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# Phytochemical and Biological Evaluation of *Urospermum Picroides*

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**Keywords**
*Urospermum picroides*; phytochemical evaluation; antioxidant; antimicrobial; cytotoxicity.

**Abstract** The chromatographic separation of the dried aerial part extracts of *Urospermum picroides* (L.) SCOP.ex.F.W. Schmidt afforded urospermal A **(1)** and 8,15-dihydroxygermacra-1(10),4-dien-(12,6)-olide-14-al **(2)**, quercetin 3-O-β-D-glucopyranosid **(3)** and kampferol 3-O-β-D-glucopyranoside **(4)**. GC/MS analysis of petroleum ether fraction from aerial parts gave α-amyrine, β-amyrine, 12-oleanen-3-yl acetate, olean-18-en-3β-ol, moretenol, campesterol, stigmasterol, 14β-H-pregnae and stigmast-5-en-3 β-ol, in addition to many known compounds.

Inhibited of the free radicals were found to be 86.9%, 85.3%, 86.1%, and 82.4% for seeds butanol fraction, seeds ethyl acetate fraction, aerial parts ethyl acetate fraction, and aerial parts methylene chloride fraction, respectively, compared to 88.1% inhibition by ascorbic acid. The sesquiterpene-rich sub fraction containing **1** and **2** and the flavonoid-rich sub fraction containing **3** and **4** inhibited 84.7% and 83.2%, respectively, which indicated the probable synergetic effect of other constituents in their main fractions.

The antimicrobial activity was found to be (68.2%, 70.8% and 69.2%), (54.5%, 50.0% and 50.0%), for aerial parts ethyl acetate fraction, and compounds **3** and **4** towards the Gram positive bacteria *S. aureus*, the Gram negative bacteria *E. coli* and the yeast *C. albicans*, respectively. The seeds butanol fraction and the seeds ethyl acetate fraction had activities of 68.2% and 50.50% towards Gram positive *S. aureus*, respectively.

The seeds butanol fraction was found to be very strong cytotoxic towards MCF-7 (9.4±0.37) and strong towards HePG-2 (14.7±0.85). The aerial parts ethyl acetate fraction was found to be very strong towards MCF-(78.8±0.47) and strong towards HePG-2 (10.1±0.88).

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## 1. Introduction

*Urospermum picroides* (L.) SCOP.ex.F.W. Schmidt (Compositae) (Aufs., 1795 & Boulos, 2002) is used as a medicinal plant for treatment of many diseases such as reducing the risk of chronic inflammatory diseases (Strzelecka *et al.*, 2005), transcription factors as target of the anti-inflammatory treatment (Stalinska *et al.*, 2005), and reducing postprandial plateletaggregation in patients with metabolic syndrome (Fragopoulou *et al.*, 2012). *U. picroides*is sold in the markets of Dalmatia (Southern Croatia) as wild vegetables (Luczaj *et al.*, 2013).

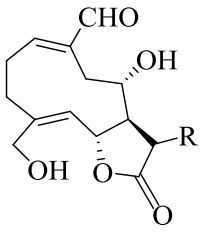
The phytochemical investigation of *U. picroides* showed the presence of luteolin-7-glucoside, quercetin, quercetin-3-galactoside, kaempferol-3-galactoside, chlorogenic and isochlorogenic acid (Giner *et al.*, 1992) as well as sesqueiterpene lactones and glucosides (Balboul *et al.*, 1997).

This article presents the results of the phytochemical, as well as biological reinvestigation.

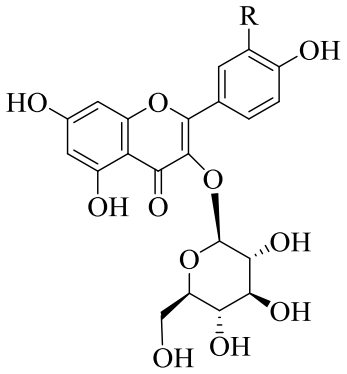
## 2. Results and Discussion

### 2.1. Phytochemical Evaluation

The chromatographic separation of the dried aerial part extracts of *U. picroides* afforded urospermal A **(1)** and 8,15-dihydroxygermacra-1(10),4-dien-(12,6)-olide-14-al **(2**), (Balboul *et al.*, 1997), as well as quercetin 3-O-β-D-glucopyranosid **(3**) and kampferol-3-O-β-D-glucopyranoside **(4**), which were previously reported from the same species (Giner *et al.*, 1992; Islam *et al.*, 2012 and Cai *et al.*, 2006). Compounds **1**-**4** were identified by comparing their $^{1}$H NMR spectra with those of the corresponding compounds, or literature data. GC/MS analysis of petroleum ether fraction from aerial part gave α-amyrine, β-amyrine, 12-oleanen-3-yl acetate,olean-18-en-3-ol, moretenol, campesterol, stigmasterol, 14β-H-pregnane and stigmast-5-en-3-ol, in addition to many known compounds as described in the experimental section.




R = α-methyl, urospermal A (**1**)
R = β-methyl,8,15-dihydroxygermacra-1(10),4-dien-(12,6)-olide-14-al (**2**)




R = OH, quercetin 3-O- β-D-glucopyranoside (**3**)
R = H, kampferol-3-O-β-D-glucopyranoside (**4**)

### 2.2. Biological Evaluation

*2.2.1 Antioxidant activity*

The antioxidant activity of petroleum ether fraction, methylene chloride fraction, ethyl acetate fraction, butanol fraction, the sesquiterpenoid mixture of compounds **1**,**2**, the flavonoid mixture of compounds **3**,**4** was assessed using ABTS method (Lissi *et al.*, 1999). A control experiment and another one using ascorbic acid as a reference antioxidant material were conducted. Table **1** showed that the seeds butanol fraction had inhibited (86.9%) of the free radicals, followed by the seeds ethyl acetate fraction (85.3%) which were very near to ascorbic acid (88.1%). The aerial parts ethyl acetate fraction had inhibited 86.1% of the free radicals, followed by the aerial parts methylene chloride fraction (82.4%). The sesquiterpene-rich sub-fraction containing compounds **1** and **2** and the

flavonoid-rich sub fraction containing compounds **3** and **4** inhibited 84.7% and 83.2%, respectively, which indicated the probable synergetic effect of other constituents in their main fractions.

**Table (1)**: Antioxidant activity assessment of fractions of aerial parts and seeds.

| Fraction or compound | Aerial part | | Seeds part | |
|---|---|---|---|---|
| | Absorbance | Inhibition % | Absorbance | Inhibition % |
| Control | 0.505 | 0% | 0.505 | 0% |
| Ascorbic acid | 0.060 | 88.1% | 0.060 | 88.1% |
| Petroleum ether fraction | 0.321 | 36.4% | 0.422 | 17.2% |
| Methylene chloride fraction | 0.089 | 82.4% | 0.225 | 55.9% |
| Ethyl acetate fraction | 0.070 | 86.1% | 0.074 | 85.3% |
| Butanol fraction | ---- | ---- | 0.067 | 86.9% |
| Compounds 1 & 2 | 0.085 | 83.2% | ---- | ---- |
| Compounds 3&4 | 0.077 | 84.7% | ---- | ---- |

*2.2.2 Antimicrobial activity*

The antimicrobial activity was assessed (Stylianakis *et al.*, 2003) using the Gram positive bacteria *Staphylococcus aureus*, and the Gram negative bacteria *Escherichia coli* and the yeast *Candida albicans*. Ampicillin and colitrimazole were used as reference antibiotics. Table **2** showed that ethyl acetate fraction of aerial parts had high antimicrobial activity 68.2%, 70.8% and 69.2%, followed by compounds **3** and **4** which had a moderate activity 54.5%, 50.0% and 50.0% towards the Gram positive bacteria *S. aureus*, and the Gram negative bacteria *E. coli* and the yeast *C. albicans*. Table **3** showed that the seeds butanol fraction had high activity 68.2% towards Gram positive *S. aureus* and ethyl acetate fraction had a moderate activity 50.50% towards Gram positive *S. aureus.*

**Table (2)**: Antimicrobial activity assessment data of ethyl acetate, methylene chloride, petroleum ether fractions, and compounds (**1, 2**) and (**3, 4**) from aerial parts.

| Fraction or compound | *S. aureus* | | *E. coli* | | *C. Albicans* | |
|---|---|---|---|---|---|---|
| | Diameter of inhibition zone (in mm) | % Activity index | Diameter of inhibition zone (in mm) | % Activity index | Diameter of inhibition zone (in mm) | % Activity index |
| Petrolum ether fraction | 4 | 18.2 | 3 | 12.5 | 5 | 19.2 |
| Methylene chloride fraction | 8 | 36.4 | 5 | 20.8 | 7 | 26.9 |
| Ethyl acetate fraction | 15 | 68.2 | 17 | 70.8 | 18 | 69.2 |
| Compounds **1**&**2** | 7 | 31.8 | 11 | 45.8 | 8 | 30.8 |
| Compounds **3**&**4** | 12 | 54.5 | 12 | 50.0 | 13 | 50.0 |
| Ampicillin | 22 | 100 | 24 | 100 | NA | NA |
| Colitrimazole | NA | NA | NA | NA | 26 | 100 |

**Table (3)**: Antimicrobial activity assessment of butanol, ethyl acetate, methylene chloride and petroleum ether from seeds.

| Compound | *S. aureus* | | *E. coli* | | *C. Albicans* | |
|---|---|---|---|---|---|---|
| | Diameter of inhibition zone (in mm) | % Activity index | Diameter of inhibition zone (in mm) | % Activity index | Diameter of inhibition zone (in mm) | % activity index |
| Petroleum ether fraction | 2 | 9.1 | NA | NA | NA | NA |
| Methylene chloride fraction | 7 | 31.8 | 4 | 16.7 | 10 | 38.5 |
| Ethyl acetate fraction | 11 | 50.50 | 10 | 41.7 | 7 | 26.9 |
| Butanol fraction | 15 | 68.2 | 11 | 45.8 | 8 | 30.8 |
| Ampicillin | 22 | 100 | 24 | 100 | NA | NA |
| Colitrimazole | NA | NA | NA | NA | 26 | 100 |

*2.2.3 Cytotoxic activity*

Cytotoxicity was expressed as the concentration that caused 50% loss of the cell monolayer ($IC_{50}$). The in vitro cytotoxicity against hepatocellular carcinoma, HePG-2 (liver) and mammary gland, MCF-7 (breast) was assessed (Denizot *et al.,* 1986). 5-Fluorouracil (5-FU) was used as a standard anticancer for comparison. Table **4** showed that butanol fraction from seeds was very strong cytotoxic towards MCF-7 (9.4±0.37) and strong towards HePG-2 (14.7±0.85). The activity of ethyl acetate fraction of aerial parts was found to be very strong towards MCF- (78.8±0.47) and strong towards (10.1±0.88) HePG-2, while the flavonoid-rich sub fraction contained compounds **3** and **4** was found to be less active.

**Table (4)**: Cytotoxic ($IC_{50}$) values of fractions of aerial parts and seeds on different cell lines.

| Fraction or compound | Aerial part | | Seeds part | |
|---|---|---|---|---|
| | In vitro Cytotoxicity $IC_{50}$ (µg/ml) | | In vitro Cytotoxicity $IC_{50}$ (µg/ml) | |
| | HePG2 | MCF-7 | HePG2 | MCF-7 |
| 5-FU | 6.6±0.24 | 4.7±0.11 | 6.6±0.24 | 4.7±0.11 |
| Butanol fraction | ---- | ---- | 14.7±0.85 | 9.4±0.37 |
| Ethyl acetate fraction | 10.1±0.88 | 8.8±0.47 | 30.2±1.83 | 18.1±0.96 |
| Methylene chloride fraction | 45.6±1.91 | 34.9±1.74 | 41.1±3.04 | 29.9±1.88 |
| Petroleum ether fraction | 79.5±3.52 | 90.6±4.56 | 88.6±4.50 | 70.3±3.92 |
| **1&2** | 36.2±1.55 | 53.1±2.30 | ---- | ---- |
| **3 &4** | 13.9±1.04 | 22.8±1.52 | ---- | ---- |

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

## 3. Experimental

**General:**

The NMR spectra were recorder on a Varian MercyryVX-300 nmr spectrometer. $^1$H-NMR spectra were run at 300 MHz in deuterated chloroform ($CDCl_3$) or dimethylsulphoxide (DMSO-$d_6$); GC/MS Analysis: **Method 1:** Aglient 6890 gas chromatograph equipped with an Aglient mass spectrometric detector, with a direct capillary interface and fused silica capillary column HP-5ms (30m x 0.32 mm x 0.25 µm film thickness). Helium was used as carrier

gas at approximate 1.0 ml/mim, pulsed splitess mode. The solvent delay was 3 min and the injection size was 1.0 µl. The mass spectrometric detector was operated in electron impact ionization mode with an ionizing energy of 70 e.v., scanning from m/z 50 to 500.The ion source temperature was 230$^{o}$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 (2 min) then elevated to 280$^{o}$C ata rate of 8$^{o}$C/min. The detector and injector temperature were set at 300 and 280$^{o}$C, respectively. Wiley and Wiley Nist mass spectral data base was used in the identification of separated peaks. (Agriculture Research Center, National Research Center, NRC, Dokki, Cairo, Egypt); **Method 2**: Avarian GC interfaced to Finnigan SSQ 7000 Mass Selective Detector (MSD) with ICIS V2.0 data system for MS identification of the GC components. The column used was DB-5 (J & W Scientific, Folosm, CA) cross–linked fused silica capillary column (30m long, 0.25mm interrnal diameter) coated with polydimethylsiloxane (0.5µm film thickness). The oven temperature was programmed from 50$^{o}$C for 3 min., at isothermal, then heating by 7$^{o}$C/ min. to 250$^{o}$C and isothermally for 10 min., at 250$^{o}$C. Injector temperature was 200$^{o}$C and the volume injected was 0.5 µl. Transition–line and ion source temperature were 250$^{o}$C and 150$^{o}$C respectively. The mass spectrometer had a delay of 3 min. to avoid the solvent peak and then scanned from m/z 50 to m/z 300. Ionization energy was set at 70 ev. (National Research Center (NRC), Dokki, Cairo); Solvents: petroleum ether (60-80$^{o}$C), diethyl ether, petroleum ether, methylene chloride, ethyl acetate, acetone, butanol and methanol were obtained from ElgomhoriaCompany; Chemical reagents for cytotoxicity activity: RPMI-1640 medium, MTT, DMSO and 5-fluorouracil (Sigma co., St. Louis, USA), fetal bovine serum (GIBCO, UK); Thin layer chromatography and preparative (TLC) were performed on silica gel (Kieselgel 60, GF 254) of 0.25 thickness.

**Plant material:**

*Urospermum picroides* was collected on April 2014 from the garden of Faculty of Science, Damietta University, and identified by Prof. Mamdouh Salem Serag, Botany Dept., Faculty of Science, Damietta University. Plant specimens were collected for pressing on herbarium sheets and later identification of species. Identification and nomenclature were followed (Tackholm, 1974 and Boulos, 1995, 2009). The voucher plant material and herbarium specimens of species recorded have been deposited in the Herbarium of Botany Dept., Faculty of Science, and Damietta University.

**Processing of plant material:**

The plant material was divided into two parts; aerial parts and seeds. Each part of them was air dried in shade at room temperature and grounded to give 400 g of aerial parts and 50 g of seeds dried powder material.

The aerial parts were extracted with $CH_2Cl_2$/MeOH (1:1) for 48 hr, after that filtrated and the solvent was evaporated. Solvent extraction by using different solvents; petroleum ether 60-80$^{o}$C, methylene chloride, ethyl acetate and butanol, respectively gave four fractions; petroleum ether fraction (6g), methylene chloride fraction (0.5g), ethyl acetate fraction (1.9g) and butanol fraction (0.9g).

A sample from petroleum ether fraction was analyzed by the GC/MS technique to give 2,6,10-trimethyl neophytadiene ($R_t$ 21.38 min, 0.36%), 2,6,10-trimethyl neophytadiene isomer ($R_t$ 21.61min, 0.63%), methyl palmitate ($R_t$ 22.21, 0.57%), palmitic acid ($R_t$22.83, 0.51%), heneicosane ($R_t$24.20, 0.96%), phytol isomer ($R_t$ 24.40, 2.25%), docosane ($R_t$ 25.31, 0.43%), tricosane ($R_t$ 26.39, 0.84%), pentacosane ($R_t$ 28.41, 0.82%), campesterol ($R_t$ 37.73, 1.06%), stigmasterol ($R_t$ 38.42, 6.36%), stigmast-5-en-3-ol ($R_t$ 39.77, 3.82%), olean-12-en-3-ol (β-amyrine) ($R_t$ 40.61, 5.12%) , urs-12-en-3-ol (α-amyrine) ($R_t$ 41.81, 5.02%), 12-oleanen-3-yl acetate ($R_t$

43.51, 8.28%), olean-18-en-3-ol ($R_t$ 43.79, 3.17%) and moretenol ($R_t$ 44.44, 2.09%).

A sample from the methylene chloride fraction was analyzed by the GC/MS technique to give 2-butoxyethanol ($R_t$ 5.22, 2.19%), tetrahydrothiophene1,1-dioxide ($R_t$ 11.59, 33.47%), (E)-4-(3-hydroxybut-1-en-1-yl)-3,5,5trimethylcyclohex-2-en-1-one ($R_t$18.59, 8.29%), (E)-4-(4-hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]heptan-1-yl)but-3-en-2-one ($R_t$ 19.16, 1.87%), eicosane ($R_t$ 23.02, 1.57%), heneicosane ($R_t$ 24.19, 1.76%), docosane ($R_t$ 25.31, 3.10%), tricosane ($R_t$ 26.39, 3.45%), tetracosane ($R_t$ 27.42, 4.36%), pentacosane ($R_t$ 28.41, 2.74%), hexacosane ($R_t$ 29.36, 3.10%), heptacosene ($R_t$ 29.91, 2.30%) and heptacosane ($R_t$ 29.91, 2.17%).

The ethyl acetate fraction was subjected to silica gel column chromatography using ethyl acetate /methanol with increasing polarity. Fractions 13-16 were combined and evaporated to give a residue (0.2g), which was re-chromatographed over silica gel column with ethyl acetate /methanol (97:3) to give a yellow solid compound (**3**) followed by a mixture of compounds (**3**) and (**4**) (Giner *et al.*, 1992).

The Butanol fraction 0.9g was subjected to silica gel column chromatography using ethyl acetate /methanol solvent system with increasing polarity. The fraction obtained by ethyl acetate /methanol (9:1) as eluent gave a mixture of compound (**1**) and compound (**2**) (Balboul *et al.*, 1997).

The seeds were extracted by a sohxlet extractor using different solvents; petroleum ether 60-80$^{o}$C, methylene chloride, ethyl acetate and butanol respectively to give four fractions; a sample of pet. ether extract and another one of methylene chloride extract were analyzed by the GC/MS technique.

The sample of petroleum ether extract gave 2-decenal ($R_t$ 12.64, 0.20%), tridecane ($R_t$ 13.29, 0.34%), 2,4-decadienal ($R_t$ 13.70, 0.24%), tetradecane ($R_t$ 15.13, 1.14%), pentadecane ($R_t$ 16.85, 2.94%), hexadecane ($R_t$ 18.48, 2.98%), 2-methylhexadecane ($R_t$ 19.41, 1.13%), heptadecane ($R_t$ 20.01, 1.52%), octadecane ($R_t$ 21.45, 3.78%), nonadecane ($R_t$ 22.83, 3.98%), eicosane ($R_t$ 24.14, 2.28%), 14β-H-pregnane and stigmast-5-en-3-ol (24.65, 1.49%), heneicosane ($R_t$ 24.41, 4.61%), 9,12-octadecadienoic acid ($R_t$ 26.11, 5.70%), docosane ($R_t$ 26.60, 4.33%), tricosane ($R_t$ 27.77, 4.71%), tetracosane ($R_t$ 28.10, 0.20%), (E)-pentacosa-1,3-diene ($R_t$ 29.16, 0.48%), heptacosane ($R_t$ 29.44, 0.22%), octacosen ($R_t$ 29.90, 3.81%), nonacosen ($R_t$ 30.89, 3.70%), henitiacontane ($R_t$ 44.07, 3.76%), dotriacontane ($R_t$ 40.93, 0.77%), tritriacontane ($R_t$ 44.07, 0.57%), (23s)-ethyl cholest-5-en-3β-ol ($R_t$ 45.25, 0.82%).

The sample of methylene chloride gave tetradecane ($R_t$ 25.33, 0.46%), hexadecane ($R_t$ 31.30, 0.88%), 2,6,10-trimethyltetradecane ($R_t$ 34.06, 0.57%), 3-methylheptadecane ($R_t$ 35.93, 0.39%), octadecane ($R_t$ 36.69, 1.22%), pytol ($R_t$ 37.70, 0.79%), nonadecane ($R_t$ 39.19, 1.21%), methyl palmitate ($R_t$ 39.91, 4.39%), eicosane ($R_t$ 41.58, 3.00%), heneicosane ($R_t$ 43.86, 8.48%), methyl-(E)-octadec-10-enoate ($R_t$ 43.97, 1.95%), docosane ($R_t$ 46.06, 7.51%), tricosane ($R_t$ 48.17, 10.73%), tetracosane ($R_t$ 50.19, 11.77%), pentacosane ($R_t$ 52.13, 10.89%), hexacosane ($R_t$ 54.00, 8.56%), heptacosane ($R_t$ 55.80, 6.44%), octacosane ($R_t$ 57.54, 4.82%), nonacosane ($R_t$ 59.22, 3.63%), triacontane ($R_t$ 60.85, 2.47%), hentriacontane ($R_t$ 62.42, 1.80%), dotriacontane ($R_t$ 64.01, 1.16%), tritriacontane ($R_t$ 65.86, 0.79%) and stigmast-5-en-3-ol ($R_t$ 66.62, 0.84%).

**Biological activity**

*Antioxidant activity:*

Antioxidant activity screening assay ABTS method. For each of the investigated fractions or compounds (2 mL) of ABTS solution (60 µM) was added to 3 mL $MnO_2$ 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). A blank sample was run without ABTS and using MeOH/phosphate buffer (1:1) instead of test material. Negative control was run with ABTS and MeOH/phosphate buffer (1:1) only (Lissi *et al.*, 1999). The % inhibition was calculated by the formula:

$$\text{Inhibition\%} = \frac{\text{Abs (control)} - \text{Abs (test)}}{\text{Abs (control)}} x100$$

*Antimicrobial activity:*

Antimicrobial fractions or compounds were individually tested against a panel of Gram positive bacteria *Staphylococcus aureus*, Gram negativebacteria, *Escherichia coli* and the yeast *Candida albicans*. The fraction or compound under investigation was dissolved in DMSO (1 mg /ml). Paper discs of Whatman filter paper were prepared with standard size (5mm) and sterilized in an autoclave. The paper discs were soaked in the desired concentration of the sample solution and placed aseptically in Petri dishes containing nutrient agar media (agar 20g + beef extract 3g + peptone 5g), seeded with *Staphylococcus aureus*, *E. coli* and *Candida albicans*. The Petri dishes were incubated at 36$^{o}$C and the inhibition zones were recorded after 24h of incubation. Each treatment was replicated three times. The antimicrobial 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 (Stylianakis *et al*., 2003). The % activity index for the test samplewas calculated by the formula:

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

*Cytotoxicity activity:*

The cell lines mentioned above were used to determine the inhibitory effects of fractions or 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. Cell lines were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/ml penicillin and 100µg/ml streptomycin at 37$^{o}$C in a 5% $CO_2$ incubator. The cell lines were seeded in a 96-well plate at a density of 1.0x104cells/well at 37$^{o}$C for 48 h under 5% $CO_2$. After incubation the cells were treated with different concentrations of fractions or 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 100 µl is added into each well to dissolve the purple formazan formed. The color intensity was measured and recorded at absorbance of 570 nm using a plate reader (EXL 800) (Denizot *et al*., 1986). The relative cell viability in percentage was calculated as:

The relative cell viability % = (A570 of treated samples/A570 of untreated sample) X 100

**Table (5):** Gas chromatography analysis of different fractions from aerial parts and seeds parts of *Urospermum picroides*.

| Compound name | MS data (m/z [identity] (relative abundance %)) |
|---|---|
| 2-butoxyethanol | 118 $[M]^+$ (1.6), 100 $[C_6H_{12}O]^+$ (3.3), 87 $[C_5H_{11}O]^+$ (23.3), 75 $[C_3H_7O_2]^+$ (10), 57 $[C_4H_9]^+$ (100). |
| tetrahydrothiophene - ,1,1-dioxide | 120 $[M]^+$ (73.3), 56 $[C_4H_8]^+$ (100). |
| 2-decenal | 154 $[M]^+$ (1.6),136 (3.3), 121 (10), 110 (20), 98 (30), 83 (66.6),70 (100), 55 (83.3). |
| tridecane | 184 $[M]^+$ (3.3), 141 $[C_{10}H_{21}]^+$ (1.6), 127 $[C_9H_{19}]^+$ (1.6), 99 $[C_7H_{15}]^+$(10), 85 $[C_6H_{13}]^+$ (43.3), 71 $[C_5H_{15}]^+$ (66.6), 57 $[C_4H_9]^+$ (100). |

| | |
|---|---|
| 2,4-decadienal | 152 $[M]^+$ (6.6),123 $[C_8H_{11}O]^+$ (3.3), 109 $[C_7H_9O]^+$ (3.3), 95 $[C_6H_7O]^+$ (10), 81$[C_5H_5O]^+$ (100), 55 $[C_3H_3O]^+$ (13.3). |
| tetradecane | 198 $[M]^+$ (6.6), 183 $[C_{13}H_{27}]^+$ (1.6), 169 $[C_{12}H_{25}]^+$ (1.6), 155 $[C_{11}H_{23}]^+$ (1.6), 141 $[C_{10}H_{21}]^+$ (1.6), 127 $[C_9H_{19}]^+$ (1.6), 99 $[C_7H_{15}]^+$(13.3), 85 $[C_6H_{13}]^+$ (70), 71 $[C_5H_{15}]^+$ (73.3), 57 $[C_4H_9]^+$ (100). |
| pentadecane | 212 $[M]^+$ (6.6), 169 $[C_{12}H_{25}]^+$ (1.6), 155 $[C_{11}H_{23}]^+$ (3.3), 141 $[C_{10}H_{21}]^+$ (3.3), 127 $[C_9H_{19}]^+$ (6.6), 113 $[C_8H_{17}]^+$ (10), 99 $[C_7H_{15}]^+$ (13.3), 85 $[C_6H_{13}]^+$ (56.6) , 71 $[C_5H_{11}]^+$ (76.6) , 57 $[C_4H_9]^+$ (100). |
| hexadecane | 226 $[M]^+$ (6.6), 155 $[C_{11}H_{23}]^+$ (20), 141 $[C_{10}H_{21}]^+$ (6.6), 127 $[C_9H_{19}]^+$ (10), 99 $[C_7H_{15}]^+$(20), 85 $[C_6H_{13}]^+$ (66.6), 71 $[C_5H_{15}]^+$ (83.3), 57 $[C_4H_9]^+$ (100). |
| (E)-4-(3-hydroxybut-1-en-1-yl)-3,5,5trimethylcyclohex-2-en-1-one | 208 $[M]^+$ (1.6), 193 $[C_{12}H_{17}O_2]^+$ (1.6), 165 $[C_{11}H_{17}O]^+$ (1.6), 152 $[C_{10}H_{16}O]^+$ (13.3), 135 $[C_{10}H_{15}]^+$ (6.6), 108 $[C_7H_9O]^+$ (100), 95$[C_6H_7O]^+$ (10), 55 $[C_4H_7]^+$ (1.6). |
| (E)-4-(4-hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]heptan-1-yl)but-3-en-2-one | 224 $[M]^+$ (1.6), 209 $[C_{12}H_{17}O_3]^+$ (1.6), 191 $[C_{12}H_{15}O_2]^+$ (1.6), 123 $[C_7H_7O_2]^+$ (100), 109 $[M–C_7H_{15}O]^+$ (6.6), 95 $[C_6H_7O]^+$ (6.6), 55 $[C_4H_7]^+$ (3.3). |
| 2-methylhexadecane | 240 $[M]^+$ (0.8), 225 $[C_{16}H_{33}]^+$ (3.3), 210 $[C_{15}H_{30}]^+$ (0.8), 197 $[C_{14}H_{29}]^+$ (20), 169 $[C_{12}H_{25}]^+$ (1.6), 155 $[C_{11}H_{23}]^+$ (1.6), 141 $[C_{10}H_{21}]^+$ (13.3), 127 $[C_9H_{19}]^+$ (13.3), 113 $[C_8H_{17}]^+$ (16.6), 99 $[C_7H_{15}]^+$(26.6), 85 $[C_6H_{13}]^+$ (56.6), 71 $[C_5H_{15}]^+$ (76.6), 57 $[C_4H_9]^+$ (100). |
| heptadecane | 240 $[M]^+$ (0.8), 169 $[C_{12}H_{25}]^+$ (13.3), 155 $[C_{11}H_{23}]^+$ (6.6), 141 $[C_{10}H_{21}]^+$ (10), 127 $[C_9H_{19}]^+$ (10), 113 $[C_8H_{17}]^+$ (13.3), 99 $[C_7H_{15}]^+$(23.3), 85 $[C_6H_{13}]^+$ (66.6), 71 $[C_5H_{15}]^+$ (86.6), 57 $[C_4H_9]^+$ (100). |
| 2,6,10-trimethyl neophytadiene | 278 $[M]^+$ (13.3), 179 $[C_{13}H_{23}]^+$ (10), 123 $[C_9H_{18}]^+$(63.3),111 (26.6), 95 $[C_7H_{11}]^+$ (90),82 $[C_6H_{10}]^+$ (100),68 $[C_5H_8]^+$ (66.6), 57 $[C_4H_9]^+$ (73.3). |
| octadecane | 254 $[M]^+$ (6.6), 225 $[C_{16}H_{33}]^+$ (0.8), 211 $[C_{15}H_{31}]^+$ (1.6), 197 $[C_{14}H_{29}]^+$ (3.3), 155 $[C_{11}H_{23}]^+$ (6.6), 141 $[C_{10}H_{21}]^+$ (10), 127 $[C_9H_{19}]^+$ (13.3), 113 $[C_8H_{17}]^+$ (16.6), 99 $[C_7H_{15}]^+$(26.6), 85 $[C_6H_{13}]^+$ (73.3), 71 $[C_5H_{15}]^+$ (86.6), 57 $[C_4H_9]^+$ (100). |
| 2,6,10-trimethyl neophytadiene isomer | 278 $[M]^+$ (16.6), 179 $[C_{13}H_{23}]^+$ (10), 137 $[C_{10}H_{17}]^+$ (13.3), 123 $[C_9H_{15}]^+$(50), 109 $[C_8H_{13}]^+$ (23.3), 95 $[C_7H_{11}]^+$ (90), 81$[C_6H_9]^+$ (100), 68$[C_5H_8]^+$ (60), 57 $[C_4H_9]^+$ (66.6). |
| methyl palmitate | 270 $[M]^+$ (13.3), 239 $[C_{16}H_{31}O]^+$ (10), 227 $[C_{14}H_{27}O_2]^+$ (13.3), 185 $[C_{11}H_{21}O_2]^+$ (8.5),143 $[C_8H_{15}O_2]^+$ (23.3), 129 $[C_7H_{13}O_2]^+$ (16.6), 87 $[C_4H_7O_2]^+$(66.6), 74 $[C_3H_6O_2]^+$ (100), 57 $[C_4H_7]^+$ (36.6). |
| palmitic acid | 268 $[M]^+$ (6.6), 239 $[C_{17}H_{35}]^+$ (1.6), 225 $[C_{16}H_{33}]^+$ (3.3), 197 $[C_{14}H_{29}]^+$ (3.3), 183 $[C_{13}H_{27}]^+$ (6.6), 169 $[C_{12}H_{25}]^+$ (13.3), 155 $[C_{11}H_{23}]^+$ (6.6), 141 $[C_{10}H_{21}]^+$ (10), 127 $[C_9H_{19}]^+$ (13.3), 113 $[C_8H_{17}]^+$ (16.6), 99 $[C_7H_{15}]^+$(26.6) , 85 $[C_6H_{13}]^+$ (76.6), 71 $[C_5H_{15}]^+$ (90), 57 $[C_4H_9]^+$ (100). |
| nonadecane | 256 $[M]^+$ (23.3), 213 $[C_{13}H_{25}O_2]^+$ (33.3), 171 $[C_{10}H_{19}O_2]^+$ (20), 129 $[C_7H_{13}O_2]^+$ (36.6), 97 $[C_6H_9O]^+$ (30), 73 $[C_3H_5O_2]^+$ (100), 57 $[C_4H_9]^+$ (73.3). |
| eicosane | 282 $[M]^+$ (3.3) , 239 $[C_{17}H_{35}]^+$ (1.6) , 211 $[C_{15}H_{31}]^+$ (1.6), 197 $[C_{14}H_{29}]^+$ (1.6) , 141 $[C_{10}H_{21}]^+$ (6.6) , 127 $[C_9H_{19}]^+$ (6.6) , 113 $[C_8H_{17}]^+$ (10) , 99 $[C_7H_{15}]^+$ (20) , 85 $[C_6H_{13}]^+$ (56.6) , 71 $[C_5H_{11}]^+$ (80) , 57 $[C_4H_9]^+$ (100 |
| heneicosane | 296 $[M]^+$ (3.3), 253 $[C_{18}H_{37}]^+$ (1.6), 239 $[C_{17}H_{35}]^+$ (1.6), 183 $[C_{13}H_{27}]^+$ (3.3), 155$[C_{11}H_{23}]^+$ (6.6), 141 $[C_{10}H_{21}]^+$ (6.6), 127 $[C_9H_{19}]^+$ (10), 113 $[C_8H_{17}]^+$ (10), 99 $[C_7H_{15}]^+$ (20), 85 $[C_6H_{13}]^+$(70), 71 $[C_5H_{11}]^+$ (86.6), 57 $[C_4H_9]^+$(100). |
| phytol isomer | 278 $[M\text{-}H_2O]^+$ (1.6), 207 $[C_{15}H_{27}]^+$ (1.6), 196 $[C_{13}H_{24}O]^+$ (1.6), 165 $[C_{12}H_{21}]^+$ (1.6), 137 $[C_{10}H_{17}]^+$ (1.6), 123 $[C_9H_{15}]^+$ (36.6), 95 $[C_7H_{11}]^+$ (33.3), |

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| | 81 $[C_6H_9]^+$ (33.3), 71 $[C_4H_7]^+$ (33.3), 57 $[C_4H_7]^+$ (33.3). |
| 14β-H-pregna | 288 $[M]^+$ (0.8),206 (73.3),165 (30), 141 (20),111 (33.3),85 (53.3), 57 (100). |
| docosane | 310 $[C_{22}H_{46}]^+$ (3.3), 281 $[C_{20}H_{41}]^+$ (1.6), 267 $[C_{19}H_{39}]^+$ (1.6), 253 $[C_{18}H_{37}]^+$ (1.6),239 $[C_{17}H_{35}]^+$ (1.6), 169 $[C_{12}H_{25}]^+$ (3.3), 141 $[C_{10}H_{21}]^+$ (10), 127 $[C_9H_{19}]^+$ (10), 113 $[C_8H_{17}]^+$ (13.3), 99 $[C_7H_{15}]^+$ (20), 85 $[C_6H_{13}]^+$ (60),71 $[C_5H_{11}]^+$ (80), 57 $[C_4H_9]^+$ (100). |
| 9,12-octadecadienoic acid | 280 $[M]^+$ (23.3), 236 $[C_{15}H_{24}O_2]^+$ (6.6), 165 $[C_{12}H_{21}]^+$ (13.3), 123 (26.6), 81 $[C_6H_9]^+$ (100), 55 $[C_4H_7]^+$ (86.6). |
| tricosane | 324 $[M]^+$ (3.3), 295$[C_{21}H_{43}]^+$ (1.6), 281 $[C_{20}H_{41}]^+$ (1.6), 267 $[C_{19}H_{39}]^+$ (1.6), 253 $[C_{18}H_{37}]^+$ (1.6), 239 $[C_{17}H_{35}]^+$ (1.6), 169 $[C_{12}H_{25}]^+$ (3.3), 141 $[C_{10}H_{21}]^+$ (10), 127 $[C_9H_{19}]^+$ (10), 113 $[C_8H_{17}]^+$ (13.3), 99 $[C_7H_{15}]^+$ (20), 85 $[C_6H_{13}]^+$ (60),71 $[C_5H_{11}]^+$ (80), 57 $[C_4H_9]^+$ (100). |
| tetracosane | 338 $[M]^+$ (3.3), 323 $[C_{23}H_{47}]^+$ (1.6), 295$[C_{21}H_{43}]^+$ (1.6), 281 $[C_{20}H_{41}]^+$ (1.6), 267 $[C_{19}H_{39}]^+$ (1.6), 253 $[C_{18}H_{37}]^+$ (1.6), 239 $[C_{17}H_{35}]^+$ (1.6), 169 $[C_{12}H_{25}]^+$ (3.3), 141 $[C_{10}H_{21}]^+$ (10), 127 $[C_9H_{19}]^+$ (10), 113 $[C_8H_{17}]^+$ (13.3), 99 $[C_7H_{15}]^+$ (20), 85 $[C_6H_{13}]^+$ (60),71 $[C_5H_{11}]^+$ (83.3), 57 $[C_4H_9]^+$ (100). |
| pentacosane | 352 $[M]^+$ (3.3), 281 $[C_{20}H_{41}]^+$ (10), 239 $[C_{17}H_{35}]^+$ (1.6), 225 $[C_{16}H_{33}]^+$ (1.6), 183 $[C_{13}H_{27}]^+$ (6.6), 155$[C_{11}H_{23}]^+$ (6.6), 85 $[C_6H_{13}]^+$(63.3), 71 $[C_5H_{11}]^+$ (80), 57 $[C_4H_9]^+$(100). |
| (E)-pentacosa-1, 3-diene | 348 $[M]^+$ (1.6) , 225 $[C_{16}H_{33}]^+$ (6.6) , 113 $[C_8H_{17}]^+$ (23.3) 197 $[C_{14}H_{29}]^+$ (6.6) , 169 $[C_{12}H_{25}]^+$ (10) , 141 $[C_{10}H_{21}]^+$ (16.6) , 85 $[C_6H_{13}]^+$ (70) , 57 $[C_4H_9]^+$ (100) |
| hexacosane | 366 $[M]^+$ (1.6), 323 $[C_{23}H_{47}]^+$ (1.6), 295$[C_{21}H_{43}]^+$ (1.6), 281 $[C_{20}H_{41}]^+$ (1.6), 267 $[C_{19}H_{39}]^+$ (1.6), 253 $[C_{18}H_{37}]^+$ (1.6), 239 $[C_{17}H_{35}]^+$ (1.6), 169 $[C_{12}H_{25}]^+$ (3.3), 141 $[C_{10}H_{21}]^+$ (10), 127 $[C_9H_{19}]^+$ (10), 113 $[C_8H_{17}]^+$ (13.3), 99 $[C_7H_{15}]^+$ (20), 85 $[C_6H_{13}]^+$ (60),71 $[C_5H_{11}]^+$ (80), 57 $[C_4H_9]^+$ (100). |
| octacosen | 392 $[M]^+$ (0.8), 309 $[C_{22}H_{45}]^+$ (3.3), 281 $[C_{20}H_{41}]^+$ (6.6), 253 $[C_{18}H_{37}]^+$ (6.6), 197 $[C_{14}H_{29}]^+$ (6.6), 169 $[C_{12}H_{25}]^+$ (10), 141 $[C_{10}H_{21}]^+$ (20), 113 $[C_8H_{17}]^+$ (30), 85 $[C_6H_{13}]^+$ (90), 57 $[C_4H_9]^+$ (100). |
| 1-heptacosene | 378 $[M]^+$ (1.6), 295$[C_{21}H_{43}]^+$ (1.6), 281 $[C_{20}H_{41}]^+$ (1.6), 267 $[C_{19}H_{39}]^+$ (1.6), 253 $[C_{18}H_{37}]^+$ (1.6),239 $[C_{17}H_{35}]^+$ (1.6), 169 $[C_{12}H_{25}]^+$ (3.3), 141 $[C_{10}H_{21}]^+$ (10), 127 $[C_9H_{19}]^+$ (10), 113 $[C_8H_{17}]^+$ (13.3), 99 $[C_7H_{15}]^+$ (20), 85 $[C_6H_{13}]^+$ (60),71 $[C_5H_{11}]^+$ (80), 57 $[C_4H_9]^+$ (100). |
| heptacosane | 380 $[M]^+$ (1.6), 364 $[C_{26}H_{53}]^+$ (1.6), 351 $[C_{25}H_{51}]^+$ (1.6), 337 $[C_{24}H_{49}]^+$ (1.6), 323 $[C_{23}H_{47}]^+$ (1.6),295$[C_{21}H_{43}]^+$ (1.6), 281 $[C_{20}H_{41}]^+$ (1.6), 267 $[C_{19}H_{39}]^+$ (1.6), 253 $[C_{18}H_{37}]^+$ (1.6),239 $[C_{17}H_{35}]^+$ (1.6), 169 $[C_{12}H_{25}]^+$ (3.3), 141 $[C_{10}H_{21}]^+$ (10), 127 $[C_9H_{19}]^+$ (10), 113 $[C_8H_{17}]^+$ (13.3), 99 $[C_7H_{15}]^+$ (20), 85 $[C_6H_{13}]^+$ (56.6),71 $[C_5H_{11}]^+$ (80), 57 $[C_4H_9]^+$ (100). |
| 1-nonacosen | 406 $[M]^+$ (0.8), 323 $[C_{23}H_{47}]^+$ (3.3), 295 $[C_{21}H_{43}]^+$ (3.3), 267 $[C_{19}H_{39}]^+$ (6.6), 225 $[C_{16}H_{33}]^+$ (6.6), 197 $[C_{14}H_{29}]^+$ (10), 169 $[C_{12}H_{25}]^+$ (10), 141 $[C_{10}H_{21}]^+$ (20), 113 $[C_8H_{17}]^+$ (30), 85 $[C_6H_{13}]^+$ (70), 57 $[C_4H_9]^+$ (100). |
| 3-methylheptadecane | 254 $[M]^+$ (2.1), 225 $[C_{16}H_{33}]^+$ (13), 169 $[C_{12}H_{25}]^+$ (4.3), 141 $[C_{10}H_{21}]^+$ (8.6), 113 $[C_8H_{17}]^+$ (13), 99 $[C_7H_{15}]^+$ (15.2), 85$[C_6H_{13}]^+$ (43.4), 71$[C_5H_{11}]^+$ (60), 57 $[C_4H_7]^+$ (100). |
| pytol isomer | 278 $[M\text{-}H_2O]^+$ (2.1), 207 $[C_{15}H_{27}]^+$ (1), 151 $[C_{11}H_{19}]^+$ (4.3), 137 $[C_{10}H_{17}]^+$ (10.8), 123 $[C_9H_{15}]^+$ (58.6), 95 $[C_7H_{11}]^+$ (100), 81 $[C_6H_9]^+$ (63), 53 $[C_4H_5]^+$(86.9). |
| campesterol | 400 $[M]^+$ (23.3), 385 $[C_{27}H_{45}O]^+$ (13.3), 367 $[C_{27}H_{43}]^+$ (6.6), 315 $[C_{22}H_{35}O]^+$ (13.3), 281 $[C_{21}H_{29}]^+$ (36.6), 207 $[C_{15}H_{27}]^+$ (100), 145 $[C_{11}H_{13}]^+$ (23.3), 57 $[C_4H_9]^+$ (23.3). |
| stigmasterol | 412 $[M]^+$ (83.3), 394 $[C_{29}H_{46}]^+$ (13.3), 369 $[C_{26}H_{41}O]^+$ (20), 351 $[C_{26}H_{39}]^+$ (23.3), 300 $[C_{21}H_{32}O]^+$ (36.6), 271 $[C_{19}H_{27}O]^+$ (66.6), 255 $[C_{19}H_{27}]^+$ (66.6), |

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|---|---|
| | 159 $[C_{12}H_{15}]^+$ (56.6), 83 $[C_6H_{11}]^+$ (80), 55 $[C_4H_7]^+$ (100). |
| stigmast-5-en-3-ol | 414 $[M]^+$ (60), 381 $[C_{28}H_{45}]^+$ (23.3), 329 $[C_{23}H_{37}O]^+$ (46.6), 281 $[C_{21}H_{29}]^+$ (50) , 207 $[C_{15}H_{27}]^+$ (100), 145 $[C_{11}H_{13}]^+$ (53.3), 55 $[C_4H_7]^+$ (33.3). |
| olean-12-en-3-ol (β-amyrine) | 426 $[M]^+$ (3.3), 411 $[C_{29}H_{47}O]^+$ (1.6), 218 $[C_{16}H_{26}]^+$ (100), 203 $[C_{15}H_{23}]^+$ (63.3), 203 $[C_{15}H_{23}]^+$ (63.3), 135 $[C_{10}H_{15}]^+$ (10), 119 $[C_9H_{11}]^+$ (10), 95 $[C_6H_7O]^+$ (13.3). |
| urs-12-en-3-ol (α-amyrine) | 426 $[M]^+$ (10), 411 $[C_{29}H_{47}O]^+$ (6.6), 393 $[C_{29}H_{45}]^+$ (1.6), 218 $[C_{16}H_{26}]^+$ (100), 203 $[C_{15}H_{23}]^+$ (26.6), 189 $[C_{14}H_{21}]^+$ (26.6), 175 $[C_{13}H_{19}]^+$ (13.3), 135 $[C_{10}H_{15}]^+$ (30), 121 $[C_9H_{15}]^+$ (26.6), 95 $[C_6H_7O]^+$ (26.6). |
| 12-oleanen-3-yl acetate | 468 $[M]^+$(3.3), 393 $[C_{29}H_{45}]^+$ (1.6), 218 $[C_{16}H_{26}]^+$ (100), 189 $[C_{14}H_{21}]^+$ (16.6), 135 $[C_{10}H_{15}]^+$ (10), 119 $[C_9H_{11}]^+$ (10), 95 $[C_6H_7O]^+$ (10). |
| olean-18-en-3-ol | 426 $[M]^+$(1.6), 393 $[C_{29}H_{45}]^+$ (3.3), 204 $[C_{15}H_{24}]^+$ (90), 189 $[C_{14}H_{21}]^+$ (100), 135 $[C_{10}H_{15}]^+$ (26.6), 119 $[C_9H_{11}]^+$ (26.6), 95 $[C_6H_7O]^+$ (40), 55 $[C_4H_7]^+$ (23.3). |
| 10-octadecenoic acid,methyl ester | 296 $[M]^+$(2.1), 264 $[C_{18}H_{32}O_2]^+$ (13), 222 $[C_{15}H_{26}O]^+$ (6.5), 180 $[C_{12}H_{20}O]^+$ (8.6), 111 $[C_8H_{15}]^+$ (26), 69 $[C_5H_9]^+$ (73.9), 55 $[C_4H_7]^+$ (100). |
| moretenol | 426 $[M]^+$ (10), 327 $[C_{24}H_{39}]^+$ (1.6),207 $[C_{14}H_{23}O]^+$ (100), 189 $[C_{14}H_{21}]^+$ (50), 175 $[C_{13}H_{19}]^+$ (13.3), 135 $[C_{10}H_{15}]^+$ (26.6), 121 $[C_9H_{13}]^+$ (26.6), 55 $[C_4H_7]^+$ (13.3). |
| (23S)-ethyl cholest-5-en-3β-ol | 414 $[M]^+$ (70), 371 $[C_{26}H_{43}O]^+$ (1.6), 273 $[C_{19}H_{29}O]^+$ (26.6), 213 $[C_{16}H_{21}]^+$ (46.6), 145 $[C_{11}H_{13}]^+$ (50), 57 $[C_4H_9]^+$ (100). |
| triacontane | 422 $[M]^+$ (2.1), 379 $[C_{27}H_{55}]^+$ (1), 351 $[C_{25}H_{51}]^+$ (1), 309 $[C_{22}H_{45}]^+$ (1), 281 $[C_{20}H_{41}]^+$ (1), 253 $[C_{18}H_{37}]^+$ (1), 197 $[C_{14}H_{29}]^+$ (2.1), 169 $[C_{12}H_{25}]^+$ (4.3), 141 $[C_{10}H_{21}]^+$ (6.5), 113 $[C_8H_{17}]^+$ (13), 99 $[C_7H_{15}]^+$ (17.3), 85 $[C_6H_{13}]^+$ (54),71 $[C_5H_{11}]^+$ (78.2), 57 $[C_4H_9]^+$ (100). |
| hentriacontane | 436 $[M]^+$ (2.1), 309 $[C_{22}H_{45}]^+$ (1), 281 $[C_{20}H_{41}]^+$ (4.3), 253 $[C_{18}H_{37}]^+$ (2.1), 113 $[C_8H_{17}]^+$ (13), 85 $[C_6H_{13}]^+$ (54),71 $[C_5H_{11}]^+$ (78.2), 57 $[C_4H_9]^+$ (100). |
| dotriacontane | 450 $[M]^+$ (1), 295 $[C_{21}H_{43}]^+$ (1), 281 $[C_{20}H_{41}]^+$ (8.6), 253 $[C_{18}H_{37}]^+$ (4.3), 225 $[C_{16}H_{33}]^+$ (2.1), 169 $[C_{12}H_{25}]^+$ (4.3), 85 $[C_6H_{13}]^+$ (54),71 $[C_5H_{11}]^+$ (78.2), 57 $[C_4H_9]^+$ (100). |
| tritriacontane | 464 $[M]^+$ (2.1),295 $[C_{21}H_{43}]^+$ (1), 281 $[C_{20}H_{41}]^+$ (13), 253 $[C_{18}H_{37}]^+$ (6.5), 85 $[C_6H_{13}]^+$ (54),71 $[C_5H_{11}]^+$ (78.2), 57 $[C_4H_9]^+$ (100). |

**Mass spectra of some compounds**

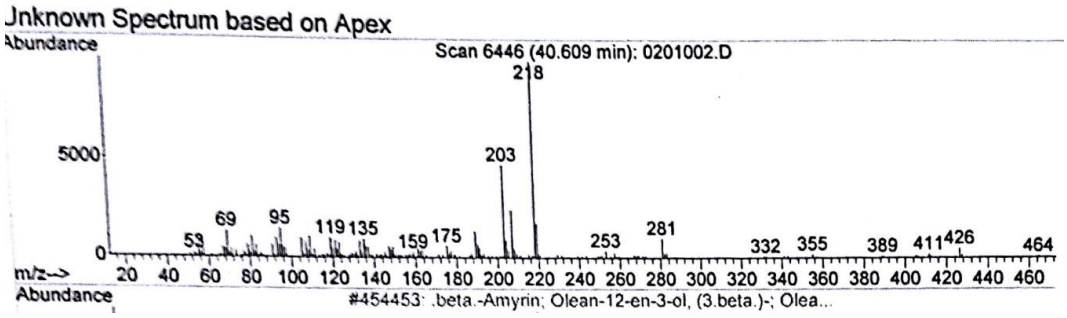


**Fig. 1:** Mass spectrum of β-amyrine.

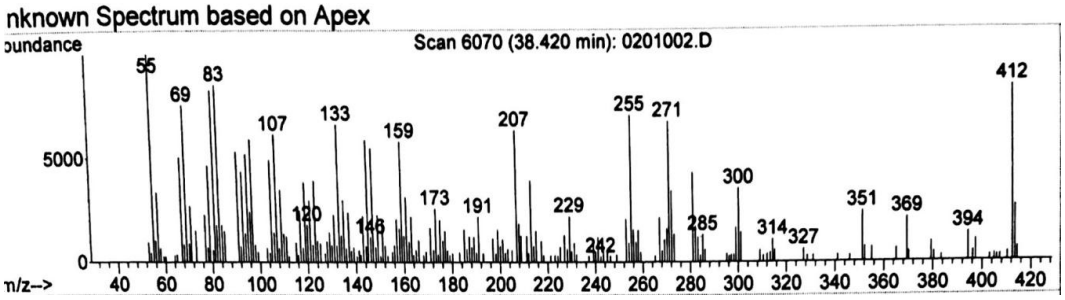


**Fig. 2:** Mass spectrum of stigmasterol.

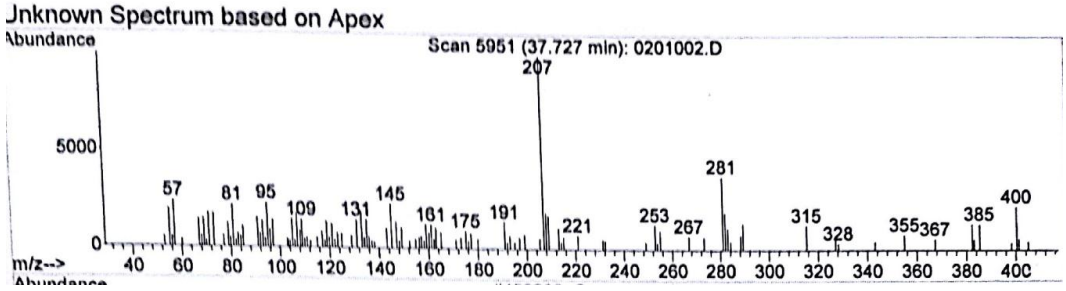


**Fig. 3:** Mass spectrum of campesterol..

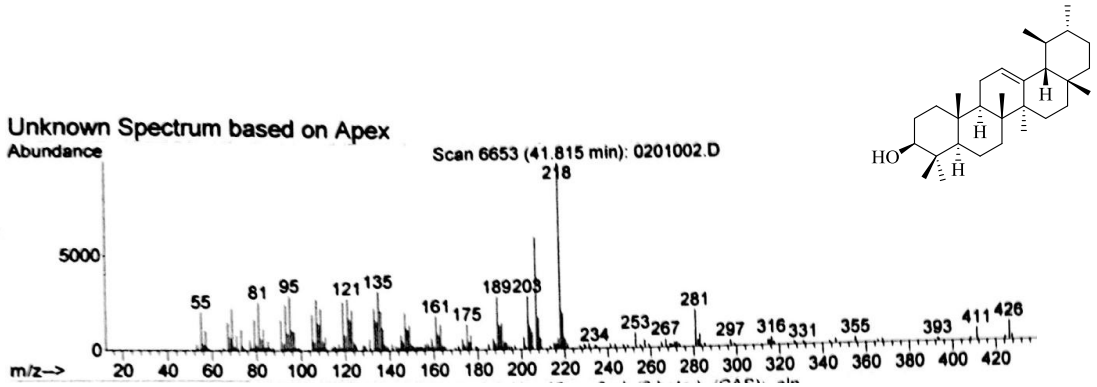


**Fig. 4:** Mass spectrum of α-amyrine

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# التقييم الفيتوكيميائى والبيولوجى لنبات السليس

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أدى فصل خلاصات الأجزاء الهوائية لنبات "يوروسبرمم بكرويدز" إلى الحصول على اثنين من نوع سسكويتربين لاكتون، وهما يوروسبرمال A (**١**) و ٨، ١٥- دايهيدروكسي جيرماكرا-١(١٠)، ٤-دايين-٦، ١٢-أولايد- ١٤-آل (**٢**)، واثنين من نوع فلافونويد جلايكوزايد، وهما كوارستين-٣-O-بيتا-D-جلوكوبيرانوزايد (**٣**) وكامفيرول-٣-O-بيتا-D-جلوكوبيرانوزايد (**٤**). تحليل عينة من خلاصة الإيثر البترولي للأجزاء الهوائية بتقنية كروماتوجرافيا الغاز المقترن بمطياف الكتلة نتج عنها تعريف ألفا أميرين، وبيتا أميرين، ١٢-أوليانين-٣-ويل أسيتات، ١٨-أوليانين-٣-بيتا-أول، موريتينول، كامبستيرول، استجماستيرول، ١٤-بيتا-H-برجنان، استجماست-٥-إين-٣-بيتا-أول، بالإضافة إلى العديد من المركبات الشائعة.

وقد وجد أن خلاصات بيوتانول للبذور، وإيثايل أسيتات للبذور، وإيثايل أسيتات للأجزاء الهوائية، وكلوريد الميثيلين للأجزاء الهوائية تثبط الشقوق الحرة بنسب 86.9%، 85.3%، 86.1%، 82.4% على الترتيب، مقارنة بتثبيط 88.1% بحمض الأسكوربك. بينما أدت الجزئية الغنية بالسسكويتربينات **١** و**٢** والجزئية الغنية بالفلافونويدات **٣** و**٤** إلى نسب تثبيط 84.7% و83.2% على اترتيب، مما يوضح التأثير التناغمي المحتمل للمكونات الأخرى.

وجد أن الفعالية ضد الميكروبات (متمثلةً في البكتريا موجبة الجرام استافيلوكوكس أوريوس والبكتريا سالبة الجرام اشرشيا كولاي واخميرة كانديدا ألبيكانز) لخلاصة إيثايل أسيتات للأجزاء الهوائية والجزئية الغنية بالفلافونويدات **٣** و**٤** هي (68.2%، 70.8% و69.2%) للأولى و(54.5%، 50.0% و50.0%) للأخيرة. كما وجد أن فعالية خلاصة بيوتانول للبذور وخلاصة إيثايل أسيتات للبذور تجاه البكتريا موجبة الجرام استافيلوكوكس أوريوس هي 68.2% و50.50% على الترتيب.

كما وجد أنه لخلاصة البيوتانول للبذور سمومية قوية جداً لخلايا سرطان الثدى MCF-7 (قيمتها 0.37±9.4) وسمومية قوية لخلايا سرطان الكبد HePG-2 (قيمتها 0.85±14.7)، ولخلاصة إيثايل أسيتات للأجزاء الهوائية سمومية قوية جداً لخلايا سرطان الثدى MCF-7 (قيمتها 0.47±78.8) وسمومية قوية لخلايا سرطان الكبد HePG-2 (قيمتها 0.88±10.1).