.33), 57 $[C_4H_6]^+$(100).
tetracosane	338 $[M^+]$ (5.56), 309 $[C_{22}H_{45}]^+$ (0.83), 281 $[C_{20}H_{41}]^+$ (3.33), 253 $[C_{18}H_{37}]^+$ (4.83), 225 $[C_{16}H_{33}]^+$ (6.16), 197 $[C_{14}H_{29}]^+$ (7.46), 169 $[C_{12}H_{25}]^+$ (9.96), 141 $[C_{10}H_{21}]^+$ (13.23), 113 $[C_8H_{17}]^+$ (22.13), 85 $[C_6H_{13}]^+$ (76.66), 57 $[C_4H_6]^+$ (100).
pentacosane	352 [M^+] (5,23), 338 $[C_{24}H_{50}]^+$ (0.83), 323 $[C_{23}H_{47}]^+$ (1.13), 309 $[C_{22}H_{45}]^+$ (3.16), 295 $[C_{21}H_{43}]^+$ (4.6), 281 $[C_{20}H_{41}]^+$ (4.6), 267 $[C_{19}H_{39}]^+$ (5.86), 253 $[C_{18}H_{37}]^+$ (6.11), 239 $[C_{17}H_{35}]^+$ (6.63), 225 $[C_{16}H_{33}]^+$ (6.66), 211 $[C_{15}H_{31}]^+$ (7.36), 197 $[C_{14}H_{29}]^+$ (8.83), 183 $[C_{13}H_{27}]^+$ (9.63), 169 $[C_{12}H_{25}]^+$ (11.43), 155 $[C_{11}H_{23}]^+$ (12.46), 141 $[C_{10}H_{21}]^+$ (15.46), 127 $[C_9H_{19}]^+$ (17.73), 113 $[C_8H_{17}]^+$ (22.43), 99 $[C_7H_{15}]^+$(30.36), 85 $[C_6H_{13}]^+$ (80.76), 71 $[C_5H_{11}]^+$ (83.33), 57 $[C_4H_6]^+$ (100).
hexacosane	366 $[M^+]$ (5.16), 242 (0.83), 323 $[C_{23}H_{47}]^+$ (1.66), 295 $[C_{21}H_{43}]^+$ (4.13), 267 $[C_{19}H_{39}]^+$ (4.76), 239 $[C_{17}H_{35}]^+$ (6.63), 224 $[C_{16}H_{32}]^+$ (5.13), 197 $[C_{14}H_{29}]^+$ (7.66), 169 $[C_{12}H_{25}]^+$ (11.83), 141 $[C_{10}H_{21}]^+$ (15.53), 113 $[C_8H_{17}]^+$ (22.22), 85 $[C_6H_{13}]^+$ (82.93), 57 $[C_4H_6]^+$ (100).
heptacosane	380 $[M^+]$ (5.53), 351 $[C_{25}H_{51}]^+$ (0.83), 323 $[C_{23}H_{47}]^+$ (3.66), 304 (3.23), 281 $[C_{20}H_{41}]^+$ (2.96), 253 $[C_{18}H_{37}]^+$ (5.63), 225 $[C_{16}H_{33}]^+$ (5.96), 197 $[C_{14}H_{29}]^+$ (6.63), 169 $[C_{12}H_{25}]^+$ (9.96), 141 $[C_{10}H_{21}]^+$ (16.26), 113 $[C_8H_{17}]^+$ (24.16), 85 $[C_6H_{13}]^+$ (82.96), 57 $[C_4H_6]^+$ (100).
octacosane	394 $[M^+]$ (1.66), 372 (0.41), 351 $[C_{25}H_{51}]^+$ (0.83), 323 $[C_{23}H_{47}]^+$ (1.66), 295 $[C_{21}H_{43}]^+$ (3.33), 267 $[C_{19}H_{39}]^+$ (4.16), 239 $[C_{17}H_{35}]^+$ (4.96), 217 (3.33), 197 $[C_{14}H_{29}]^+$ (5.86), 169 $[C_{12}H_{25}]^+$ (8.33), 141 $[C_{10}H_{21}]^+$ (13.33), 113 $[C_8H_{17}]^+$ (18.43), 85 $[C_6H_{13}]^+$(76.16), 57 $[C_4H_6]^+$ (100).
hentriacontane	436 $[M^+]$ (1.66), 414 (0.41), 393 $[C_{28}H_{57}]^+$ (0.83), 365 $[C_{26}H_{53}]^+$ (1.66), 337 $[C_{24}H_{59}]^+$ (2.11), 309 $[C_{22}H_{45}]^+$ (2.33), 281 $[C_{20}H_{41}]^+$ (2.66), 253 $[C_{18}H_{37}]^+$ (2.96), 225 $[C_{16}H_{33}]^+$ (3.33), 197 $[C_{14}H_{29}]^+$ (4.22), 169 $[C_{12}H_{25}]^+$ (6.63), 141 $[C_{10}H_{21}]^+$ (15.26), 113 $[C_8H_{17}]^+$ (22.33), 85 $[C_6H_{13}]^+$ (68.33), 57 $[C_4H_6]^+$ (100).
2-methylundecane	170 $[M^+]$ (1,66), 148 (15,26), 132 (13.33), 119 (39.4), 104 (25.26), 85 $[C_6H_{13}]^+$ (60,66), 71 $[C_5H_{11}]^+$ (82.33), 57 $[C_4H_6]^+$ (100).
2-methyldodecane	184$[M^+]$ (3.66), 173 (1,66), 165 (14.16), 155 $[C_{11}H_{23}]^+$ (0.83), 146 (19.16), 138 (5.11), 131 (27.16), 123 (4.83), 113 $[C_8H_{17}]^+$ (16.66), 105 (16.53), 97 $[C_7H_{13}]^+$(13.33), 85 $[C_6H_{13}]^+$ (64.43), 71 $[C_5H_{11}]^+$ (83.33), 57 $[C_4H_6]^+$ (100).
4,6-dimethyldodecane	198 $[M^+]$ (1.66), 176 (3.26), 165 (3.33), 155 $[C_{11}H_{23}]^+$ (1.66), 146 (11.73), 131 (17.73), 113 $[C_8H_{17}]^+$ (16,63), 103 (10.33), 91(37.73), 81 (17.46), 71 $[C_5H_{11}]^+$ (93), 57 $[C_4H_6]^+$ (100).
2,6,10-trimethylpentadecane	254 $[M^+]$ (0.40), 232 (1.66), 216 (0.83), 201(1.66), 190(4.30), 181$[C_{13}H_{25}]^+$(6.33), 169 $[C_{12}H_{25}]^+$ (13.26), 152 (4.22), 143(4.96), 133 (11.33), 122 (6.63), 109 (22.96), 100 $[C_7H_{16}]^+$ (17.83), 91 (41.11), 81 (16.63), 71 $[C_5H_{11}]^+$ (60, 66),57 (100).
squalene	410 $[M^+]$ (1.66), 386 (0.83), 367 $[C_{27}H_{43}]^+$ (1.66), 341 $[C_{25}H_{41}]^+$ (3.33), 323 (0.83), 299 $[C_{22}H_{35}]^+$ (1.66), 273 $[C_{20}H_{33}]^+$ (0.83), 239 $[C_{18}H_{22}]^+$ (1.96), 217 (1.66), 191 $[C_{14}H_{23}]^+$ (4.16), 175 $[C_{13}H_{19}]^+$ (4.81), 159 $[C_{12}H_{15}]^+$ (5.33), 149 (12.13), 137 $[C_{10}H_{17}]^+$ (20.73), 121 $[C_9H_{13}]^+$ (17.66), 95 (28.22), 69 (100), 53 $[C_4H_5]$ (29.23).
7-ethyl-1,4-dimethylazulene	184 $[M^+]$ (57.44), 169 $[C_{13}H_{13}]^+$ (73.33), 153 $[C_{12}H_9]^+$ (21.66), 141 $[C_{11}H_9]^+$ (9.33), (26.33) 128 $[C_{10}H_8]^+$ (27,11), 115 $[C_9H_7]^+$, (18.76), 105 (37.4), 92 $[C_7H_8]^+$ (100).
tetradecyne	154 $[M^+]$ (29.96), 183 (5.11), 174 (10.70), 165 $[C_{12}H_{21}]^+$ (23.66), 159 (30.33), 149 (24.11), 138 $[C_{10}H_{18}]^+$ (40.63), 123 $[C_9H_{15}]^+$ (81), 109 $[C_8H_{13}]^+$ (49.66), 95 $[C_7H_{11}]^+$ (29.66) , 81 $[C_6H_9]^+$ (74.63), 69 $[C_5H_9]^+$ (74.63), 55 $[C_4H_7]^+$ (100) .
1,2-dicyclohexylethane	194 $[M^+]$ (5.11), 186 (0.40), 179 $[C_{13}H_{24}]^+$ (19.96), 171 (15.16), 159 (54.33), 147 (27.83), 139 $[C_{10}H_{19}]^+$ (4.33), 123 $[C_9H_{16}]^+$ (17.33), 119 (26.63), 111 $[C_8H_{15}]^+$ (16.33), 109 (20.63), 97 $[C_7H_{13}]^+$ (27.96), 90 (22.33), 83 $[C_6H_{11}]^+$ (100), 76 (14.13), 69 $[C_5H_9]^+$ (48), 62 (1.66), 55 $[C_4H_7]^+$ (48).
9-methylanthracene	192 $[M^+]$ (66.63), 177 $[C_{14}H_9]^+$ (6.66), 165 $[C_{13}H_9]^+$ (16.26), 151 $[C_{12}H_7]^+$ (8.71) ,138 $[C_{11}H_6]^+$ (1.66), 125 $[C_{10}H_5]^+$ (10.33), 112 $[C_9H_4]^+$ (6.33), 105 (100).
benzoic acid	119 $[M^+]$ (46.33), 112 (11.46), 105 $[C8H_9O]^+$ (38.66), 98 (26.63), 91 $[C7H7]^+$ (53.33), 77 (53.33), 69 (27.11), 57 (67.66), 50 (13.33).
p-methylcinnamic acid	162 $[M^+]$ (100), 147 (66.33), 133 (15.26), 119 (9.96), 105 $[C_8H_9]^+$ (41.11), 91 $[C_7H_7]^+$ (40.66), 77 $[C_6H_5]^+$ (22.96), 65 $[C_5H_5]^+$ (9.93), 51 $[C_4H_3]^+$ (11.33).
tetradecanoic acid	228 $[M^+]$ (18.66), 213 $[C_{13}H_{25}O_2]^+$ (0.66), 199 $[C_{12}H_{23}O_2]^+$ (9.63), 185 $[C_{11}H_{21}O_2]^+$ (44.33), 171 $[C_{10}H_{19}O_2]^+$ (17.40), 157 $[C_9H_{17}O_2]^+$ (9.23), 143 $[C_8H_{15}O_2]^+$ (17.96), 129 $[C_7H_{13}O_2]^+$ (66.63), 111 $[C_7H_{11}O]^+$ (20.53), 97 $[C_7H_{13}]^+$ (29.96), 83 $[C_6H_{11}]^+$ (35.96),73 (100).
hexadecanoic acid	256 $[M^+]$ (3.70), 239 (0.40), 227 $[C_{14}H_{27}O_2]^+$ (11.16), 227 $[C_{13}H_{25}O_2]^+$ (35.83), 199 $[C_{12}H_{23}O_2]^+$ (10.33), 185 $[C_{12}H_{23}O_2]^+$ (11.21), 171 $[C_{10}H_{19}O_2]^+$ (22.11), 160 (22.33), 149 (25.86), 129 (73.33), 112 $[C_8H_{16}]^+$

	(16.63), 97 $[C_7H_{13}]^+$ (32.93), 85 $[C_6H_{13}]^+$ (47.66), 73 (100).
octadecanoic acid	284 $[M^+]$ (33.33), 267 (0.40) , 255 $[C_{16}H_{31}O_2]^+$ (6.63), 241 $[C_{15}H_{29}O_2]^+$ (32.76), 227 $[C_{14}H_{27}O_2]^+$(11.66), 213 $[C_{13}H_{25}O_2]^+$ (12.33), 199 $[C_{12}H_{23}O_2]^+$ (12.66), 185 $[C_{11}H_{21}O_2]^+$ (35.66), 171 $[C_{10}H_{19}O_2]^+$ (15.90), 157 $[C_9H_{17}O_2]^+$ (6.63), 143 $[C_8H_{15}O_2]^+$ (12.53), 129 $[C_7H_{13}O_2]^+$ (70.73), 115 $[C_6H_{11}O_2]^+$ (17.66), 101 $[C_5H_9O_2]^+$ (10.33), 87 $[C_4H_7O_2]^+$ (22.93), 73 $[C_3H_5O_2]^+$ (23.96), 57 (100).
phenol	150 $[M^+]$ (56), 135 $[C_9H_{11}O]^+$ (100), 121 $[C_8H_9O]^+$ (38.66), 107 $[C_7H_7O]^+$ (35.23), 91 $[C_6H_3O]^+$ (67.43), 79 (35.11), 67 (12.13), 55 (13.33).
1,5,5-trimethylbicyclo [2.2.1]heptan-2-ol	154 $[M^+]$ (7.33), 146 (6.63), 139 $[C_9H_{15}O]^+$ (99.33), 127 (10.73), 119 (20.71), 109 $[C_7H_9O]^+$ (23.33), 99 (18.83), 91 $[C_7H_7]^+$ (100), 81 (33.33), 71 (61), 64 $[C_5H_4]^+$ (1.66) 57 (93).
9,12,15-octadecatrien-1-ol	264 $[M^+]$ (2.83), 249 $[C_{17}H_{29}O]^+$ (3.11), 222 $[C_{15}H_{26}O]^+$ (9.96), 205 $[C_{15}H_{25}]^+$ (9.96), 189 (0.66), 71 $[C_{12}H_{19}]^+$ (4.11), 149 $[C_{11}H_{17}]^+$ (10.66), 135 $[C_{10}H_{15}]^+$ (15.43), 121 $[C_9H_{13}]^+$ (18.11), 95 (39.96), 79 $[C_6H_7]^+$ (100).
1-heneicosyl formate	340 $[M^+]$ (0.40), 306 (0/66), 292 (1.66), 280 $[C_{20}H_{40}]^+$(2.33), 267 $[C_{19}H_{39}]^+$ (2.33), 256 (5.66), 236 (6.63) , 224 $[C_{16}H_{32}]^+$ (7.33), 208 (8.90), 193 (11.55), 179 (9.66), 165 (13.33) 152 (9.33), 139 $[C_{10}H_{19}]^+$ (18.16), 127 $[C_9H_{19}]^+$ (23.66), 111 $[C_8H_{15}]^+$ (5.83), 97 $[C_7H_{13}]^+$ (66.33), 83 $[C_6H_{11}]^+$ (63.11), 71 $[C_5H_{11}]^+$ (55.96), 57 $[C_4H_9]^+$ (100).
(-)myrtenal	150 $[M^+]$ (4.33), 138 (13.22), 120 $[C_8H_8O]^+$ (3.66), 117 (7.33), 107 $[C_7H_7O]^+$ (94.33), 91 (97), 81$[C_5H_5]^+$ (100), 67 $[C_4H_3O]^+$ (39.23), 55 $[C_3H_3O]^+$ (55.33).
phytol	296 $[M^+]$ (0.33), 281 $[C_{19}H_{37}O]^+$ (0.40), 266 $[C_{18}H_{34}{}^{O}]^+$ 252 $[C_{17}H_{32}{}^{O}]^+$ (0,66), 238 $[C_{16}H_{30}O]^+$ (0,69), 223 $[C_{16}H_{30}]^+$ (0,66), 207 $[C_{16}H_3O]^+$ (0,83), 139 $[C_{14}H_{25}]^+$ (0,83), 179 $[C_{13}H_{24}]^+$ (0,99), 165 $[C_{12}H_{21}]^+$ (0,99), 151 $[C_{11}H_{19}]^+$ (1,66), 137 $[C_{10}H_{17}]^+$ (3.66), 123 $[C_9H_{16}]^+$ (32.33), 97 (19.33), 71 (100).
3-hydroxy-14βH-pregnane	318 $[M^+]$ (16.11), 288 $[C_{28}H_{46}O_2]^+$ (0.83), 270 $[C_{18}H_{22}O_2]^+$ (0.83), 255 $[C_{18}H_{23}O]^+$ (0.83), 239 $[C_{18}H_{23}]^+$ (10.33), 207 (0.77), 182 (88), 157 $[C_{12}H_{13}]^+$ (10.33), 134 $[C_{10}H_{14}]^+$ (10.33), 77 $[C_6H_5]^+$ (10.33), 57 (100).
α-tocopherol	430 $[M^+]$ (76.33), 414 $[C_{28}H_{46}O_2]^+$ (0.83), 388 $[C_{26}H_{43}O_2]^+$ (0.83), 369 (0.83), 344 $[C_{23}H_{36}O_2]^+$ (0.83), 327 $[C_{23}H_{35}O]^+$ (0.83), 302 $[C_{20}H_{30}O_2]^+$ (0.83), 281 (0.83), 253 (0.83), 221 $[C_{16}H_{29}]^+$ (0.83), 205 $[C_{15}H_{25}]^+$ (10.33), 189 $[C_{14}H_{21}]^+$ (1.66), 165 $[C_{12}H_{21}]^+$ (100), 137 $[C_{10}H_{17}]^+$ (1.66), 121 (4.11), 105 (2.23), 83 $[C_6H_{11}]^+$ (2.73), 69 $[C_5H_9]^+$ (1.66), 43 $[C_3H_7]^+$ (30,66) .
6-methoxy-3-methyl-1H-indole	161 $[M]^+$ (98.66), 146 $[C_9H_8NO]^+$ (2.73), 154 (0.66), 132 $[C_8H_6NO]^+$ (27.33), 125 (0.83),117 $[C_8H_4O]^+$ (46.63), 103 $[C_7H_3O]^+$ (4.11), 91 $[C_6H_3O]^+$ (22.16), 77 $[C_5HO]^+$ (22.16), 65 $[C_4HO]^+$ (46.63), 43 (100).
campesterol	400 $[M^+]$ (65.83), 284 $[C_{27}H_{45}O]^+$ (22.46), 357 $[C_{25}H_{41}O]^+$ (2.11), 342 $[C_{24}H_{48}O]^+$ (1.66), 229 $[C_{23}H_{37}O]^+$ (1.66), 215 $[C_{22}H_{35}O]^+$ (53.33), 301 $[C_{21}H_{33}O]^+$ (1.66), 289 (71.66), 273 $[C_{19}H_{29}O]^+$ (23.86), 255 $[C_{19}H_{27}]^+$ (42.66), 231 (32.36), 201 $[C_{15}H_{21}]^+$ (3.66), 185 $[C_{14}H_{17}]^+$ (23,33), 197 (24.03), 178 (23.33),145 (100), 161 $[C_{12}H_{17}]^+$ (49.96), 147 $[C_{11}H_{15}]^+$ (38.86), 123 $[C_9H_{15}]^+$ (38.66), 107 $[C_8H_{12}]^+$ (69.96), 95 $[C_7H_{11}]^+$ (68.33), 81 $[C_6H_9]^+$ (61.66), 55 $[C_4H_7]^+$ (55.66).
stigmasta-5,22-dien-3-ol	412 $[M^+]$ (1.66), 397 $[C_{28}H_{45}O]^+$ (7.46), 382 $[C_{27}H_{42}O]^+$ (6.66), 369 $[C_{26}H_{41}O]^+$ (16.22), 351 (32.66), 340 $[C_{24}H_{36}O]^+$ (0.83), 327 $[C_{23}H_{35}O]^+$ (5.93), 313 $[C_{22}H_{33}O]^+$ (17.44), 300 $[C_{21}H_{32}O]^+$ (46.33), 385 $[C_{20}H_{29}O]^+$ (16.63), 271 (72.66), 255 $[C_{19}H_{27}]^+$ (79.66), 229 $[C_{17}H_{25}]^+$ (18.66), 213 (38.91) ,201 $[C_{15}H_{21}]^+$ (12.3), 175 $[C_{13}H_{19}]^+$ (17.11), 162 $[C_{12}H_{17}]^+$ (31.22), 147 $[C_{11}H_{15}]^+$ (31.22),133 (63.33), 121 $[C_9H_{13}]^+$ (63.33), 105 (51.63), 81 $[C_6H_9]^+$ (83), 55 $[C_4H_7]^+$ (96.33), 43 (100).
stigmast-4-en-3-one	414 $[M^+]$ (20.43), 396 (53.33), 383 $[C_{27}H_{43}O]^+$ (51.11), 370 $[C_{26}H_{42}O]^+$ (10.73), 353 $[C_{25}H_{37}]^+$ (8.66), 327 (9.23), 300 $[C_{21}H_{32}O]^+$ (8.11),281 (26.26), 253 (8.66), 229 $[C_{17}H_{25}O]^+$ (8.11), 207 (39.53), 175 $[C_{13}H_{19}O]^+$ (8.11), 147 $[C_{11}H_{15}O]^+$ (8.11), 124 (100), 95 $[C_7H_{11}O]^+$ (8.11), 81 $[C_6H_9O]^+$ (8.11), 55 $[C_4H_7O]^+$ (8.11) .
γ-sitosterol	414 $[M^+]$ (96.66), 396(53.33), 383 $[C_{27}H_{43}O]^+$ (51.11), 371 $[C_{26}H_{43}O]^+$ (2.66), 354 $[C_{25}H_{38}O]^+$ (11.66), 329 $[C_{23}H_{37}O]^+$ (8.11), 303 (66.53), 273 $[C_{19}H_{29}O]^+$ (36.33), 255 $[C_{19}H_{27}]^+$ (44.13), 231 (35.13), 213 $[C_{16}H_{21}]^+$ (61.41), 187 $[C_{14}H_{19}]^+$ (23.33), 161 $[C_{12}H_{17}]^+$ (23.33), 145 (66.33), 123 (61.41), 107 $[C_8H_{11}]^+$ (60), 81 $[C_7H_9]^+$ (45.66), 55 $[C_4H_7]^+$ (45), 43 (100).
1,19-eicosadiene	278 $[M^+]$ (0.83), 208 $[C_{15}H_{28}]^+$ (1.66), 179 (9.96), 152 $[C_{11}H_{20}]^+$ (1.66), 140 $[C_{10}H_{20}]^+$ (1.66), 123 (97), 111 $[C_8H_{15}]^+$ (42.33), 95 (85), 68 $[C_5H_9]^+$ (100), 41 $[C_3H_5]^+$ (0.83).
tetradecyloxirane	240 $[M^+]$ (0.83), 226 $[C_{15}H_{30}O]^+$ (0.83), 218 (32.46), 211 $[C_{15}H_{30}O]^+$ (19.96), 199 $[C_{13}H_{26}O]^+$ (21.42), 183 $[C_{12}H_{23}O]^+$ (0.83), 169 $[C_{11}H_{21}O]^+$ (0.83), 155 $[C_{10}H_{20}O]^+$ (0.83), 141 $[C_9H_{17}O]^+$ (0.83), 123 (97.93), 95 (100), 85 $[C_5H_9O]^+$ (68).
sulfolane	120 $[M^+]$ (68.53), 105 $[C_4H_9OS]^+$ (0.83), 98 (0.83), 92 $[C_2H_4O_2S]^+$ (3.33), 78 $[CH_2O_2S]^+$ (3.33), 71 (0.83), 64 (3.66), 56 (100).

Antimicrobial activity assessment (disc diffusion assay)

Extracts were individually tested against a panel of Gram positive *Staphylococcus aureus*, Gram negative *Escherichia coli* bacterial and *Candida albicans*. Each of the compounds was dissolved in DMSO and

solution of the (5 cm^3) 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 20 g + beef extract 3 g + peptone 5 g) seeded with *Staphylococcus aureus*, *E. coli*and, *Candida albicans.* The Petri dishes were incubated at 36 ^{0}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 [11]. The % activity index for the complex was calculated by the formula:

$$\% \ Activity \ Index = \frac{Zone \ of \ inhibition \ by \ test \ extract \ (diametre) \ x \ 100}{Zone \ of \ inhibition \ by \ standard \ (diametre)}$$

Antioxidant activity assessment

In antioxidant activity screening assay ABTS method, each of the investigated extract (2 mL), ABTS solution (60 µM) were added to 3 mL MnO_2 solution (25 mg/mL), all prepared in (5 mL) aqueous phosphate buffer solution (pH 7, 0.1 M). The mixture were shaken, centrifuged, filtered and the absorbance of the resulting green blue solution (ABTS radical solution) at 734 nm was adjusted to approx. 0.5. Then, 50 µl of (2 mM) solution of the tested extract in spectroscopic grade MeOH/phosphate buffer (1:1) was added. The absorbance was measured and the reduction in color intensity was expressed as inhibition perecentage. L–ascorbic acid was used as standard antioxidant (positive control). Blank sample was run without ABTS using MeOH/phosphate buffer (1:1) instead of tested extracts. Negative control was run with ABTS and MeOH/phosphate buffer (1:1) only.

Cytotoxicity MTT assay

The different cell lines mentioned above were used to determine the inhibitory effects of compounds 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. The cells were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/ml penicillin and 100 µg/ml streptomycin at 37^{0}C in a 5% CO_2 incubator. The cells were seeds in a 96-well plate at a density of 1.0x104 cells/well. [14] at 37^{0}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 100 µl 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) [15, 16**]**. The relative cell viability in percentage was calculated as:

$$\% \ Relative \ Cell \ Viability \ = \frac{A570 \ of \ treated \ samples \ x \ 100}{A570 \ of \ untreated \ sample}$$

ACKNOWLEDGEMENT

The authors are indebted to Chemistry Department, Faculty of Science, Mansoura University for providing some research facilities.

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