ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Phytochemical and Biological Evaluation of *Tephrosia apollinea.*

Khattab Omer Azeez, Noha M Shaker, Maha M ElShamy[2], and Mamdouh Abdel Mogib.

[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 *Tephrosia apollinea* (Leguminosae), an important medicinal plant. The phytochemical investigation resulted in the separation and identification of the flavones *semiglabrin* **1**, *pseudosemiglabrin* **2**, *glabratephrin* **3** and *apollinine* **4,** from methylene chloride fraction, in addition to the identification of the volatile constituents of petroleum ether and methylene chloride by GC/MS analysis. Structures of separated compounds were elucidated by spectral analysis. Additionally, the antimicrobial, antioxidant activities and cytotoxicity of different fractions of *T. apollinea* were evaluated. The antimicrobial activity index of methylene chloride extract decreased in the following order: against *Syncephalastrum racemosum* (**61.92%**) > against *Geotricum candidum* (**60.27%**) > against *Candida albicans* (**59.44%**) > against *Aspergillus fumigates* (**56.11%**). The activity index of methylene chloride extract (Ta2), ethyl acetate extract (Ta3) and petroleum ether extract (Ta1) against *Streptococcus pneumonia* were found to be **78.15%, 58.40%** and **47.89%**, respectively, and against *Bacillis subtillis*, their activity indexes were **62.65%, 52.77%, 41.49%**, respectively. While the activity index of (Ta2), (Ta 1), (Ta 3) against *Escherichia coli* were **68.34%, 58.29%** and **52.26%**, respectively. The radical scavenging activity of the extracts and standard were found to be in the following order: ascorbic acid, butanol (Ta 4), ethyl acetate (Ta 3), methylene chloride (Ta 2) and petroleum ether (Ta 1), respectively. The IC50 values against **HePG2** indicated that the cytotoxicity of extracts decreased in the order: (Ta 1) was “strong”, (Ta 2) was “moderate”, (Ta 3) and (Ta 4) were “weak”. The cytotoxicity against **HCT-116** (Ta 1) was “strong”, (Ta 2), (Ta 4) were “moderate” and (Ta 3) was “weak”, The cytotoxicity against **PC3** of (Ta 1) was “very strong”, (Ta 2), (Ta 3) and (Ta 4) were “moderate”.

Keywords: Leguminosae, *Tephrosia apollinea*, flavones, *semiglabrin*, *pseudosemiglabrin*, *glabratephrin*, *tephroglabrin*, *apollinine*.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Tephrosia (Leguminosae), is a large tropical and subtropical genus. It includes 350 species. *Tephrosia apollinea* is distributed in Iran, Pakistan, northwest India and northeast Africa (Egypt, Sudan, Ethiopia, Eritrea, Djibouti and Somalia) [1, 2].

The extracts of *T. apollinea* has piscicidal, insecticidal, insect repellent, anti-cancer, antioxidant, antiangiogenic and cytotoxic activities [2, 3].

The literature survey indicated that *T. apollinea* is characterized by the presence of rotenoids, isoflavones, flavanones, chalcones, flavonols, and prenylated flavonoids [2].

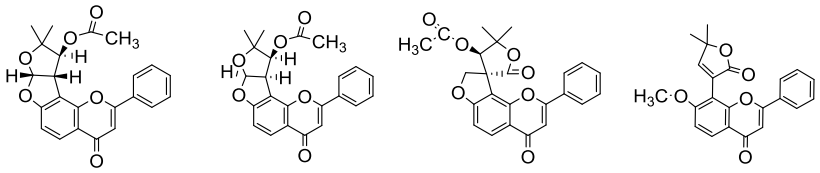
In this article, we present the results of the phytochemical reinvestigation, as well as the antimicrobial, antioxidant and antitumor activities of *T. apollinea*.

RESULT AND DISCUSSION

Phytochemical evaluation

The separation of extracts of *T. apollinea* afforded known natural products, including, semiglabrin **1**, pseudosemiglabrin **2**, glabratephrin **3,** and apollinine **4**. The structures of the separated compounds were proven by 1D and 2D-NMR data, GC/MS analysis which agreed with the corresponding analogues from the literature [4, 5].

Petroleum ether and methylene chloride extracts were analyzed by GC/MS. A sample from petroleum ether extract afforded 33compounds, representing 80.47% from the sample, with n-eicosane (8.84%), n-hexadecane (6.74%) and n-nonadecane (6.55%) being the major components. A sample from methylene chloride extract afforded 22 compounds, representing 82.18% from the sample, with n-hexadecanoic acid (12.54%), 9-octadecenoic acid (10.36%), and tetracosane (9.09%) being the major components.



semiglabrin (**1**) pseudosemiglabrin (**2**) glabratephrin (**3**) apollinine (**4**)

Antimicrobial activity assessment

Table 1: the inhibition zone in mm of extracts of *T. apollinea* compared to standard antibiotics

Sample Test Microorganisms	*Ta* 1	*Ta* 2	*Ta* 3	*Ta* 4	Standard antibiotic
fungi					*Amphotericin B*
Aspergillus fumigates (RCMB 02568)	NA	13.3 ± 1.21	NA	NA	23.7 ± 0.1
Syncephalastrum racemosum (RCMB 05922)	NA	12.2 ± 0.25	NA	NA	19.7 ± 0.2
Geotricum candidum (RCMB 05097)	NA	17.3 ± 0.38	NA	NA	28.7 ± 0.2
Candida albicans (RCMB 05036)	NA	15.1 ± 0.72	NA	NA	25.4 ± 0.1
Gram positive bacteria					*Ampicillin*
Streptococcus pneumonia (RCMB 010010)	11.4 ± 0.72	18.6 ± 1.2	13.9 ± 0.43	NA	23.8 ± 0.2
Bacillis subtillis (RCMB 010067)	13.6 ± 0.58	20.3 ± 0.63	17.1 ± 0.58	NA	32.4 ± 0.2
Gram negative bacteria					*Gentamicin*
Pseudomonas aeruginosa (RCMB 010043)	NA	NA	NA	NA	17.3 ± 0.1
Escherichia coli (RCMB 010052)	11.6 ± 0.72	13.6 ± 0.63	10.4 ± 0.58	NA	19.9 ± 0. 3

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

The results indicated that the activity index of methylene chloride extract decreased in the following order: against *Syncephalastrum racemosum* (**61.92%**) > against *Geotricum candidum* (**60.27%**) > against

Candida albicans (**59.44%**) > against *Aspergillus fumigates* (**56.11%**). The activity index of methylene chloride extract (Ta2), ethyl acetate extract (Ta3) and petroleum ether extract (Ta1) against *Streptococcus pneumonia* were found to be **78.15%, 58.40%** and **47.89%**, respectively, and against *Bacillis subtillis*, their activity indexes were **62.65%, 52.77%, 41.49%**, respectively. While the activity index of (Ta2), (Ta 1), (Ta 3) against *Escherichia coli* were **68.34%, 58.29%** and **52.26%**, respectively (Table 1).

Free radical scavenging activity assessment

The IC50 values of the extracts of *T. apollinea* were presented in Table 2, and Fig. 1. The free radicals of 2,2-diphenyl-1-picrylhydrazyl (DPPH) is used for detection of the antioxidant activity of the extracts [10]. Butanol extract (Ta 4) had the highest scavenging activity. The radical scavenging activity of the extracts and standard decreased in the following order: ascorbic acid, butanol (Ta 4), ethyl acetate (Ta 3), methylene chloride (Ta 2) and petroleum ether (Ta 1) (Table 2 and Fig. 1), respectively.

Table 2: Antioxidant activity of the extracts of *T. apollinea* by DPPH

Extract	IC50 µg/ml.
ascorbic acid	14.20
Petroleum ether (Ta 1)	298.4
Methylene chloride (Ta 2)	201.47
Ethyl acetate (Ta 3)	33.76
Butanol (Ta 4)	19.00

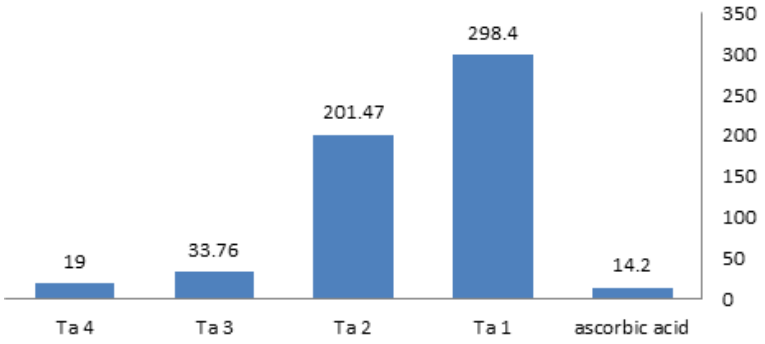


Figure 1: The IC50 values of the extracts of *T. apollinea*

Cytotoxic activity assessment

The IC50 values against **HePG2** (Table 3) indicated that the cytotoxicity of extracts decreased in the order: petroleum ether extract (Ta 1) was "strong", methylene chloride extract (Ta 2) was "moderate", ethyl acetate extract (Ta 3) and butanol extract (Ta 4) were "weak". The cytotoxicity against **HCT-116** of the petroleum ether extract (Ta 1) was "strong", methylene chloride extract (Ta 2), butanol extract (Ta 4) were "moderate" and ethyl acetate extract (Ta 3) was "weak", The cytotoxicity against **PC3** of the petroleum ether extract (Ta 1) was "very strong", methylene chloride extract (Ta 2), ethyl acetate extract (Ta 3) and butanol extract (Ta 4) were "moderate".

Table 3: Cytotoxic activity assessment of *T. apollinea* extracts against human tumor cells HePG2, HCT-116 and PC3

Extracts	In vitro cytotoxicity IC50 (µg/ml)•		
	HePG2	HCT-116	PC3
5-fluorouracil	6.6±0.24	8.4±0.20	9.6±0.27
Petroleum ether (Ta 1)	19.90	11.70	9.11
Methylene chloride (Ta 2)	48.00	23.10	23.90
Ethyl acetate (Ta 3)	67.30	59.90	47.90
Butanol (Ta 4)	83.90	47.00	44.20

•IC50 (µg/ml): 1 – 10 (very strong). 11 – 20 (strong). 21 – 50 (moderate). 51 – 100 (weak) and above 100 (non-cytotoxic).

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 ($CDCl_3$) 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^{o}C and the quadrupole temperature was 150^{o}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^{o}C then elevated to 280^{o}C at rate of 8^{o}C/min. and 10 min .hold at 280^{o}C the detector and injector temperature were set at 280^{o}C and 250^{o}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; The cell linesHePG-2, hepatocellular carcinoma (liver), MCF-7, mammary gland (breast) and HCT-116 cell line human colon carcinoma 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 (sigma co., St. Louis, USA), Fetal Bovine serum (GIBCO, UK).

Plant material

Tephrosia apollinea (Del.) Link was collected from Alkharga Oasis, Alwady Algaded on March, 2014. The plant species was identified by by Prof. Dr. Ibrahim Mashaly, Botany Department, Faculty of Science, Mansoura University.

Processing of the plant material

The aerial parts were dried in an oven at 40^{o}C for 24 hour and been grinded to give 253 g of dried powdered material, which was extracted by a soxhlet extractor using different solvents; petroleum ether (60-80^{o}C), methylene chloride, ethyl acetate and methanol, respectively to give four fractions; petroleum ether fraction (12.00g, 4.74% w/w), methylene chloride fraction (3.00 g, 1.18% w/w), ethyl acetate fraction (2.70g, 1.06% w/w) and methanol fraction (22 g,8.69% $_{w}/_{w}$). The methanol fraction was portioned in a separatory funnel between water and butanol to give a butanol fraction (17 g, 6.71% $_{w/w}$).

A sample of the petroleum ether fraction was analysis by GC/MS. The methylene chloride fraction (3 g) was separated by silica gel CC eluted by hexane /ethyl acetate with increasing polarity. The fraction eluted by hexane /ethyl acetate 7:3 gave a mixture of semiglabrin **1** and pseudosemiglabrin **2** (11:10, 5 mg). The fraction eluted by hexane /ethyl acetate 1:1 gave glabratephrin **3** (5 mg). The fraction eluted by hexane /ethyl acetate 7:13 gave apollinine **4** (6 mg). Additionally, a sample of the methylene chloride fraction was analysis by GC/MS The separation of both ethyl acetate fraction and butanol fraction did not succeed to give separated compounds.

GC/MS analysis of petroleum ether fraction (Ta 1) afforded undecane (R_t 9.29 min., 0.33%), dodecane (R_t 11.40 min., 1.41%), tridecane (R_t 11.68 min., 0.22%), 2-methyldodecane (R_t 12.65 min., 0.37%), 2,6-

dimethyldodecane (R_t 12.82 min., 0.88%), eugenol (R_t 14.54 min., 1.04%), tetradecane (R_t 15.31 min., 3.41%), pentadecane (R_t 17.07 min., 4.75%), hexadecane (R_t 18.68 min., 6.74%), 2-methylheptadecane (R_t 19.35 min., 0.61%), heptadecane (R_t 20.27 min., 6.55%), octadecane (R_t 21.67 min., 2.35%), nonadecane (R_t 23.08 min., 4.25%), eicosane (R_t 24.23 min., 8.84%), 3-hydroxy,14-βH-pregnan-20-one (R_t 24.81 min., 0.53%), heneicosane (R_t 25.64 min., 4.58%), olicacid (R_t 26.65 min., 3.76%), docosane (R_t 26.86 min., 2.97%), tricosane (R_t 27.97 min., 3.97%), 2-methyltricosane (R_t 28.56 min., 0.49%), tetracosane (R_t 29.03 min., 3.35%), pentacosane (R_t 30.03 min., 2.47%), 2-methylpentacosane (R_t 30.53 min., 0.62%), hexacosane (R_t 31.00 min., 2.67%), 2-methylhexacosane (R_t 31.57 min., 0.42%), heptacosane (R_t 32.06 min., 2.15%), octacosane (R_t 33.26 min., 1.77%), squalene (R_t 33.74 min., 1.09%), nonacosane (R_t 34.67 min., 1.04%), triacontane (R_t 36.34 min., 0.46%), hentriacontane (R_t 38.43 min., 0.89%), and dl-alpha-tocopherol (R_t 39.67 min., 0.95%).

GC/MS analysis of methylene chloride fraction (Ta 2) gave thiophene (R_t 11.75 min., 2.45%), eugenol (R_t 14.50 min., 0.99%), shyobunol (R_t 17.35 min., 1.27%), myristic acide (R_t 20.88 min., 0.90%), nonadecane (R_t 21.19 min., 0.27%), longifolenaldehyde (R_t 21.85 min., 0.63%), 7,11,15-trimethyl-3-methylenehexadec-1-ene (R_t 21.92 min., 0.81%), n-hexadecanoicacid (R_t 23.69 min., 12.54%), eicosane (Rt 24.03 min.,0.72%) 9-octadecenoic acid (R_t 25.92 min., 10.36%), pregnane (R_t 26.14 min., 2.45%), eicosane (R_t 26.33 min., 4.99%), heneicosane (R_t 26.47 min., 1.99%), docosane (R_t 26.90min., 1.90%), tricosane (R_t 27.61min., 3.90%), 2-methyltricosane (R_t 27.71 min., 7.27), tetracosane (R_t 28.72 min., 9.09%), pentacosane (R_t 29.76 min., 5.54%), hexacosane (R_t 30.95 min., 5.99%), heptacosane (R_t 31.91 min 6.90%), octacosane (R_t 33.13 min., 4.27% and triacontane (R_t 36.26 min., 3.36%). The MS data of compounds identified by the GC/MS was presented in Table 5.

Table 5: MS data of compounds identified by GC/MS from petroleum ether and methylene chloride fractions

Name of compound	MS data: m/z [identity] (rel. abound.%)
undecane	156 $[M^+]$ (7.5), 139 (0.83), 127 $[C_9H_{19}]^+$, 113 $[C_8H_{17}]^+$ (3.33), 98 $[C_7H_{14}]^+$ (8.33), 85 $[C_6H_{13}]^+$ (8.33), 71$[C_5H_{11}]^+$ (33.33S), 57 $[C_4H_9]^+$ (100).
dodecane	170 $[M^+]$ (6.89), 148 (0.83),133 (3.33),112 $[C_8H_{16}]^+$ (5), 85 $[C_6H_{13}]^+$ (41.66), 57 $[C_4H_9]^+$ (100).
tridecane	184 $[M^+]$ (0.83), 169 $[C_{12}H_{25}]^+$ (0.83), 148 (3.33), 133 (6.66), 120 (21.66) , 98$[C_7H_{14}]^+$ (16.66), 85 $[C_6H_{13}]^+$ (16.66), 71 $[C_5H_{11}]^+$ (66.66), 57 $[C_4H_9]^+$ (100).
2-methyldodecane	184 $[M^+]$ (0.83), 180 (0.83), 165 (16.66), 141 $[C_{10}H_{21}]^+$ (15), 119 (10), 99 $[C_7H_{13}]^+$ (20), 85 $[C_6H_{13}]^+$ (16.66), 57 $[C_4H_9]^+$ (100).
2,6-dimethyldodecane	198 $[M^+]$ (1.66), 180 (3.33), 165 (8.33), 146 (11.66), 131(16.66), 113 $[C_8H_{17}]^+$ (18.33), 97 $[C_7H_{13}]^+$ (10.0), 85 $[C_6H_{13}]^+$ (23.33), 71$[C_5H_{11}]^+$ (86.66), 57 $[C_4H_9]^+$ (100).
Eugenol	164 $[M^+]$(96.66), 149 $[C_9H_9O_2]^+$(20), 131 $[C_9H_8O_2]^+$ (33.33), 113 (16.66), 99 (23.33), 85 (60), 71(90), 57(100).
tetradecane	198 $[M^+]$ (13.33), 169 $[C_{12}H_{25}]^+$ (3.33), 141 $[C_{10}H_{21}]^+$ (20), 113 $[C_8H_{17}]^+$ (10), 85 $[C_6H_{13}]^+$ (66.66), 57 $[C_4H_9]^+$ (100).
pentadecane	212 $[M^+]$ (13.33), 183 (3.33), 168 $[C_{12}H_{26}]^+$ (6.66), 155 $[C_{11}H_{23}]^+$ (10),141 $[C_{10}H_{21}]^+$ (10.43), 113 $[C_8H_{17}]^+$ (13.33), 99 $[C_7H_{15}]^+$ (33.33), 85$[C_6H_{13}]^+$ (79.66), 71 $[C_5H_{11}]^+$ (90),57 $[C_4H_9]^+$ (100).
hexadecane	226 $[M^+]$ (16.66), 202 (3.33), 184 $[C_{13}H_{28}]^+$ (10), 170 $[C_{12}H_{26}]^+$ (16.66), 155 $[C_{11}H_{23}]^+$ (26.66), 141 $[C_{10}H_{21}]^+$ (13.33), 127 $[C_9H_{19}]^+$ (13.33), 113 $[C_8H_{17}]^+$ (16.66), 99 $[C_7H_{15}]^+$ (26.66), 85 $[C_6H_{13}]^+$ (96.66), 71$[C_5H_{10}]^+$ (106,66), 57 $[C_4H_9]^+$ (1 00).
2-methylheptadecane	254 $[M^+]$ (0.83), 232 (10), 216 (3.33), 189 (10), 169 $[C_{12}H_{25}]^+$ (36.66), 152 (13.33), 113 $[C_8H_{17}]^+$ (20), 85 $[C_6H_{13}]^+$ (53.33), 57 $[C_4H_9]^+$ (100).
heptadecane	240 $[M^+]$ (13.33), 211 $[C_{15}H_{31}]^+$ (3.33), 184 $[C_{13}H_{28}]^+$ (15), 169 $[C_{12}H_{25}]^+$(18.33), 141 $[C_{10}H_{21}]^+$ (13.33), 113 $[C_8H_{17}]^+$ (26.66), 85 $[C_6H_{13}]^+$ (96.66), 57 $[C_4H_9]^+$ (100).
octadecane	254 $[M^+]$ (13.33),228 (3.33), 212 $[C_{15}H_{32}]^+$ (5),197 $[C_{14}H_{29}]^+$ (10),178 (50), 157 (10), 141 $[C_{10}H_{21}]^+$ (16.66), 113 $[C_8H_{17}]^+$ (23.33), 85 $[C_6H_{13}]^+$ (86.66), 57 $[C_4H_9]^+$ (100).
nonadecane	268 $[M^+]$ (10), 256 (66.66), 239 $[C_{17}H_{35}]^+$, 213 (56.66), 185 (33.33),157 (26.66), 129 (53.33), 111 (23.33), 85 $[C_6H_{13}]^+$ (96.66), 57 $[C_4H_9]^+$ (100).
eicosane	282 $[M^+]$ (10), 256 (66.66), 213 (56.66), 185 (33.33), 157 (26.66), 129 (53.33),111 (26.66), 85 $[C_6H_{13}]^+$ (96,66), 57 $[C_4H_9]^+$ (100).
3-hydroxy,14-βHpregnan-20-one	332$[M^+]$ (0.83), 324 (0.83), 302 $[C_{20}H_{30}O_2]^+$(0.83), 274 $[C_{18}H_{26}O_2]^+$(16.66), 238 (26.66), 206 (60), 165$[C_{11}H_{17}O]^+$ (43.33), 141 $[C_9H_{17}O]^+$ (26.66),111$[C_7H_{11}O]^+$ (40), 85$[C_5H_9O]^+$(66.66), 57 $[C_4H_9]^+$ (100).
heneicosane	296 $[M^+]$ (13.33), 274 (10), 254 $[C_{18}H_{38}]^+$ (6.66), 238 $[C_{16}H_{34}]^+$ (10), 211 $[C_{15}H_{31}]^+$ (13.33), 191 (13.13), 169 $[C_{12}H_{25}]$ (15) $^+$, 141 $[C_{10}H_{21}]^+$ (16.66), 113 $[C_8H_{17}]^+$ (26,66),85 $[C_6H_{13}]^+$ (96.66), 57 $[C_4H_9]^+$ (100).
oleic acid	282 $[M^+]$ (10), 280 (11.66), 264 $[C_{18}H_{32}O]^+$(0.83), 240 (10), 220 (98.33), 189 (26.66), 165 (26.66), 141(13.33), 123 (23.33), 97 $[C_7H_{15}]^+$ (63.33), 81(56.33), 55 $[C_4H_7]^+$ (66.66).
docosane	310 $[M^+]$ (16.66), 288 (10), 270 (6.66), 253 $[C_{16}H_{32}]^+$ (6.66), 237(6.66), 220 (33.33), 203(13.33), 183 $[C_{13}H_{27}]^+$ (13.33), 155 $[C_{11}H_{23}]^+$ (16.66), 127 $[C_9H_{19}]^+$ (20), 99 $[C_7H_{15}]^+$ (40),

	71 $[C_5H_{11}]^+$ (100), 54 (26.66).
tricosane	324 $[M^+]$ (13.33), 302 (10), 281 $[C_{20}H_{41}]^+$(16.99), 253 $[C_{18}H_{37}]^+$ (10), 225 $[C_{16}H_{33}]^+$ (13.33), 207 (3.33), 183 $[C_{13}H_{27}]^+$ (13.33), 155$[C_{11}H_{23}]^+$ (20), 127 $[C_9H_{19}]^+$ (25), 99$[C_7H_{15}]^+$ (38.33), 71 $[C_5H_{11}]^+$ (100), 57 $[C_4H_9]^+$ (26.66).
2-methyltricosane	338 $[M^+]$ (3.33),337 (3.33), 316 (10), 295 $[C_{21}H_{43}]^+$ (16.99), 264 (16.66), 234 (30), 203 (20), 183 $[C_{13}H_{27}]^+$ (20), 165 (20), 141 $[C_{10}H_{21}]^+$ (2.3), 111 (43.3), 85 $[C_6H_{13}]^+$ (9), 57 $[C_4H_9]^+$ (100).
tetracosane	338 $[M^+]$ (13.33), 309 $[C_{22}H_{45}]^+$ (3.33), 281 $[C_{20}H_{41}]^+$ (10), 264 (16.66), 239 $[C_{16}H_{35}]^+$ (10), 211 $[C_{15}H_{31}]^+$ (10), 183 $[C_{13}H_{27}]^+$ (13.33), 155 $[C_{11}H_{23}]^+$ (20), 127 $[C_9H_{19}]^+$ (26.66), 99$[C_7H_{15}]^+$ (43.33), 71 $[C_5H_{11}]^+$ (100), 57 $[C_4H_9]^+$ (26.66).
pentacosane	352 $[M^+]$ (10), 323 $[C_{23}H_{47}]^+$ (3.33), 295 $[C_{21}H_{43}]^+$ (10), 267 $[C_{19}H_{39}]^+$ (10), 239 $[C_{17}H_{35}]^+$ (10), 215 (10), 197 $[C_{14}H_{29}]^+$ (13.33), 169 $[C_{12}H_{25}]^+$ (16.66), 141 $[C_{10}H_{21}]^+$ (20), 113 $[C_8H_{17}]^+$ (30),85 $[C_6H_{13}]^+$ (96.66), 57 $[C_4H_9]^+$ (100).
2-methylpentacosane	366 $[M^+]$ (3.33),344 (6.66) , 323 $[C_{23}H_{47}]^+$ (10), 304 (5), 279 (13.33), 260 (9,16), 239 $[C_{17}H_{35}]^+$ (10), 211$[C_{15}H_{31}]^+$ (10),189 (10), 167 (30),149 (33.33), 131 (6.66), 111 (33.33), 85 $[C_6H_{13}]^+$ (66.66), 57 $[C_4H_9]^+$(100).
hexacosane	366 $[M^+]$ (3.33),337 $[C_{24}H_{49}]^+$ (2.5), 309 $[C_{22}H_{45}]^+$ (6.66),281 $[C_{20}H_{41}]^+$ (6.66),253 [C18H37]+ (8.33), 225 $[C_{16}H_{33}]^+$ (8.33), 197 $[C_{14}H_{29}]^+$ (10), 169 $[C_{12}H_{25}]^+$ (13.33), 141 $[C_{10}H_{21}]^+$ (16.66), 113 $[C_8H_{17}]^+$ (30), 85$[C_6H_{13}]^+$ (86.66) 57 $[C_4H_9]^+$ (100).
2-methylhexacosane	380 $[M^+]$ (0.83), 358 (3.33), 337 $[C_{24}H_{49}]^+$ (13.33), 306 (3.33), 281$[C_{20}H_{41}]^+$ (3.33), 258 (8.33), 239 $[C_{17}H_{35}]^+$ (10), 215 (8.33), 197 $[C_{14}H_{29}]^+$ (10), 169 $[C_{12}H_{25}]^+$ (13.33), 141 $[C_{10}H_{21}]^+$ (16.66), 111 (30), 85 $[C_6H_{13}]^+$ (70), 57 $[C_4H_9]^+$ (100).
heptacosane	380 $[M^+]$ (4.16), 358 (1.66), 337 $[C_{24}H_{49}]^+$ (3.33), 309 $[C_{22}H_{45}]^+$ (3.33), 281 $[C_{20}H_{41}]^+$ (4.16), 253 $[C_{18}H_{37}]^+$ (6.66), 225 $[C_{16}H_{33}]^+$ (6.66),, 197 $[C_{14}H_{29}]^+$ (10), 169 $[C_{12}H_{25}]^+$ (10.83), 141 $[C_{10}H_{21}]^+$ (16.66), 113 $[C_8H_{17}]^+$ (30), 85$[C_6H_{13}]^+$ (83.33), 57 $[C_4H_9]^+$ (100).
octacosane	394 $[M^+]$ (3.33), 372 (0.83), 351$[C_{25}H_{51}]^+$ (1.66), 323 $[C_{23}H_{47}]^+$ (2.5),302 (0.83), 281 $[C_{20}H_{41}]^+$ (3.33), 253 $[C_{18}H_{37}]^+$ (3.33), 225 $[C_{16}H_{33}]^+$ (3.33), 197 $[C_{14}H_{29}]^+$ (6.66), 169 $[C_{12}H_{25}]^+$ (10), 141 $[C_{10}H_{21}]^+$ (13.33), 113 $[C_8H_{17}]^+$ (20), 85$[C_6H_{13}]^+$ (76.66), 57 $[C_4H_9]^+$ (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 (0.83), 217 (1.66), 191 $[C_{14}H_{23}]^+$ (4.16), 161 (3.33), 137 $[C_{10}H_{17}]^+$ (16.66), 119 (13.33), 95 (21.66),69 $[C_5H_9]$ (100), 51 (0.83).
nonacosane	408 $[M^+]$ (2.5), 386 (0.83), 365 $[C_{26}H_{53}]^+$ (0.83), 337 $[C_{24}H_{49}]^+$ (3.33), 309 $[C_{22}H_{45}]^+$ (3.33), 281 $[C_{20}H_{41}]^+$ (3.33), 253 $[C_{18}H_{37}]^+$ (3.33), 225 $[C_{16}H_{33}]^+$ (4.16), 197 $[C_{14}H_{29}]^+$ (5), 169 $[C_{12}H_{25}]^+$ (6.66), 141 $[C_{10}H_{21}]^+$ (10), 113 $[C_8H_{17}]^+$ (20), 85 $[C_6H_{13}]^+$ (73.33), 57 $[C_4H_9]^+$ (100).
triacontane	422 $[M^+]$ (1.66), 398 (0.83), 379 $[C_{27}H_{55}]^+$ (0.83), 351$[C_{25}H_{51}]^+$ (0.83), 332 (0. 309 $[C_{22}H_{45}]^+$ (1.66), 281 $[C_{20}H_{41}]^+$ (3.33), 253 $[C_{18}H_{37}]^+$ (3.33), 225 $[C_{16}H_{33}]^+$ (3.33), 197 $[C_{14}H_{29}]^+$ (4.16), 169 $[C_{12}H_{25}]^+$ (6.66), 141 $[C_{10}H_{21}]^+$ (10), 113 $[C_8H_{17}]^+$ (16.66), 85 $[C_6H_{13}]^+$ (70), 57 $[C_4H_9]^+$ (100).
hentriacontane	436 $[M^+]$ (0.83), 414 (0.83), 394 $[C_{28}H_{58}]^+$ (0,83), 365 $[C_{26}H_{53}]^+$ (0.83), 337$[C_{24}H_{46}]^+$ (1.66), 309 $[C_{22}H_{45}]^+$ (1.66), 281 $[C_{20}H_{41}]^+$ (3.33), 253 $[C_{18}H_{37}]^+$ (3.33), 239 $[C_{17}H_{35}]^+$ (3.33), 225 $[C_{16}H_{33}]^+$ (3.33), 197 $[C_{14}H_{29}]^+$ (5), 169 $[C_{12}H_{25}]^+$ (6.66), 141 $[C_{10}H_{21}]^+$ (10), 113 $[C_8H_{17}]^+$ (20), 85 $[C_6H_{13}]^+$ (70), 57 $[C_4H_9]^+$ (100).
dl-alpha-tocopherol	430 $[M^+]$ (96.66), 408 (0.83), 388 $[C_{28}H_{44}O_2]$+ (0.63), 368 (0.63), 344 $[C_{23}H_{36}O_2]$+ (0.63), 316 $[C_{21}H_{32}O_2]$+ (0.63), 298 (0.63), 281(0.83), 263(1.66), 246 $[C_{16}H_{24}O_2]$+ (0.63), 223 (0.63), 205 $[C_{13}H_{17}O_2]$+ (12.5), 187 (1.66),165 (100),145 (1.66), 121(3.33), 103 (1.66), 83 (1.66), 57 $[C_4H_9]^+$ (5).
thiophene	120 $[M^+]$ (73.33), 105 $[C_4H_9OS]^+$ (0.83), 92 $[C_2H_4O_2S]^+$ (3.33), 78$[CH_2O_2S]^+$ (3.33), 69 (0.83), 56 (100).
shyobunol	222 $[M^+]$ (3.33), 204 $[C_{15}H_{24}]^+$ (3.33), 189 $[C_{21}H_{21}]^+$ (13.33), 179 (10), 161 $[C_{12}H_{17}]^+$ (46,66), 147$[C_{11}H_{17}]^+$ (10), 136 (36.66), 121 $[C_9H_{13}]^+$ (70), 109 (53.33), 93 $[C_7H_{10}]^+$ (73.33), 81$[C_6H_9]^+$ (100), 69 (60), 55 (46.66),
myristic acide	228 $[M^+]$ (16.66), 217 (3.33), 199 $[C_{12}H_{23}O_2]$ (0.83), 185 $[C_{11}H_{21}O_2]$ (36.66), 171$[C_{10}H_{19}O_2]$ (13.33), 149 (16.66), 129 $[C_7H_{13}O_2]$ (50), 111 (23.33), 97 (43.33), 85$[C_6H_{13}]^+$ (63.33), 71 $[C_5H_{11}]^+$ (80), 57 $[C_4H_9]^+$ (100).
longifolenaldehyde	222[M+] (6.66), 205$[C_{14}H_{21O}]^+$ (13.33), 191$[C_{13}H_{19O}]^+$ (10),177 $[C_{12}H_{17O}]^+$ (20), 162 $[C_{12}H_{18}]^+$ (23.33), 149 $[C_{11}H_{17}]^+$ (66.66), 137 $[C_{10}H_{17}]^+$ (23.33), 125 (53.33), 109 $[C_8H_{13}]^+$ (70), 95 $[C_7H_{11}]^+$ (96.66), 81 $[C_6H_9]^+$ (100), 69 $[C_5H_9]^+$ (93.33), 55 $[C_4H_7]^+$ (86.66.0)
-3-7,11,15-trimethyl-methylenehexadec-1-ene	278 $[M^+]$ (3.33), 263 $[C_{19}H_{35}]^+$ (1.66), 246 (3.33), 222 $[C_{16}H_{30}]^+$ (0.83), 208 $[C_{15}H_{28}]^+$ (3.33), 193 $[C_{14}H_{25}]^+$ (3.33), 179 (10), 165 $[C_{12}H_{21}]^+$ (10), 151 $[C_{11}H_{19}]^+$ (15.0), 137 $[C_{10}H_{17}]^+$ (16.66), 123 $[C_9H_{15}]^+$ (63.33), 109 (40), 95 $[C_7H_{11}]^+$ (100), 71 $[C_5H_{11}]^+$ (66.66), 57 $[C_4H_9]^+$ (96.66).
n-hexadecanoicacid	256 $[M^+]$ (23.33), 241 $[C_{15}H_{29}O_2]$ (0.83), 227 $[C_{14}H_{27}O_2]$ (10), 213$[C_{13}H_{25}O_2]$ (23.33), 199 $[C_{12}H_{23}O_2]$ (10), 185 $[C_{11}H_{21}O_2]$ (13.33), 171 $[C_{10}H_{19}O_2]$ (13.33), 149 (100), 129 $[C_7H_{13}O_2]$ (31.66), 115 $[C_8H_{11}O_2]$ (11.66), 97 $[C_6H_9O_2]$ (20), 73 $[C_5H_{13}]$ (86.66), 57 $[C_4H_9]^+$ (43.33).
pregnane	288 $[M^+]$ (0.83), 284 (20), 241 (16.66), 211 (3.33), 185 (16.66), 155 (3.33), 129 (26.66), 85$[C_6H_{13}]^+$ (60), 57 $[C_4H_9]^+$ (100).
9-octadecenoic acid	282$[M^+]$ (1.66), 280 (3.33), 264 $[C_{18}H_{32}O]$ (16.66), 249 $[C_{18}H_{33}]$ (1.66), 222 $[C_{16}H_{30}]^+$ (10), 207 $[C_{15}H_{27}]$ (3.33), 180 $[C_{13}H_{24}]^+$ (3.33), 165 $[C_{12}H_{21}]^+$ (6.66), 149 (16.66), 123 $[C_9H_{15}]^+$ (21.66), 97 $[C_7H_{13}]$ (63.33), 69 (81.66), 55 (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 fungal (*Candida albicans*) strains. Each of the extracts was dissolved in DMSO and solutions of the concentration 1 mg /ml were prepared separately. Paper discs of Whatman filter paper discs with standard size (5 mm) were cut and sterilized in an autoclave. The paper discs were soaked in the desired concentration of the extract solution and placed aseptically in the petridishes containing nutrient agar media (agar 20 g + beef extract 3g + peptone 5g) seeded with *Staphylococcus aureus*, *E. coli* and *Candida albicans*. The petridishes were incubated at 36^{o}C and the inhibition zones were recorded after 24 h of incubation. Each treatment was replicated three times. The antibacterial activities of a common standard antibiotics, ampicillin, gentamicin and amphotericin B was also recorded using the same procedure as above at the same concentration and solvents [6, 7, 8]. The % activity index for the complex was calculated by the formula:

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

Antioxidant activity assessment

Free radical scavenging method (DPPH)

The antioxidant activity of extracts were 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 10^{0}C in the dark. A methanol solution of the test compound was prepared. A 40 ul aliquot of the methanol solution was added to 3 ml 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:

$$PI = [\{(AC - AT)/ AC\} \times 100] \quad (1)$$

Where *A*C = Absorbance of the control at t = 0 min and *A*T = absorbance of the sample + DPPH at t = 16 min [9-11]**.**

Cytotoxicity *MTT assay*

The cell line mentioned above was used to determine the inhibitory effects of compounds on cell growth using the MTT assay [12, 13]. 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 seeds in a 96-well plate at a density of 1.0x10^{4} cells/well [14] at 37^{o}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 5mg/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 (A_{570} of treated samples/A_{570} of untreated sample) X 100.

ISSN: 0975-8585

ACKNOWLEDGEMENT

The authors acknowledge Ministry of Education in Iraq for funding, and Chemistry Department, Faculty of Science, Mansoura University for providing some research facilities.

REFERENCES

[1] Boulos L. flora of Egypt. Al Hadara Publishing, Cairo, Egypt. 3 (2002): 229-231.
[2] Waterman P. and Khalid S. Phytochem 1980;19: 909.
[3] Sinha B, Natu A and Nanavati D. Phytochem 1982;21:1468.
[4] Bell E, Lackey J and Polhill R. Biochem Syst Ecol 1978;6:201.
[5] Wenkert E and Gottlieb H. Phytochem 1977;16:1811.
[6] Stylianakis A, Kolocouris N, Kolocouris G, Fytas G, Foscolos E, Padalko J, Neyts D and Clerq E. Bioorg Med Chem Lett 2003;13:1699 – 1703.
[7] Murray R., Rosenthal S., Kobayashi S.and Pfaller A. Medical Microbiology. 3rd ed. St. Louis: Mosby, p.161. (1998).
[8] Sardari A, Gholamreza M and Daneshtalab M. Pharm Biol 1998;36:180-188.
[9] Kitts D, Wijewickreme A and Hu C. Mol Cell Biochem 2000;203:1–10.
[10] Sanchez M, Larrauri A and Saura Calixto F. J Sci Food Agr 1998;76:270 – 276.
[11] Lissi E, Modak B, Torres R, Escobar J, and Urza A. Free Radical Res 1999;30:471 – 477.
[12] Mosmann T. Immunol Methods 1983;65:55-63.
[13] Denizot F. and Lang R. Immunol Methods 1986;89:271-277.
[14] Mauceri H, et al. Nature 1998;394:287-291.