TRITERPENES, PHENOLICS AND HEPATOPROTECTIVE ACTIVITY OF *ORIGANUM SYRIACUM* L. SUBSP. *SINAICUM* GREUTER AND BURDET AND *O. MAJORANA* L. HERBS

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Received:22-3-2009
Accepted:19-5-2009

Abstract

*Compounds **1-8** were isolated from the aerial parts and roots of Origanum syriacum L. subsp. sinaicum Greuter and Burdet and O. majorana L. They were identified as β-sitosterol **1**, four triterpene acids: ursolic acid **2**, 3β-hydroxyurs-12-en-27-oic acid **3**, 3α-hydroxyolean-12-en-27-oic acid **4**, 2α-hydroxyursolic acid **5**, a flavone: apigenin **6**, a flavonol: quercetin **7** and a phenol acid: rosmarinic acid **8**. Identification of these compounds was carried out on bases of physicochemical characters and spectroscopic analyses. The total flavonoid content, as determined colorimetrically, amounted to 0.92 % and 0.87 %, calculated as quercetin per dry weight in both species, respectively. Quantification of flavonoids and phenolic acids was carried out adopting HPLC technique. Major identified components in both species were caffeic, rosmarinic and ferulic acids. Acute toxicity studies showed that O. syriacum L. subsp. sinaicum Greuter and Burdet and O. majorana L. are safe having an LD_{50} of up to 10 g/kg b.wt. of aqueous and ethanol extracts and 8.4 and 9.3 g/kg b.wt. of the volatile oils, respectively. Chronic toxicity studies showed safety of both herbs upon long term use. The aqueous and fractionated ethanol extracts of both herbs exhibited a pronounced hepatoprotective, as well as, hepatocurative activity.*

INTRODUCTION

The genus *Origanum* (Family Lamiaceae) comprises about 30 species of perennial herbs native to the countries bordering the Mediterranean Sea [(1)]. Members of the genus have been widely utilized a long time ago to treat various ailments [(2, 3)]. Recent uses in folk medicine include treatment of respiratory problems, jaundice and hepatoses [(4, 5)]. Moreover, it represents a major component in several pharmaceutical preparations in the Egyptian market. Two *Origanum* species growing in Egypt are chosen for thorough investigations, namely, *Origanum syriacum* L. subsp. *sinaicum* Greuter and Burdet [(6)] and *O. majorana* L. A comparative botanical description of the two species has been recently reported [(7)]. In another publication, the authors investigated the influence of seasonal variation on the essential oil composition of *O. syriacum* L. subsp. *sinaicum* Greuter and Burdet and evaluated its tocolytic effect on the uterus of non-pregnant rats [(8)]; furthermore, a similar study on *O. majorana* L. is underway [(9)].

Several reports were traced on the flavonoid content of *Origanum* species [(10-12)], few only on the two species under investigation [(13, 14)]. Dorman and co-workers examined the phenolic acid content of the aqueous extracts of several *Origanum* species, among which were *O. syriacum* L. and *O. majorana* L. [(15)]. Meanwhile, there is neither published data regarding the sterol and triterpene content nor the qualitative nor quantitative analyses of the phenolic compounds of these two species, growing in Egypt. Hence, it was interesting to pursue the work on *O. syriacum* L. subsp. *sinaicum* Greuter and Burdet and *O. majorana* L. herbs. Fractionation of the ethanol extracts, quantitative estimation of the flavonoids and phenolic acids of the fore mentioned species using HPLC, as well as, studies on the hepatoprotective and hepatocurative effects are presented in this note.

EXPERIMENTAL

Plant material

Samples of the aerial parts and roots of *O. syriacum* L. subsp. *sinaicum* Greuter and Burdet and *O. majorana* L. were obtained from plants cultivated in the Experimental Station of Medicinal Plants, Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Giza, as previously reported by the same authors [(7)].

General

UV lamp (Spectroline Model CM-10, New York, USA) was used for location of fluorescent spots. UV spectra were determined in methanol and after addition of different reagents on a Hewlett Packard 8452A diode array spectrophotometer in the region of 200-500 nm. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) were measured on Varian Mercury-VX-300 NMR spectrophotometer; NMR spectra were recorded in CD_3OD, DMSO-d_6 and chemical shifts were given in δ (ppm) relative to TMS as internal standard. HPLC apparatus Agilent series 1100 equipped with Quaternary pump and a UV detector

series 1100 was used for HPLC quantification of phenolic constituents. Melting points, uncorrected were determined on electrothermal 9100 (UK).

For vacuum liquid chromatography (VLC), silica gel H (E-Merck, Darmstadt, Germany) was used. For column chromatography (CC), silica gel 60 (Fluka, 70-230 mesh ASTM, Germany), Sephadex LH-20 (Pharmacia, Uppsala, Sweden), in addition to, argentation silica gel (prepared in the dark by addition of 10% silver nitrate solution in distilled water to the silica support (Silica gel 60, Fluka, 70-230 mesh ASTM, Germany) and drying at 110°C for 18-20 hr [16], were used. Thin-layer chromatography (TLC) was performed on silica gel GF_{254} precoated plates (Fluka, Germany) and on argentation silica gel TLC, prepared by development of silica gel GF_{254} precoated plates in 10% silver nitrate solution in distilled water and drying in an oven at 110°C.

Solvent systems: **S_1**, chloroform-methanol (9.8:0.2 v/v); **S_2**, chloroform-methanol (9.5:0.5 v/v); **S_3**, *n*-hexane-ethyl acetate (7:3 v/v); **S_4**, *n*-hexane-ethyl acetate-formic acid (7:3: four drops v/v); **S_5**, benzene-ethyl acetate-formic acid-water (5.5:4.5:1:0.5 v/v); **S_6**, ethyl acetate-methanol-water-formic acid (100:16:14: two drops v/v) were used. TLC plates were visualized under visible and UV light before and after exposure to ammonia vapor. Spray reagents: I, p-anisaldehyde-sulfuric acid was used for detection of sterols and triterpenes and II, aluminium chloride for flavonoids [17].

Standards of flavonoid aglycones: quercetin, kaempferol, luteolin, naringenin, apigenin, and phenol acids: chlorogenic, caffeic, ferulic and rosmarinic acids were obtained from Fluka, Sigma, Germany.

Extracts for phytochemical study

The ethanol extract was prepared by cold percolation of 1.0 kg of the air-dried aerial parts and 0.5 kg of the root of *O. syriacum* L. and 100 g of each of aerial parts and root of *O. majorana* L. with 90 % ethanol till exhaustion. The extract in each case was evaporated to dryness to yield 189.5 g and 14.0 g from aerial parts and root of *O. syriacum* L., 23.5 g and 3.25 g from aerial parts and root of *O. majorana* L., respectively. Each extract was suspended in distilled water and successively partitioned with *n*-hexane, chloroform, ethyl acetate and *n*-butanol to yield 37.5g, 21.2 g, 18.6g, 36.6g and 4.09g, 2.85 g, 1.88 g, 3.54 g from the *n*-hexane, chloroform, ethyl acetate and *n*-butanol of the aerial parts of each of *O. syriacum* L., and *O. majorana* L. respectively; while the root extractives, similarly obtained, reached 4.55 g, 2.25 g, 0.64 g,1.25 g for *O. syriacum* L., and 1.17 g, 0.52 g, 0.16 g, 0.39 g for *O. majorana* L., respectively.

Isolation of major sterols and triterpenes

The *n*-hexane (35 g) and chloroform (20 g) fractions of the **aerial parts of *O. syriacum* L.**, were separately fractionated by vacuum liquid chromatography column (VLC) using gradient elution with *n*-hexane followed by *n*-hexane-chloroform, chloroform and ethyl acetate mixtures. The *n*-hexane fraction yielded one major fraction (2 g, eluted with 72% chloroform in *n*-hexane); which on rechromatography afforded 144.8 mg of white needle crystals of **compound 1** (R_f 0.44 in solvent system S_1), m.p. 140-142 °C. Meanwhile, the chloroform fraction of *O. syriacum* L. yielded one major fraction (9.56 g, eluted with 5 % chloroform in ethyl acetate); rechromatography of 300 mg of which afforded 170 mg of white lustrous prisms of **compound 2** (R_f 0.53 in solvent system S_2), m.p. 280-283 °C.

The *n*-hexane fraction (3g) of the **root of *O. syriacum* L.**, on fractionation by CC (silica gel, 75 g, 3.5 x 26 cm) and gradient elution with *n*-hexane followed by *n*-hexane-chloroform, chloroform and ethyl acetate mixtures, yielded one major fraction, (200 mg, eluted with 90% chloroform in *n*-hexane) which, on rechromatography over a silica gel column showed two major spots [R_f 0.45 and 0.32/ argentation silica gel TLC plates/ solvent system S_4]. Repeated column chromatography on argentation silica gel columns (20 g, 22 x 1.5 cm, gradient elution) afforded 35 mg of white needle prisms of **compound 3**, and 9 mg of white needle crystals of **compound 4**. The chloroform fraction (2 g) was subjected to fractionation by CC (silica gel, 50 g, 11.2 x 3 cm) and yielded one major fraction (70 mg, eluted using 40 % ethyl acetate in chloroform); purification of the residue yielded **compound 5** as 28 mg of translucent plates (R_f 0.38 in solvent system S_2), m.p. 286-288 °C .

Isolation of major phenolic components

The ethyl acetate fraction of the **aerial parts of *O. syriacum* L.** (14 g) was chromatographed over a vacuum liquid chromatography column (VLC, Si gel H, 200 g, 5 x 10 cm). Gradient elution was carried out using ethyl acetate-chloroform and ethyl acetate-methanol mixtures with increasing polarity. One major fraction was obtained (2.2 g, eluted by 27.5% ethyl acetate-chloroform mixture); which on repeated rechromatography (sephadex LH- 20) yielded 9.2 mg of **compound 6** (R_f 0.63 in solvent system S_5) and 82 mg of **compound 7** (R_f 0.39 in solvent system S_5), m. p. 314-316 °C .

The ethyl acetate extract of the **root of *O. syriacum* L.** (0.5 g) was chromatographed over several sephadex LH-20 columns using ethanol-water 1:1 as an eluent to yield 56 mg of yellowish white powder of **compound 8** (R_f 0.77 in solvent system S_6), m.p. 168-170 °C.

Colorimetric determination of the flavonoid content

The method based on measuring the intensity of the color developed when flavonoids are complexed with aluminium chloride, was adopted. The percentage was calculated as quercetin with reference to a pre-established calibration curve using aliquots (0.2-1.4 ml) of quercetin solution in ethanol 95% (equivalent to 4-24 µg quercetin). The solvent

was evaporated to dryness and the residue, in each case, mixed with 5 ml of 0.1M $AlCl_3$ solution. The intensity of the developed yellow color was measured at 420 nm. For each concentration, three determinations were carried out.

Samples (1 gram each) of the air dried powdered aerial parts of both plant species were defatted with petroleum ether (40-60°C), then exhaustively extracted with ethanol 95%. The ethanol extract in each case, was transferred to a 100 ml measuring flask and the volume adjusted with ethanol. Aliquots of the extracts (0.5 ml, each) were separately transferred to test tubes, evaporated to dryness, and processed as for the standard sample.

HPLC quantification of polyphenols

The amount of major flavonoid and phenol acid constituents in the hydrolyzed aerial parts of both plant species was determined by HPLC adopting the procedure of Mattila *et al* [18]. One gram of each of the air-dried aerial parts was weighed into a 100 ml conical flask then dispersed in 40 ml of aqueous methanol (62.5%). The mixture was then ultrasonicated for 5 min. To this extract 10 ml of 6 M HCl were added. The flask containing the mixture was placed in a shaking water bath at 90°C for 2 hours. After hydrolysis, the sample was allowed to cool, filtered, made up to 100 ml with methanol, and ultrasonicated again for 5 min. The sample was filtered through a 0.2 µm membrane filter into the sampler vial for injection. HPLC analysis was performed on a Hypersil-ODS (4.6x 250 mm, 5µm) column. Isocratic elution was employed using acetonitrile-15% acetic acid (40:60 v/v) as mobile phase. The flow rate of the mobile phase was 1 ml/min. and the injection volumes were 40 µl for the standards and analyzed extracts. Detection was carried out by a UV detector set at 270 nm for phenolic acids and 330 nm for flavonoids.

The major components of the samples were identified by comparing their retention times to those obtained for the standards (prepared as 50-600 µg/ml solutions in methanol). Quantification was based on measuring the peak areas of both standards and samples by adopting the external standard method. Results were the average of triplicate experiments and are recorded (Table 1).

Experimental animals

Adult male albino rats (130-150 g) were obtained from the animal-breeding unit of National Research Center, El-Dokki, Giza, Egypt. All animals were fed on a standard laboratory diet under hygienic conditions and water supplied *ad libitum*.

For chronic toxicological study, 80 adult male albino rats were used in the experiment and divided into 8 groups (each of 10). One group was kept as non treated control; the second group received **compound 8 (**rosmarinic acid), orally, in a dose of 25 mg/kg body wt daily. Groups three to eight received 100 mg/kg body wt, orally, of aqueous and ethanol extracts and volatile oils of *O. syriacum* L. and *O. majorana* L. respectively for 8 weeks.

For testing the effect on the liver, 150 adult male albino rats were used in the experiment and divided into fifteen groups (each of 10). The aqueous and ethanol extracts, *n*-hexane, ethyl acetate, *n*-butanol fractions, essential oils (100 mg/kg body wt) of *O. syriacum* L. and *O. majorana* L. and rosmarinic acid (25 mg/kg body wt) were tested for their hepatoprotective activity. The tested extracts were administered at a daily dose of 100 mg/kg body weight for one month before induction of liver damage[19] using silymarin as a reference drug. The extracts, as well as, the reference drug were administered to the rats for another month after liver damage.

Samples for biological study

The previously prepared solvent-free dried extracts were separately dissolved in a concentration of 5 % in distilled water by the aid of few drops of tween 80%. The essential oils, prepared by hydro distillation of 500 g of the dried aerial parts [8, 9], was separately similarly treated. The aqueous extracts were prepared by boiling 0.5 kg of the minced fresh aerial parts of each of *O. syriacum* L. and *O. majorana* L. with water followed by lyophilization; the dried residues, 21.8 g and 10.56 g, were separately dissolved in distilled water in concentration of 5 % w/v immediately before use.

Chemicals and kits

The following chemicals and kits were purchased from their respective sources: carbon tetrachloride (Analar, El-Gomhoreya Co., Cairo, Egypt, for induction of liver damage [19], 5 ml / kg of 25% carbon tetrachloride in liquid paraffin, IP), Silymarin (Sedico Pharmaceutical Co., 6 October City, Egypt, standard hepatoprotective drug (25 mg / kg body weight), and biochemical transaminase kits (Bio-Meriéux Co., Durham, NC, USA) for assessment of blood glucose level, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase enzymes (ALP).

Determination of median lethal dose LD_{50}

LD_{50} of the volatile oils, aqueous and ethanol extracts of the two herbs was determined according to the procedures developed by Karber [20].

Chronic toxicity studies

Blood samples were collected at zero time and every 4 weeks from the retro orbital venous plexus of anaesthetized rats and, in each case, divided for analysis of total cholesterol [21], triglycerides, glucose level [22], creatinine [23], blood urea [24], aspartate aminotransferase (AST/SGOT), and alanine aminotransferase (ALT/SGPT) [25] (Table 2).

Measurement of AST, ALT and ALP serum levels

The levels of aspartate aminotransferase (AST) [25], alanine aminotransferase (ALT) [25] and alkaline phosphatase (ALP) enzymes [26] were measured in the blood of each group at zero time, after one month of receiving the tested drug, 72 hours after induction of liver damage and after one month of treatment with the tested samples (Tables 3-5).

Statistical analysis

All data were expressed as mean ± SE and the statistical significance was evaluated by student's t test (Snedecor and Cochran, 1989) [27].

RESULTS AND DISCUSSION

General

Colorimetric estimation of the flavonoid content in *O. syriacum* L. and *O. majorana* L. cultivated in Egypt was carried out depending on the yellow color produced when flavonoid compounds react with aluminium chloride. The flavonoid content was calculated as quercetin by applying the least square method [28] from the regression equation: y=bx+c, where (y)= absorbance, (b)=slope= 0.0211, (x)=concentration, (c)=intercept and the correlation of coefficient (R^2)=0.9871.

The total flavonoid content of *O. syriacum* L. and *O. majorana* L. amounted to 0.92 % and 0.87 %, respectively, calculated as quercetin, on dry weight base.

The results of HPLC analysis of the hydrolyzed methanol extracts of the two plant species (Table 1) revealed a variation in the phenolic composition. Although qualitative variation was mild, among the major phenol acids and flavonoid components, yet quantitative variation was obvious. Identified constituents were caffeic, rosmarinic, ferulic, and chlorogenic acids, as well as, quercetin, kaempferol, apigenin and luteolin. The amount of identified phenol acids amounted to 136.6486 mg/100 g in *O. syriacum* L. while reaching about double that figure in *O. majorana* L. (265.581 mg/100 g) with the predominance of chlorogenic and ferulic acids. On the other hand, that amount of caffeic and rosmarinic acids was slightly less. Concerning the flavonoid constituents (expressed as their corresponding aglycones), they showed a marked decrease in *O. majorana* L. to about half the total amount in *O. syriacum* L. with a parallel decrease in the percentage of apigenin in *O. syriacum* L. and absence of kaempferol in *O. majorana* L.

It is noteworthy to mention that this is the first report on evaluation of the total flavonoid and phenolic acid contents in *O. syriacum* L. and *O. majorana* L. cultivated in Egypt.

Column chromatographic fractionation of the *n*-hexane, chloroform and ethyl acetate fractions of the ethanol extract of the aerial parts and root of *O. syriacum* L. allowed the isolation of eight major compounds which were characterized through their physicochemical and spectral data. Compounds **1, 2, 6** and **7** were obtained from the aerial parts and **3, 4, 5** and **8** from the root.

Compounds **1-8** were identified as β-sitosterol [16], ursolic acid [29, 30], 3β-hydroxyurs-12-en-27-oic acid, and 3α-hydroxyolean-12-en-27-oic acid [31, 32], 2α-hydroxyursolic [30], apigenin, quercetin [33] and rosmarinic acid [34], respectively. The isolated compounds **1-5** gave positive Liebermann's and Salkowki's tests indicating their possible steroidal or triterpenoidal nature. Two triterpenes, *viz.*, ursolic acid **2**, and 2α-hydroxyursolic acid **5**, as well as, a steroidal compound, namely, β-sitosterol **1** were identified by their m. p., co-TLC comparison to authentic reference samples, and by comparison of MS, ^{1}H-NMR and ^{13}C-NMR data to previously reported ones [29, 30 & 16]. 3β-hydroxyurs-12-en-27-oic acid **3** and 3α-hydroxyolean-12-en-27-oic acid **4** were identified through comparison of their MS, ^{1}H-NMR, DEPT and ^{13}C-NMR spectra to those previously published reported data [31, 32]. All eight compounds were detected (TLC) in the corresponding extracts of *O. majorana* L.

Compounds **2**, **5** and **8** were previously reported from *O. majorana* [35, 36], and compounds **5** and **7** from *O. syriacum* [15]. However, this is the first report for the presence of compounds **3** and **4** in the two plants under investigation, as well as, of all the isolated constituents in these Egyptian cultivars.

Biological study

The LD_{50} was up to 10g / kg b. wt. for the aqueous and ethanol extracts of *O. syriacum* L. and *O. majorana* L. herbs, 8.4 and 9.3 g/kg b. wt. for the essential oils and 6.1 g /kg b.wt. for rosmarinic acid, respectively. The tested extracts are thus of high safety margin at the tested dose level [37].

At the same time, administration of 100 mg/kg b.wt. of aqueous and ethanol extracts of both plant species, as well as, of the essential oils and 25 mg/kg b. wt. of rosmarinic acid caused nearly no effect on serum cholesterol, triglycerides, glucose, creatinine, and urea. However, administration of same dose showed a significant decrease in serum AST level. In addition, aqueous and ethanol extracts caused a significant reduction of serum glucose and ALT levels after eight weeks of administration. All these effects reveal safety upon long term use (Table 2).

Previous studies on flavonoids showed their significant hepatoprotective effect [38]. In addition, phenolic acids were reported to exhibit a similar activity [39]. The present chemical investigation, as well as, those published on the essential oils [8, 9] revealed the presence of appreciable amounts of phenolics in these two Egyptian cultivars, thus stimulating the assessment of their hepatoprotective activity. In this respect, the aqueous and ethanol extracts, as well as, the volatile oils, *n*-hexane, ethyl acetate and *n*-butanol fractions were tested. Induction of liver damage led to a significant rise in AST, ALT and ALP. A daily dose of all the tested extracts

(100 mg/kg b. wt.) showed no significant change in AST, ALT and ALP levels after one month of administration. Yet, administration of these extracts for another month after induction of liver damage led to a significant decrease in the liver enzymes levels from the control group. It is obvious (Tables 3-5) that the ethanol extract of *O. syriacum* L. was the most active among the tested samples producing an effect more or less similar to that of rosmarinic acid. Moreover, the ethanol extracts of *O. syriacum* L. and *O. majorana* L. were found more active in reduction of liver enzymes than their polar and non-polar fractions and this may be attributed to the synergistic effect of triterpenoids and phenolic constituents [40]. Quercetin, rosmarinic, caffeic and ursolic acids were reported to exert a hepatoprotective effect [39]. The overall hepatoprotective activity of the ethanol extracts of both plant species may thus be attributed to the presence of these constituents.

These findings support the folk medicinal use of *Origanum* species in hepatoses and liver problems as previously reported [5].

It is noteworthy to mention that this is the first report on the hepatoprotective and hepatocurative effects of the aqueous, ethanol, ethyl acetate, *n*-butanol and *n*-hexane extracts and oil of *O. syriacum* L. and rosmarinic acid as tested on rat liver *in vivo*.

Table 1. Phenolic constituents identified by HPLC analysis in the hydrolyzed extracts of the aerial parts of *O. syriacum* L. and *O. majorana* L.

Rt*	Constituent	Concentration mg %	
		***O. syriacum* L.**	***O. majorana* L.**
6.51	Chlorogenic acid	7.568	8.133
6.072	Caffeic acid	72.741	136.060
8.666	Ferulic acid	11.838	14.295
9.074	Rosmarinic acid	44.502	107.090
	Total identified phenol acids	136.649	265.578
10.603	Quercetin	28.117	0.074
10.659	Luteolin	0.069	12.801
11.738	Apigenin	5.313	16.410
11.805	Kaempferol	13.482	Nd**
	Total identified flavonoids	46.982	29.285

*Rt; Retention time in min., Nd**; Not detected.

Table 2. Effects of long term administration of the different extracts, essential oils of *O. syriacum* L., *O. majorana* L. and rosmarinic acid on cholesterol, triglycerides, glucose, creatinine, urea and liver enzymes (AST and ALT) in rats.

	Group	Time weeks	Biochemical changes of serum level						
			Cholesterol mg/dl	Triglycerides mg/dl	Glucose mg/dl	Creatinine mg/dl	Urea mg/dl	AST u/l	ALT u/l
	Control	Zero	79.3±1.2	68.2±1.4	82.1±1.6	1.2±0.1	24.6±1.1	28.7±0.9	30.6±0.4
		4	78.2±1.4	69.4±1.1	83.2±1.5	1.1±0.2	23.8±1.2	29.1±1.1	30.7±0.6
		8	77.9±1.5	67.8±2.2	84.5±2.3	1.1±0.1	24.2±1.1	28.5±0.7	30.8±0.7
O. syriacum L.	Aqueous extract 100mg/kg	Zero	78.8±1.1	71.6±2.1	89.4±1.5	1.3±0.2	24.5±1.3	29.3±0.4	29.6±0.4
		4	76.1±1.4	72.2±1.9	82.2±1.3*•	1.2±0.3	22.9±1.1	26.8±0.6	28.2±0.2
		8	74.3±1.6	70.4±1.6	80.1±1.6*•	1.3±0.1	23.6±1.2	24.1±0.3*•	28.1±0.1
	Ethanol extract 100mg/kg	Zero	84.2±2.1	77.2±2.5	87.6±2.1	1.1±0.04	23.7±0.9	32.4±0.9	31.4±0.7
		4	81.6±2.4	78.3±2.2	81.2±1.4*•	1.3±0.03	22.9±1.9	29.6±0.8	28.7±0.3
		8	80.4±2.5	76.9±2.1	78.3±1.7*•	1.2±0.1	23.2±0.8	23.4±0.9*•	24.1±0.8*•
	Essential oil 100mg/kg	Zero	85.1±1.9	75.9±2.3	86.5±1.3	1.2±0.1	24.1±0.6	31.2±1.1	32.1±1.1
		4	80.2±2.6	76.1±2.4	81.4±1.8*•	1.1±0.2	23.9±0.8	26.8±0.5*•	28.6±0.3
		8	78.1±1.8	74.2±1.2	75.3±1.9*•	1.2±0.1	24.2±0.7	24.1±0.3*•	24.1±0.6*•
O. majorana L.	Aqueous extract 100mg/kg	Zero	72.3±2.1	69.7±2.4	81.7±2.6	1.2±0.1	22.8±1.4	28.7±0.8	29.8±0.6
		4	69.8±2.6	66.2±3.8	74.3±3.1	1.1±0.03	23.1±1.2	26.1±0.6	29.1±0.8
		8	66.4±2.9	67.1±4.1	71.2±2.9	1.1±0.1	23.4±1.6	24.3±0.5*•	28.4±0.9
	Ethanol extract 100mg/kg	Zero	75.8±3.2	65.8±2.8	88.4±3.9	1.2±0.04	23.9±1.1	28.1±1.1	30.1±0.9
		4	72.9±3.5	63.1±3.1	83.2±2.9*•	1.2±0.03	23.1±1.3	27.2±0.4	29.3±1.1
		8	70.1±3.6	62.8±3.6	79.8±2.6*•	1.1±0.01	23.5±1.5	25.4±0.7*•	28.6±0.8
	Essential oil 100mg/kg	Zero	81.5±3.1	67.2±3.2	87.2±4.1	1.1±0.04	23.9±1.3	28.7±0.6	26.7±0.3
		4	78.5±3.5	66.2±2.9	88.1±3.9	1.1±0.02	23.9±1.1	27.2±0.7	25.2±1.1
		8	76.1±4.1*•	65.3±2.7	86.7±3.4	1.1±0.03	23.6±0.9	27.1±0.9	24.1±0.9
	Rosmarinic acid 25mg/kg	Zero	79.7±3.4	74.6±3.8	88.9±3.2	1.2±0.1	22.6±1.1	27.8±0.8	29.1±0.8
		4	76.8±3.9	74.9±4.1	89.1±4.1	1.1±0.1	23.4±1.4	26.3±0.9	24.2±0.7
		8	75.1±3.7*•	74.1±3.6	86.4±4.4	1.1±0.1	22.9±1.2	26.1±0.4	24.5±0.6*•

*statistically significantly different from control group at $p<0.01$

• statistically significantly different from zero time at $p<0.01$

Table 3. Effects of the different extracts, essential oils of *O. syriacum* L., *O. majorana* L., rosmarinic acid and silymarin on the serum AST level (u/L).

Group		Zero time	30 days[a]	72 h[b]		30 days[b]	
		Mean±S.E.	Mean±S.E.	Mean±S.E.	% change	Mean±S.E.	% change
	Control	29.7±0.8	30.1±0.9	136.4±6.1	–	149.3±7.2	–
O. syriacum L.	Aqueous Extract	26.9±0.5	25.3±0.6	61.8±3.1*•	54.69	34.7±0.9*•	76.76
	Ethanol Extract	28.1±0.8	26.2±1.3	58.3±2.1*•	57.26	30.5±0.9*•	79.57
	n-Hexane Fraction	30.4±0.9	30.1±1.2	79.5±1.9*•	41.72	51.1±1.7*•	65.77
	Ethyl acetate Fraction	30.2±1.1	29.7±0.8	49.6±1.3*•	63.64	37.4±0.8*•	74.95
	n-Butanol Fraction	28.7±0.8	28.5±0.9	72.8±2.4*•	46.63	45.3±1.2*•	69.66
	Essential Oil	29.2±0.6	28.6±0.7	39.5±0.9*•	71.04	33.5±1.1*•	77.56
O. majorana L.	Aqueous Extract	27.4±0.8	26.2±1.1	52.3±2.1*•	59.24	39.7±1.3*•	73.74
	Ethanol Extract	30.2±1.2	28.9±1.1	48.6±1.7*•	62.12	33.5±0.7*•	77.84
	n-Hexane Fraction	31.9±1.2	30.7±1.3	70.3±2.8*•	45.21	49.8±2.5*•	67.06
	Ethyl acetate Fraction	27.6±0.4	27.4±0.6	51.6±2.1*•	59.78	35.2±1.1*•	76.72
	n-Butanol Fraction	26.8±0.5	26.5±0.7	66.9±2.4*•	47.86	41.7±2.2*•	72.42
	Essential Oil	32.2±1.4	31.4±1.3	41.2±2.1*•	67.89	30.7±1.5*•	79.7
	Rosmarinic acid	30.6±1.2	30.2±1.1	42.8±1.3	68.62	32.6±1.2	78.16
	Silymarin	30.1±1.2	28.5±0.9	36.4±0.8*•	73.31	25.3±0.7*•	83.05

*statistically significant different from control group at p<0.01
• statistically significant different from zero time at p<0.01
[a] pre-treated with tested samples
[b] after induction of liver damage

Table 4. Effects of the different extracts, essential oils of *O. syriacum* L., *O. majorana* L., rosmarinic acid and silymarin on the serum ALT level (u/L).

Group		Zero time	30 days[a]	72 h[b]		30 days[b]	
		Mean±S.E.	Mean±S.E	Mean±S.E.	% change	Mean±S.E.	% change
	Control	28.7±0.8	28.1±1.2	128.3±5.2	-	151.2±6.9	-
O. syriacum L.	Aqueous Extract	27.4±0.8	26.2±1.1	52.3±2.1*•	59.24	39.7±1.3*•	73.74
	Ethanol Extract	30.2±1.2	28.9±1.1	48.6±1.7*•	62.12	33.5±0.7*•	77.84
	n-Hexane Fraction	31.9±1.2	30.7±1.3	70.3±2.8*•	45.21	49.8±2.5*•	67.06
	Ethyl acetate Fraction	27.6±0.4	27.4±0.6	51.6±2.1*•	59.78	35.2±1.1*•	76.72
	n-Butanol Fraction	26.8±0.5	26.5±0.7	66.9±2.4*•	47.86	41.7±2.2*•	72.42
	Essential Oil	32.2±1.4	31.4±1.3	41.2±2.1*•	67.89	30.7±1.5*•	79.7
O. majorana L.	Aqueous Extract	28.6±0.6	28.4±0.8	64.1±2.4*•	50.04	51.6±3.2*•	65.87
	Ethanol Extract	32.1±0.5	31.6±0.7	61.4±2.9*•	52.14	44.6±1.4*•	70.50
	n-Hexane Fraction	27.1±0.4	27.9±1.1	82.1±3.2*•	36.00	61.7±3.1*•	59.19
	Ethyl acetate Fraction	29.3±0.7	28.6±0.8	65.3±2.6*•	49.10	59.3±2.9*•	60.78
	n-Butanol Fraction	28.2±0.9	28.3±0.6	86.1±4.2*•	32.89	78.2±2.7*•	48.28
	Essential Oil	26.9±0.8	26.5±0.6	59.1±2.4*•	53.94	33.6±1.1*•	77.77
	Rosmarinic acid	27.3±0.8	26.9±0.7	56.3±2.3*•	56.12	36.2±1.3*•	76.06
	Silymarin	29.8±0.3	27.1±0.4	41.2±1.6*•	67.89	26.9±1.1*•	82.21

*statistically significant different from control group at p<0.01
• statistically significant different from zero time at p<0.01
[a] pre-treated with tested samples
[b] after induction of liver damage

Table 5. Effects of the different extracts, essential oils of *O. syriacum* L., *O. majorana* L., rosmarinic acid and silymarin on the serum ALP level (KAU).

	Group	Zero time	30 days[a]	72 hr[b]		30 days[b]	
		Mean±S.E	Mean±S.E	Mean±S.E	% change	Mean±S.E	% change
	Control	6.9±0.1	6.8±0.2	42.1±1.3	–	46.4±1.7	–
O. syriacum L.	Aqueous Extract	7.2±0.1	6.9±0.1	12.3±0.7*•	70.78	8.4±1.05*•	81.9
	Ethanol Extract	7.4±0.2	7.1±0.1	11.2±0.4*•	73.4	7.6±0.05*•	83.62
	n-Hexane Fraction	7.6±0.1	7.5±0.4	21.2±0.7*•	49.64	15.6±0.5*•	66.38
	Ethyl acetate Fraction	7.5±0.1	7.2±0.3	14.3±0.9*•	66.03	9.2±0.4*•	80.17
	n-Butanol Fraction	7.3±0.2	7.1±0.1	19.8±0.6*•	52.97	13.1±0.1*•	71.77
	Essential Oil	6.8±0.1	7.1±0.1	10.9±0.6*•	74.11	7.8±0.04*•	83.19
O. majorana L.	Aqueous Extract	6.9±0.1	6.8±0.1	16.1±0.1*•	61.75	12.8±0.6*•	72.41
	Ethanol Extract	7.1±0.4	6.9±0.3	15.1±0.1*•	64.13	11.6±0.5*•	75.00
	n-Hexane Fraction	7.5±0.1	7.3±0.1	17.2±0.9*•	59.14	12.7±1.1*•	72.63
	Ethyl acetate Fraction	7.4±0.2	7.1±0.2	13.7±0.9*•	67.45	10.7±0.4*•	76.93
	n-Butanol Fraction	6.4±0.1	6.3±0.1	18.1±0.7*•	57.00	16.7±0.8*•	64.01
	Essential Oil	7.8±0.2	7.5±0.3	14.6±0.8*•	65.32	10.2±0.4*•	78.02
	Rosmarinic acid	7.3±0.4	7.1±0.4	13.1±0.9	68.88	9.1±0.3	80.38
	Silymarin	7.3±0.4	7.1±0.2	9.2±0.7*•	78.15	6.8±0.2*•	85.34

*statistically significant different from control group at $p<0.01$
• statistically significant different from zero time at $p<0.01$
[a] pre-treated with tested samples
[b] after induction of liver damage

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التربينات الثلاثية، والفينوليات والنشاط الواقى للكبد لنباتى أوريجانم سيرياكم ل.تحت نوع سينايكم جروتر و بردت و أوريجانم ماجورانا ل.

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**قسم الفارماكولوجي - المركز القومي للبحوث – الدقي

أسفرت الدراسة الفيتوكيميائية لنبات أوريجانم سيرياكم ل.تحت نوع سينايكم جروتر و بردت و أوريجانم ماجورانا ل. عن فصل ثمانى مركبات من خلاصة الكحول الايثيلى من كل من الجزء الهوائي و الجذر و هى : β-sitosterol **1**, ursolic acid **2**, 3β-hydroxyurs-12-en-27-oic acid **3,** 3α-hydroxyolean-12-en-27-oic acid **4**, 2α-hydroxyursolic acid **5,** apigenin **6**, quercetin**7,** rosmarinic acid **8**. وقد تم التعرف علي المركبات بمقارنتها بعينات أصيله وتعيين درجة الانصهار واستخدام الطرق الطيفية المختلفة مثل طيف الأشعة الفوق بنفسجية، الرنين النووي المغناطيسي ومطياف الكتلة.

وقد تم تعيين كمية الفلافونيدات في النباتين باسخدام الطريقة اللونية التي تعتمد على تفاعل هذه المواد مع كلوريد الألومونيوم. كذلك تم التعرف على و تعيين نسبة الفلافونيدات والاحماض الفينولية بواسطة استخدام كروماتوجرافيا السائل ذات الاداء العالي.ونتج عن ذلك التعرف على أحماض: chlorogenic, caffeic, rosmarinic and ferulic كذلك quercetin, luteolin, apigenin, kaempferol فى النباتين بكميات مختلفة. وقد تناول البحث دراسة التأثير البيولوجي لكل من الخلاصة المائية و الكحولية للنباتين أسفرت عن تعيين السمية لكل من المستخلص المائي والكحولي والزيت الطيار ، وقد تبين أن : الجرعة القاتلة لكل الخلاصات عالية ، ولذلك لا تتسبب في قتل الحيوانات بعد تناول النبات لمرة واحدة ، وكذلك ليس له تأثير سام على المدى البعيد.

كذلك شملت الدراسة البيولوجية بحث التأثيرات الواقية والمعالجة لكبد الفئران وهي أول دراسة يتم فيها دراسة تأثير المستخلص المائي والكحولي والزيوت الطيارة للنباتين و **8** rosmarinic acid علي وقاية ومعالجة الكبد بعد تعرضه للتلف بواسطة الكاربون تتراكلوريد بواسطة *In vivo* tests وقد اظهرت ان المستخلصات تحت الدراسة لها تأثيرفعال في خفض مستوي انزيمات الكبد بعد تعرضه للتلف بواسطة الكاربون تيتراكلوريد.