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Gas chromatography-mass spectrometry analysis, hepatoprotective and antioxidant activities of the essential oils of four Libyan herbs

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Following an ethnobotanical survey for hepatoprotective remedies, four Libyan medicinal plants from family Lamiaceae; *Ajuga iva* (L.) Schreber, *Marrubium vulgare* L., *Rosmarinus officinalis* L. and *Thymus capitatus* Hoff. et Link growing widely in Beida, Libya, were selected for our study. The chemical composition of essential oils hydrodistilled from the dried aerial parts, were analyzed by gas chromatography-mass spectrometry (GC-MS). The major constituents of the essential oils of *A. iva* and *R. officinalis* were carvacrol (35.07%) and 1,8-cineol (35.21%), respectively, thymol was the major constituent of the essential oil of *M. vulgare* (20.11%) and *T. capitatus* (90.15%). The oil of *M. vulgare* had the most powerful antioxidant activity by restoring glutathione levels in the blood of alloxan-induced diabetic rats. The rats treated with the essential oils of *M. vulgare, R. officinalis* and *T. capitatus* (50 mg/kg) showed a significant decrease in liver enzymes which were elevated by CCl_4 ($p < 0.01$). The essential oil of *M. vulgare* had the most potent hepatoprotective activity.

Key words: *Ajuga iva*, *Marrubium vulgare, Rosmarinus officinalis*, *Thymus capitatus* antioxidant, hepatoprotective.

INTRODUCTION

Despite tremendous advances in modern medicine, hepatic disease remains a worldwide health problem; thus the search for new medicines is still ongoing. Numerous formulations of medicinal plants are used to treat liver disorders in Chinese ethnomedical practice and traditional medicine. Many of these treatments act as radical scavengers, whereas others are enzyme inhibitors or mitogens (Fadhel and Amran, 2002). Many drugs and plant extracts have been evaluated for hepatoprotective and antioxidant effects against chemical-induced liver damage in a carbon tetrachloride-induced hepatotoxicity model. Carbon tetrachloride accumulates in hepatic parenchyma cells and is metabolized to CCl3• radicals by liver cytochrome P450-dependent monooxygenases (Bhathal et al., 1983; Weber et al., 2003).

In this respect, four herbs from the Libyan flora that belong to family Lamiacea were chosen for this study; *Ajuga iva* (L.) Schreber, *Marrubium vulgare* L., *Rosmarinus officinalis* L. and *Thymus capitatus* Hoff. et Link. These plants are widely used in African and Asian folk medicine, however, to the best of our knowledge, the hepatoprotective effects of their essential oils against CCl4-induced liver injury in rats has not been demonstrated.

A. iva (L.) Schreber is an annual herbaceous plant known in Moroccan pharmacopoeia as a panacea (cure-

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all) because of its numerous beneficial effects and specifically for gastrointestinal disorders, hypertension and diabetes (Ziyya et al., 1997), but nothing is known about its essential oil composition or biological activity. *M. vulgare* L. (known in Arabic as Farasion) is indigenous in Europe, the Mediterranean area and Asia. This plant is traditionally used as expectorant and antispasmodic in acute or chronic bronchitis, coughs, asthma and in general for respiratory infections. Externally, it has been used in ulcers and wounds (Blumenthal, 1998).

Rosemary, *R. officinalis* L. (in Arabic hasa El-ban) is an evergreen perennial shrub grown in many parts of the world. It has been reported to possess a number of therapeutic applications in folk medicine in curing or managing of a wide range of diseases such as diabetes, respiratory disorders, stomach problems and inflammatory diseases (Erenmemisoglu et al., 1997). Among natural antioxidants, Rosemary has been widely accepted as one of the species with the highest antioxidant activity (Peng et al., 2005). The aqueous and ethanolic extracts were reported to possess hepatoprotective effects (Amin and Hamza, 2005; Hoefler et al., 1987). *T. capitatus* Hoff. et Link. [syn., *Coridothymus capitatus* (L.) Rchb.f., *Satureja capitata* L., *Thymbra capitata* (L.) Cav.], is a perennial, herbaceous shrub commonly used as a spicy herb and locally known under the common name “zaatar”. Previous studies have shown that thyme species are exploited for their essential oil and for their reputation as medicinal herbs (Dob et al., 2006). The chemical composition of the essential oils of *M. vulgare* L., *R. officinalis* L. and *T. capitatus* Hoff. et Link. growing in different localities and their biological activities are reported in Table 1.

The present article focused on studying the chemical composition of the essential oils of these plants growing in Libya which have not been studied before and also evaluating the potential hepatoprotective activity of the essential oils of the aerial parts of the four plants on CCl_4-induced liver injury in rats and as well as their *in vivo* antioxidant activity.

MATERIALS AND METHODS

Plant

The air-dried aerial parts of *A. iva, M. vulgare, R. officinalis* and *T. capitatus* were collected from Khedar Mountain, Beida, Libya during August, 2009. Samples were identified by herbarium of Faculty of Science, Botany department, Garyounis University, Libya. Voucher specimens (A-22/313, M-23/313, R-24/313 and T-25/313, of each plant, respectively) were kept at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University.

Preparation of the essential oils

The air dried aerial parts (500 g) of the plants were separately subjected to hydrodistillation according to E.P (1984). The distillation was continued until the essential oil volume in the graduated stem of the apparatus remained constant for at least one hour. The obtained oil was dried over anhydrous sodium sulphate, weighed and then kept in the refrigerator at -20°C in an amber coloured vials till analysis.

Determination of physical constants of the essential oil samples

The percentage (v/w) of the prepared essential oils relative to the dry weight of the plants, the colour and odour of the prepared essential oils were also described. The specific gravity of each oil was determined at 25°C and the refractive indices were determined for each sample according to Egyptian Pharmacopeia methods (1984) using Abbe refractometer at 20°C. Results are recorded in (Table 2).

Gas chromatography/mass spectrometry

The GC mass analysis was conducted on an Agilent (USA) GC-MS system, model 6890, fitted with an Agilent mass spectroscopic detector (MSD), model 5937, as well as a 30 m long, cross-linked 5% phenyl polysiloxane (HP-5MS, Hewlett Packard, USA) fused-silica column (i.d. 0.25 mm, film thickness 0.25 µm). The initial temperature was 80°C, kept isothermal for 3 min, then increased to 260°C at 8°C/min, and the final temperature was kept isothermal for 15 min. The ion source temperature was 230°C and the quadrupole temperature was 150°C. The carrier gas was helium adjusted at a flow rate of 0.1 ml/min. Ionization energy was 70 eV, and scan range was 40 to 500 *m/z* at 3.62 amu/scan. The identification of the oil components was based on a Wiley MS Data Library (6th ed), followed by comparison of MS data with published literature (Adams, 2004). Results are recorded in (Table 3).

Material for biological activity

Silymarin (Sedico Pharmaceutical Co., 6th October City, Egypt) and Carbon tetrachloride (analar, El-Gomhoreya Co., Cairo, Egypt). Vitamin E (Pharco Pharmaceutical Co., Egypt), alloxan (Sigma Co., USA). Transaminase Kits (Bio-Meriéux Co.): biochemical kits for assessment of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) enzymes. Adult male albino rats of Sprauge Dawely strain weighing 100 to 150 g and albino mice (20 to 25 g). All animals were kept on standard laboratory diet and under hygienic conditions.

Assessment of the antioxidant activity

Antioxidant activity was assessed by measuring the ability of the tested oils to restore glutathione levels in the blood of alloxan-induced diabetic rats after the oral administration of 50 mg/kg body weight of each of the tested sample using Beutler et al. (1963) method. The doses of the oils were determined as 50 mg/kg/day from preliminary short-term pilot study with a range of variable doses in our laboratory. Induction of diabetes mellitus was carried out according to the method described by Eliasson and Samet (1969). Vitamin E was used as a standard. The results are recorded in Table 4.

Assessment of the hepatoprotective activity

The essential oils under investigation were tested for hepatoprotective acitivity at a dose of 50 mg/kg body weight. The tested samples were administered daily for 15 days before induction of liver damage by intraperitoneal injection of 5 ml/kg of 25% carbon tetrachloride (CCl_4) in liquid paraffin according to the method described by Klassan and Plaa (1969) using silymarin 25

Table 1. The reported constituents and biological activities of the essential oils under investigation.

Plant	Major constituents	Country	Reported uses of the essential oil
M. vulgare	Eugenol (50%) and β-Bisabolene (29 %)	Algeria (Belhattab et al., 2006)	Molluscidal and mosquitocidal (Salama et al., 2012) and Antibacterial (González and Marioli 2010)
	Carvacrol (32.98%) and thymol (32.82%)	Egypt (Salama et al., 2012)	
	β-bisabolene (25.4%), *β*-caryophyllene (11.6%), germacrene D (9.7%) and *E-β* -farnesene (8.3%)	Iran (Mahnaz et al., 2005)	
R. officinalis	Cineole<1,8> (29.5%), 2-ethyl-4,5-dimethylphenol (12.0%) and camphor (11.5%)	Algeria (Touafek et al., 2004)	Antimicrobial and antioxidant (Zaouali et al., 2010), Insecticidal (Lahlou and Berrada 2003), acetylcholinesterase and butyrylcholinesterase inhibitory (Orhan et al., 2008), hyperglycemic (Al-Hader et al., 1994), Relaxant effect on tracheal smooth muscle (Aqel 1991)
	Verbenone (12.3%), camphor (11.3%), bornyl acetate (7.6%) and limonene (7.1%)	Saint Catherine, Egypt (Soliman et al., 1994)	
	Camphor (14.9%), α-pinene (9.3%), and 1,8-cineol (9.0%)	Sinai, Egypt (Soliman et al., 1994)	
	α-pinene(14.9%), 1,8-cineol (7.43%) and linalool (14.9%)	Iran (Gachkar et al., 2007)	
	1,8-cineol (46.6%), comphor (36.9%), α-pinene (11.3%) and camphene (10.7%)	Tunisia (Marzouk et al., 2006)	
	1,8-cineol (40%), α-pinene (15.82%), camphor (14.38%), borneol (6.33%) and camphene (3.16%)	Morocco (Lahlou and Berrada 2003)	
T. capitatus	Thymol (34.55 %)	Egypt (Salama et al., 2012)	Molluscidal (Salama et al., 2012), Mosquitocidal (Salama et al., 2012; Mansour et al., 2000), antibacterial (Bouzuita et al., 2003), antioxidant (Bounatirou et al., 2007)
	Thymol (91%) and carvacrol (9%)	Italy (Solinas et al., 1981)	
	Carvacrol (36.3%), *β*-caryophylene (2.8%) and α-elemol (1.4%)	Sicily (Solinas et al., 1981)	
	Carvacrol (77.93%)	Turkey (Ozek et al., 1995)	
	Carvacrol (70.92%), γ-terpinene (4.92%), *p*-cymene (6.34%), linalool (3.86%) and *β*-caryophyllene (3.57%)	Morocco (Amarti et al., 2008)	
	Carvacrol (53.8%), *p*- cymene (8.7%), γ-terpinene (16.4%), *β*-caryophyllene (7.6%) and α-terpinene (2.2%)	Tunisia (Hedhili et al., 2002)	

mg/kg body weight as a reference drug. The tested samples as well as the reference drug were further administered to the rats for another 15 days after liver damage. The levels of aspartate aminotransferase (AST) (Thewfweld, 1974), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) (Kind and King, 1954) enzymes were measured in the blood of each group at zero time, after 15 days of receiving the tested sample, 72 h after induction of liver damage and after 15 days of treatment with the tested samples. The results are presented in Table 5.

RESULTS AND DISCUSSION

The percentage yields of the essential oils obtained by hydrodistillation from the aerial parts of *A. iva, M. vulgare, R. officinalis* and *T. capitatus*, were 0.50, 1.00, 1.62, and 1.61%, respectively on dry weight basis. Their specific gravities were 0.8615, 0.9350, 0.8889, and 0.9350 g/cm^3 at 25°C, respectively. Refractive indices recorded at 20°C were 1.517, 1.451, 1.526, and 1.351, respectively. All oil samples exhibited nearly the same physical characters being oily, pale yellow to orange in colour with aromatic odor and readily soluble in 70% ethanol (Table 2).

Gas chromatography coupled to mass spectrometry (GC-MS) allowed the determination of the chemical composition of the essential oils which are summarized in Table 3. In *A. iva*, 48 compo-

Table 2. Physical properties and Percentage yield of the tested essential oils.

Plants	Colour	Odour	%	SG	RI
A. iva	Orange	Faint	0.50	0.8615	1.517
M. vulgare	Orange	Aromatic	1.00	0.9350	1.451
R. officinalis	Pale yellow	Aromatic	1.62	0.8889	1.526
T. capitatus	Pale yellow	Aromatic	1.61	0.9350	1.351

Percentage yield v/w dry plant; SG, specific gravity at 25°C; RI, refractive index at 25°C.

Table 3. Identified components in the hydrodistillated essential oils of the dried aerial parts of *A. iva* (L). schreb, *M. vulgare* L., *R. officinalis* (L) and *T. capitatus* (L) Hoff. et Link.

Identified components	RI[a]	RI[b]	*A. iva*	*M. vulgare*	*R. officinalis*	*T. capitatus*
Isocitronellene	924	924	-	-	-	0.02
Pinene <α->	939	939	-	-	1.53	0.05
Camphene	953	954	-	-	0.42	-
Pinene <β->	979	979	-	0.36	-	0.20
Myrecene <β->	991	991	-	-	-	0.17
Phellandrene <α->	1003	1003	-	-	-	0.03
Terpinene <α->	1017	1017	-	-	-	0.08
Cymene <*p*->	1025	1025	-	-	-	0.10
1,8-Cineol	1031	1031	1.04	-	35.21	-
Terpinolene	1193	1089	-	-	1.11	1.74
Linalool	1099	1097	1.51	-	4.15	-
Sabina Ketone <dehydro->	1123	1121	-	-	-	0.43
Thujanol <*iso*-3>	1139	1138	-	-	-	0.02
Camphor	1143	1146	1.21	-	6.35	-
Camphene hydrate	1149	1150	-	-	-	0.76
Thujanol <*neo*-3->	1157	1154	-	-	-	0.44
Isoborneol	1164	1162	-	-	4.76	1.38
Menthol <*neo*-3->	1171	1166	-	-	-	0.24
Borneol	1175	1169	0.73	-	10.98	-
Isopulegol <*neo-iso*>	1176	1171	-	-	-	0.23
Terpinen-4-ol	1179	1177	1.25	-	-	-
Lavandulol	1183	1181	-	-	4.93	-
Dihydro carveol <*neo*->	1194	1194	-	-	-	0.21
Methyl chavicol	1199	1196	6.50	4.57	-	-
Verbanol	1198	1198	-	-	-	0.05
Dihydro carvone <*E*->	1201	1201	-	-	-	0.06
Verbenone	1207	1205	-	-	24.71	-
Citronellol	1227	1226	-	-	0.48	-
Ocimenone<*Z*->	1229	1229	-	-	-	0.07
Carvone	1244	1243	2.14	-	-	-
Carvotanaceton	1245	1247	1.35	-	-	-
Anethol<*Z*->	1254	1253	0.80	-	-	-
Anethol<*E*->	1288	1285	-	1.01	-	-
Thymol	1291	1290	0.73	20.11	-	90.15
p-Mentha-1-en-9-ol	1297	1295	-	-	0.89	-
Geranyl formate	1299	1298	-	-	5.59	-
Carvacrol	1300	1299	35.07	12.05	-	0.18
trans-Carvyl acetate	1343	1342	2.46	-	-	-

Table 3. Contd.

neo-iso-Carvomethyl acetate	1351	1350	-	-	0.76	-
α-Cubebene	1355	1351	0 .57	0.79	-	-
Cyclosativene	1372	1371	-	0.36	-	-
Longicyclene	1378	1374	0.57	-	-	-
β-Cubebene	1387	1388	-	1.20	-	-
Z-Caryophyllene	1409	1409	-	-	1.53	1.57
E-Caryophyllene	1419	1419	0.56	2.22	0.38	0.06
Lavandulyl isobutanoate	1426	1423	-	1.79	-	-
Vestitenone	1449	1447	0.78	-	-	-
Z-Pentadecanone	1454	1451	0.99	-	-	-
E -β-Farnesene	1458	1457	0.91	15.66	0.72	-
β-Santalene	1463	1460	-	-	-	0.07
allo-Aromadendrene	1465	1460	0.44	-	-	-
Germacrene D	1485	1485	-	1.69	-	-
E-β-Ionone	1488	1489	1.85	-	-	-
delta-Selinene	1495	1493	0.97	0.68	-	-
Benzyl tiglate	1499	1498	0.26	-	-	-
(*E,E*)-α-Farnsene	1509	1506	2.64	-	-	-
Spathulenol	1577	1578	3.24	-	-	-
trans-Sesquisabinene hydrate	1578	1579	0.85	-	-	-
Caryoyphyllene oxide	1580	1580	-	2.07	0.40	0.94
Globulol	1589	1585	1.45	1.04	-	-
Gleenol	1590	1587	0.94	-	-	-
Viridifloral	1595	1593	-	0.9	-	-
Guaiol	1603	1601	0.99	-	-	-
Khusimone	1607	1604	2.34	2.60	-	0.15
5-epi-7-epi-α-Eudesmol	1610	1608	-	1.3	-	-
1,10-di-epi-Cubenol	1617	1619	-	-	0.32	-
epoxy,allo-Alloaromadendrene	1641	1641	-	-	-	0.08
6-hydroxy-Isobornyl isobutanoate	1644	1645	0.71	-	-	-
β-Eudesmol	1650	1651	-	0.43	-	-
Cedr-8(15)-en-10- ol	1650	1652	1.56	-	-	-
Ishwarone	1682	1682	0.57	-	-	-
Tasmanone	1727	1727	0.61	3.93	-	-
α-Eudesmol acetate	1799	1795	-	3.29	-	-
n-Octadecane	1801	1800	10.06	-	-	-
14-Hydroxy-delta-Cadinene	1809	1804	0.98	1.05	-	-
iso-Acorone	1815	1813	-	0.72	-	-
Cryptomeridiol	1817	1814	0.93	1.11	-	-
iso-Longifolol acetate	1822	1820	-	0.99	-	-
2E,6Z-Farnesyl acetate	1825	1822	2.05	0.95	-	-
Z-Nuciferol acetate	1830	1831	-	0.94	-	-
2,7 (14),10-Bisabolatrien-1-ol-4-one	1850	1846	0.28	-	-	-
Laurenene	1881	1880	0.23	-	-	-
n-Nonadecane	1906	1900	0.39	-	-	-
Phytol	1944	1943	3.97	0.75	-	-
n-Eicosane	2006	2000	0.26	0.71	-	-
Z-Penyl ethyl anthranilate	2129	2127	-	1.63	-	-
Oroselone	2150	2153	0.5	1.05	-	-
1-Docosene	2195	2190	0.31	-	-	-
E-Methyl communate	2261	2258	-	6.18	-	-
n-Tricosane	2301	2300	0.27	0.63	-	-

Table 3. Contd.

Isopimarol	2311	2310	-	0.77	-	-
n-Tetracosane	2403	2400	0.35	0.63	-	-
Labd-13-*E*-8,15 diol	2420	2422	-	3.63	-	-
neo-Abietol	2473	2469	0.11	-	-	-
n-Pentacosane	2501	2500	0.12	-	-	-
Total monoterpene hydrocarbons (%)			-	0.36	3.06	2.39
Total oxygenated monoterpenes (%)			52.33	37.74	92.46	93.79
Total sesquiterpene hydrocarbons (%)			6.66	22.60	2.63	1.70
Total oxygenated sesquiterpenes (%)			12.78	10.25	0.72	1.02
Total non-terpene hydrocarbons (%)			11.76	1.34	-	-
Total oxygenated non-terpenes (%)			15.87	27.50	0.76	0.58
Total identified compounds (%)			99.40	99.79	99.63	99.48

RI[a], experimental retention index, RI[b]; retention index in Adams (2004).

Table 4. Antioxidant activity of essential oil of the plants under investigation and vitamine E drug in male albino rats (n = 10).

Groups	Blood glutathione mg (%)	% of change = control-Result/control × 100
Control	36.2±1.3	-
Diabetic untreated	21.3±0.4*	41.1
Diabetic + vit E (7.6 mg/kg)	35.7±0.8	1.38
Diabetic + *A.iva* (50 mg/kg)	34.7±0.6	4.1
Diabetic + *M. vulgare* (50 mg/kg)	35.2±0.8	2.7
Diabetic + *R.officinalis* (50 mg/kg)	34.1±1.1	5.8
Diabetic +*T. capitatus* (50mg/kg)	34.3±0.7	5.2

*Statistically significant from the control at $p<0.01$

nents were identified, representing 99.40% of the total oil profile, with carvacrol (35.07%) as the main constituent. In *M. vulgare*, 36 components were identified (99.79% of the total) with thymol and *E,β*-Farnesene as the main components (20.11 and 15.66%, respectively). In *R. officinalis*, 19 components were detected (99.63% of the total) with 1,8-cineol (35.21%) and verbenone (24.71%) as the main constituents. For *T. capitatus*, 27 components were identified (99.48% of the total) with thymol (90.15%) as the main component. The four investigated essential oils are characterized by high proportion of oxygenated monoterpenes. The chemical profile of the essential oils of *M. vulgare, R. officinalis* and *T. capitatus* of Libyan origin differ from those published for other countries (Table 1), and quantitative differences can be seen for some individual components. For example, the major constituent of *M. vulgare* grown in Libya and Egypt (Salama et al., 2012) differs from the plant grown in other countries.

Although 1,8-cineol and thymol are always one of the major constituents of the essential oil of *R. officinalis* and *T. capitatus* grown in different countries, respectively but their percentages vary greatly from one location to the other. It is not clear how such differences could be related to geographical location or ecophysiological conditions; thus the observed variations in oils chemical composition are often ascribed to the existence of specific chemotypes. The constituents of essential oils, often mono and sesquiterpenes, hydrocarbons with various chemical functionalities present low molecular sizes enabling effective penetration through cell membranes and walls to affect many biochemical processes (Misharina and Samusenko, 2008). Phenols which represent the main constituents in three of the investigated samples especially *A. iva*, *M. vulgare* and *T. capitatus* as well as monoterpenes which represent the major class in the four investigated essential oils are known to exhibit antioxidant properties (Misharina et al., 2009). But in many cases, the minor compounds may also play a significant role in the activity of the essential oils. The *in vivo* antioxidant activity of our Libyan essential oils was investigated by measuring their ability to restore glutathione levels in the blood of alloxan-induced diabetic rats. The four essential oils had restored the levels of glutathione in the diabetic rats (Table 4). Among all the tested samples, the essential oil from the

Table 5. Effect of the essential oils of the plants under investigation on the level of the serum enzymes of damaged liver rats (n = 10).

Enzyme	Time	Control untreated	Silymarin	*A. iva*	*M. vulgare*	*R. officinalis*	*T. capitatus*
AST (U/L)	zero	25.3±0.9	29.5±1.3	29.8±0.8	29.8±0.7	32.1±1.2	29.2±1.1
	15 days	26.1±0.8	26.8±1.2	29.3±0.9	28..2±0.6	31.6±1.4	28.3±0.9
	72 h*	131.6±5.9	51.3±2.7*	76.2±2.7*	52.4±1.3*	58.4±2.3*	73.2±2.6*
	15 days*	144.2±6.1	24.9±1.1*º	54.8±2.1*	34.7±1.8*º	41.3±2.6*	38.4±1.5*º
ALT (U/L)	Zero	28.6±1.1	27.6±1.2	31.6±1.5	27.6±0.9	30.2±1.3	32.1±0.9
	15 days	26.8±1.2	26.3±1.4	31.4±1.1	27.1±0.8	30.4±0.7	31.6±0.5
	72 h*	121.3±6.5	48.5±2.2*	79.8±2.3*	58.9±2.4*	53.9±1.8*	81.5±4.2*
	15 days*	139.2±5.8	29.2±0.9*º	61.2±2.4*	47.3±1.2*º	44.8±1.5*	45.9±2.1*º
ALP (KAU/L)	Zero	7.2±0.3	6.8±0.2*	7.9±0.1	7.6±0.1	7.2±0.2	6.9±0.4
	15 days	7.2±0.2	6.6±0.1	7.8±0.2	7.4±0.2	7.5±0.1*	7.1±0.2
	72 h*	31.8±1.1	7.9±0.7*	19.4±1.1*	19.2±0.5*	14.6±0.9*	13.2±0.9*
	15 days*	33.6±1.7*	7.1±0.3*º	15.8±1.2*	10.5±0.7*º	12.1±0.6*	11.9±0.8*º

*Statistically significant from the control at $p<0.01$, ºstatistically significant from72 hours after liver damage at $p<0.01$

aerial part of *M. vulgare* had the most powerful anti-oxidant activity which was comparable to that of vitamin E, followed by the oil of *A. iva, T. capitatus* and *R. officials*.

Among the four plants under investigation, a new terpenoid, characterized as *p*-menthane-5,6-dihydroxy-3-carboxylic acid was isolated from the aerial parts of *M. vulgare* possessed antihepatotoxic activity (Ahmad et al., 2010), also *R. officinalis* L. is commonly used in traditional medicine as a hepatoprotective, and its hepatoprotective and antioxidant activities are always attributed to the hydroxyphenolic components, including rosmarinic acid, carnosol and flavonoids (Fregoso et al., 2012).

Consequently, the hepatoprotective activity of the essential oils was assessed by measuring liver-related biochemical parameters following CCl_4-induced hepato-toxicity *in vivo* (Table 5). No toxic symptoms or mortality was observed after 15 days of administration of the tested essential oils and this indicates the absence of hepatotoxicity of these essential oils. The values of liver-related biochemical parameters observed in the control untreated group, silymarin group and essential oils treated groups are presented in Table 5. The activity of the enzymes AST, ALT and ALP were significantly increased in the control group 72 h after administration of CCl_4 compared to their levels at zero time ($p < 0.01$), this increase was insignificant in the groups pretreated with essential oils (50 mg/kg for 15 days). Administration of the oils for another 15 days at the same dose led to a further decrease in all of the parameters that were elevated by CCl_4. Based on these results, it can be concluded that the essential oils from *M. vulgare, R. officinalis* and *T. capitatus* seem to have hepatoprotective activity in rats, the essential oils of *M.vulgare* had the most potent activity which was comparable to the effect of silymarin (25 mg/kg). The CCl_4-hepatotoxicity model is extensively used to evaluate the hepatoprotective effects of drugs and plant extracts (Bhathal et al., 1983).

It has been reported that one of the principal causes of CCl_4-induced liver injury is the lipid peroxidation which is induced and accelerated by free radical derivatives of CCl_4 (Maling et al., 1974). In this study, the antioxidant activity of the essentials of the dried aerial parts of *A. iva*, *M. vulgare, R. officinalis* and *T. capitatus* were tested and our results showed that the four essential oils have potent antioxidant activity. Therefore, their hepatoprotective activity may be attributed to their antioxidant activity and especially to the presence of phenolic compounds and monoterpenes in the oils. The activities of *M. vulgare* and *T. capitatus* can also be attributed to the presence of high percentage of thymol which was reported to possess both antioxidant and hepatoprotective activities (Al-Malki, 2010).

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