WFL Publisher
Science and Technology
Meri-Rastilantie 3 B, FI-00980
Helsinki, Finland
e-mail: info@world-food.net

Journal of Food, Agriculture & Environment Vol.8 (2): 204-208. 2010 *www.world-food.net*

Pharmacological activities and chemical composition of the *Olea europaea* L. leaf essential oils from Tunisia

Ehsen Haloui [1], Zohra Marzouk [1]*, Belsem Marzouk [2], Ibtissem Bouftira [3], Abderrahman Bouraoui [4] and Nadia Fenina [1]

[1] *Unité de Pharmaco-économie et Développement des Médicaments, Laboratoire de Pharmacologie et Laboratoire de Biologie Végétale.* [2] *Laboratoire des Maladies Transmissibles et Substances Biologiquement Actives.* [3] *Laboratoire de Pharmacie Galénique,* [4] *Unité URSAM, Laboratoire de Pharmacologie, Faculté de Pharmacie de Monastir, Rue Avicenne, Monastir, Tunisie. *e-mail: zohra-marzouk@voila.fr*

Received 27 January 2010, accepted 12 April 2010.

Abstract

Leaves of *Olea europaea* L. (Oleacea) are used in Tunisian folk medicine to treat many inflammation type and bacterial infections such as icterus, otitis, gingivitis and cough.The aim of this study was to investigate the chemical composition and to screen the DPPH radical-scavenging activity and the anti-inflammatory and the analgesic activities of the essential oil of *Olea europaea* L. leaves (EOL). GC and GC-MS analysis of the essential oil resulted in the identification of 32 compounds representing 99.44% of the oil; α-pinene (52.70%) 2,6-dimethyloctane (16.57%) and 2-methoxy-3-isopropylpyrazine (6.01%) were the main components. The result of the (DPPH) assay showed that the radical-scavenging activity was concentration dependent. Intraperitoneal administration of *Olea europaea* L. essential oil at doses of 100, 200 and 300 mg/kg reduced significantly acetic acid-induced abdominal constrictions and paw edema. These results confirmed the great potential of olive leaves and validate their use in traditional medicine in Tunisia.

Key words: *Olea europaea* L., cv. Chemlali, essential oil, chemical composition, radical-scavenging activity, anti-inflammatory activity, analgesic activity, paw edema.

Introduction

Olea europaea L. (Oleaceae) is abundantly found in Tunisia by more than 50 different cultivars. Chemlali is the most abundant olive variety. It represents more than 60% of the total olive trees in Tunisia [1]. There has been increasing interest in olive products and by-products of olive tree and especially in olive leaves due to their various bioactivities.

Historically, olive leaves have been used as a remedy for fever and other diseases such as malaria [2-4]. According to Tunisian folk medicine, olive leaves are recommended in wide range of ailments including inflammatory disorders, bacterial infections, hypertension and diabetes, but modes of preparation and administration vary: earache is cured by using olive leaves tramped in hot olive oil with salt. Olive leaves juice, despite of its irritation, is recommended for curing trachoma. When chewed, this plant organ is used to relieve tooth pain and to treat lips irritation. Leaves decoction as a liquid mouthwash, is used for treating, aphthous, gingivitis and glossitis [5].

Previous studies demonstrated that olive leaves are used for their antimicrobial [6-8], gastroprotective [9], antioxidant [10-13], hypotensive[14], hypoglycaemic [15, 16], antiarrhythmic [17], anti-atherosclerotic [18], antiviral [19, 20], anti-tumor [21, 22] and anti-inflammatory properties [23].

Despite the popular usage of olive leaves in many therapeutic uses, their essential oil effects have, at the best of our knowledge, not been investigated. According to the literature, there are only reports on *Olea europaea* L. essential oil composition but it concerns Italian cultivars such as Leccino, Frantoio, Cipressino [24, 25] and Olivastra [26].

The present paper is the first step to investigate the chemical composition of the Tunisian Chemlali cultivar of *Olea europaea* L. leaves essential oil (EOL), and to evaluate its DPPH radical-scavenging activity, analgesic and anti-inflammatory effects.

Materials and Methods

Plant material: Fresh leaves of *Olea europaea* L. cv. Chemlali were collected in February 2007 from Hammamet (Tunisia). The plant was identified in the biological laboratory of the Faculty of Pharmacy of Monastir according to the flora of Tunisia [27]. A voucher specimen (O.E-01.71) was deposited in this laboratory.

Extraction, isolation and analysis of the essential oils: Fresh leaves (200 g) were subjected to a hydrodistillation for three hours with 500 ml of distilled water using a Clevenger-type apparatus. The oil obtained was separated from the distilled water and dried by anhydrous sodium sulphate. The extracts are volatile and therefore stored in sealed glass vials in a refrigerator at 4°C in order to prevent changes in chemical composition.

Essential oil composition was investigated by GC and GC-MS. GC analysis performed in a gas chromatograph, HP 5890, using two fused silica capillary columns, HP5 (non-polar) and Innovax (polar) (30 m x 0.25 mm film thickness (0.25 µm) and a flame ionization

detector (FID). Injector and detector temperatures were set at 240°C and 280°C, respectively. The oven temperature programme was 50°C for 3 min, then 50-280°C at 9°C/min and finally 280°C for 3 min. Nitrogen was the carrier gas at a flow rate of 1 ml/min. The samples were injected as 0.1 µl of 1% solution diluted in hexane in the split mode. The percentage of the constituents was calculated by electronic integration of FID peak areas and normalized without the use of response factor correction.

The essential oils were analysed by GC-MS using an HP 5972/A mass spectrometer operating at 70 eV in the same conditions as described above, except that the carrier gas was helium at 20 p.s.i. The identification of compounds was confirmed by comparison of their retention indexes (determined relatively to the retention times of a series of *n*-alkanes) and with those of authentic standards of the Wiley library search routines [28] based on fit and purity of mass spectra [29]. This analyse was carried out twice for the same sample. For the animal experimental uses, EOL were dissolved in 10% Tween 80 solution (used as a vehicle) at the desired concentration, just before the administration.

Animals: For studying the acute toxicity and the *in vivo* activities, male adult Wistar rats (150-180 g) and Swiss albino mice (18-25 g) of both sexes were obtained from the Pasteur institute (Tunis, Tunisia). They were housed in polypropylene cages with free access to standard pellet diet and water *ad libitum*. The animals were maintained under controlled conditions of humidity (40-45%), temperature (22±2°C) with a 12 h light-dark cycle.

Housing conditions and *in vivo* experiments approved according to the guidelines established by the European Union on Animal Care (CFE Council (86/609)). The rats were used for the anti-inflammatory evaluation of the essential oil while the mice were used for the analgesic investigation and for the acute toxicity testing. Animals were divided into drug-treated test and Tween 80-treated control groups of six or eight animals per group.

Evaluation of acute toxicity induced by Olea europaea essential oil: Groups of 10 mice received intraperitoneally (i.p.) single doses of EOL (50, 100, 200, 500, 1000 and 2000 mg/kg). The control group received only the vehicle (10% of Tween 80, 10 ml/kg). Animals were continuously observed during 2 h to detect changes in the autonomic or behavioural responses and then monitored for any mortality for the following 48 h. The LD_{50} was estimated according to the method described by Miller and Tainter [30].

Analgesic activity: Different groups of mice (n = 6) were treated coutaneously with 10% Tween solution, acetyl salicylate of lysine (ASL, 200 mg/kg, 10 mg/kg) and EOL (at the doses of 100, 200 and 300 mg/kg) 30 min before the experiments. The writhes were stimulated using 1% v/v acetic acid (10 ml/kg) intraperitoneally (i.p) administrated. The writhes were observed during 30 min and the analgesic activity was expressed as inhibition percent of the usual number of writhes observed in control animals. The percentages of inhibition were calculated according to the following formula [31]:

$$\% \text{ inhibition} = ((\text{number of writhes})_{control} - (\text{number of writhes})_{treated\ group}) \times 100/(\text{number of writhes})_{control}.$$

Results were given as mean ± S.E.M. during this time interval.

Anti-inflammatory activity: The anti-inflammatory activity was assessed on the basis of inhibition of paw edema induced by the injection of carrageenan (an edematogenic agent) into the subplantar region of the left hind paw of the rat [32]. Male Wistar rats were divided into different groups of six animals. The control group received 2.5 ml/kg of 10% Tween solution, the standard group received the reference drug (acetyl salicylate of lysine (ASL), 300 mg/kg) and the test groups received different doses of EOL (100-300 mg/kg). Thirteen minutes after intraperitoneal administration of different substances, 0.05 ml of 1% of carrageenan suspension was injected to all animals in the left hind paw.

The paw volume, up to tibiotarsal articulation, was measured using a plethysmometer (model 7150, Ugo Basile, Italy). The measures were determined at 0 h (V_0 before edematogenic agent injection) and 1, 2, 3, 4, 5 and 24 h intervals later (V_T). The difference between V_T (1, 2, 3, 4, 5 and 24 h) and V_0 was taken as the edema value. The percentages of inhibition were calculated according to the following formula:

$$\% \text{ inhibition} = ((V_T - V_0)_{control} - (V_T - V_0)_{treated\ group}) \times 100 / (V_T - V_0)_{control}$$

Antioxidant activity: The hydrogen atom or electron donation abilities of the compounds were measured from the bleaching of the purple-colored methanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH). This spectrophotometric assay uses the stable radical DPPH as a reagent [33, 34]. Of several concentrations of samples in methanol, 0.1 ml was added to 3.9 ml of 6.5×10^{-5} M DPPH methanol solution. After a 60 min incubation period at room temperature, the absorbance was read against a blank at 515 nm. Inhibition of free radical DPPH in percent (I, %) was calculated as follows:

$$I (\%) = (A_{blank} - A_{sample})/A_{blank} \times 100$$

where A_{blank} is the control reaction absorbance (containing all reagents except the test compound) and A_{sample} is the test compound absorbance. Tests were carried out in triplicate [35]. Butylated hydroxy toluene (BHT) was used as an antioxidant reference compound [36].

Statistical analysis: Data obtained from animal experiments were expressed as mean ± S.E.M. and as percentage. Results were statistically evaluated by ANOVA and using Student's t-test, $P \leq 0.05$ was considered significant.

Results

Yield and chemical composition of the essential oils: The hydrodistillation of fresh leaves gave colourful oil which yield is 0.05% (w/w). Twenty-one compounds, representing more than 99.23% of the essential oils, were identified, α-pinene (52.70%), 2.6- dimethyloctane (16.57%) being the most abundant components of the essential oil (Table 1). The other chemical components were 2-methoxy-3-isopropylpyrazine (6.01%), tetracosane (4.38%) and docosane (3.58%). The following chemical components occurred in trace amounts: β-pinene (2.46%), z-3-hexanol (1.51%), (E, Z)-2,6-nonadienal (1.46%), α-ionone (1.45%) and (E)-2-hexenol (1.26%). The remaining components mentioned in Table 1 are in amounts less than 1%. Monoterpene hydrocarbons represent 55.16% of the total essential oil.

Table 1. Percentage composition of the leaf essential oil of *Olea europaea* L. cv. Chemlali.

Constituent	R.I.	Yield (%)
(z)-3-hexanol	862	1.51
(E)-2-Hexenol	879	1.26
α –pinene	940	52.70
β – pinene	979	2.46
(Z)-3-Hexenyl acetate	1009	0.61
2-Methoxy-3-isopropylpyrazine	1094	6.01
(E,Z)-2,6-Nonadienal	1150	1.46
(E,Z)-2,6-Nonadienol	1154	0.74
1,4-Dimethoxybenzene	1163	0.45
Decanal	1208	1.05
2,6-Dimethyloctane	1230	16.57
2,4-Dimethyldodecane	1245	0.50
2,6,11-Trimethyldodecane	1250	0.43
α-ionone	1437	1.45
α-humulene	1461	0.84
1-Dodecanol	1478	0.78
β -Ionone	1499	0.89
hexadecanoicacid	1957	0.65
Oleic acid	2102	0.81
Docosane	2200	3.58
Tetracosane	2400	4.38

R.I.: Retention index. The identified constituents are listed in their order of elution from a non-polar column.

Table 2. Effect of *Olea europaea* L. cv. Chemlali essential oil on acetic acid-induced writhing in mice (N = 6).

Extract	Concentration (mg/kg)	Number of writhes	Inhibition of writhing (%)
Control	-	78.00 ± 1.15	-
O.E.O.	100	38.66 ± 7.76**	35.44
	200	34.66 ± 5.88**	53.16
	300	28.83 ± 1.47***	62.02
Reference drug (ASL 200 mg/kg)		28.16 ± 1.72***	64.55

Values are expressed as mean ± S.E.M (N = 6). **≤0.01. ***≤0.001 significant from control; ASL Acetyl salicylate of lysine.

Toxicity studies: *Olea europaea* L. leaf essential oil did not present acute toxicity up to a maximum dose of 2000 mg/kg. For 6 days of observation, after the experiment, the mice weights had a normal variation. LD_{50} was estimated to more than 2000 mg/kg. Thus, we supposed that EOL at the doses of 100, 200 and 300 mg/kg, i.p injected to animals, were safe.

Analgesic activity: The inhibition percentages of writhing for EOL are shown in Table 2. Acetyl salicylate of lysine (ASL), the reference drug, inhibited 64.55% of the number of writhing elicited by acetic acid. The intraperitoneally administration of EOL induced a dose-dependent antinociceptive activity. Essential oil at doses of 100, 200 and 300 mg/kg caused respectively 35.44%, 53.16% and 62.02% inhibition of writhes, and this effect was significant for the dose of 300 mg/kg ($P<0.001$).

Anti-inflammatory activity: This preliminary assessment of the anti-inflammatory activity established that EOL produced a reduction of the edema throughout the entire period of observation. In fact, EOL exhibited a dose-dependent anti-inflammatory activity on carrageenan-induced hind paw edema model in rat ranging between 47.3-57.12% at 100 mg/kg, 48.62-63.70% at 200 mg/kg and 54.12-67.38 % at 300 mg/kg doses (Table 3). EOL at 300 mg/kg gave significantly ($p< 0.001$) the highest activity (67.38% inhibition at 3 h). Standard drug, ASL at 300 mg/kg, decreased paw edema by a maximum of 81.24% at 3 h.

Antioxidant activity: The essential oil was subjected to a screening for its possible antioxidant activity by DPPH free radical scavenging system. As can be seen from Table 4, the weakest radical scavenging activity exhibited by EOL was determined as 74.44 ± 0.79% at 5 mg/ml concentration. The synthetic antioxidant BHT exhibited stronger activity (99 %).

Discussion

Little information is available in the literature on the chemical composition of *Olea europaea* L. essential oil [24-26]. These reports are on *Olea europaea* L. essential oil composition of other cultivars but there is no report on pharmacological activities of EOL. The data obtained from this study revealed that α-pinene and 2,6-

Table 3. Effects of *Olea europaea* L. cv. Chemlali essential oil and reference drug on carrageenan-induced paw edema.

Extract	Dose (mg/kg)	Mean swelling thickness (10^{-2} mm) ± S.E.M. (% inhibition) 1 h	2 h	3 h	4 h	5 h	24 h
Control 1	-	40.16±1.33	80.83±2.32	115.50±3.62	124.00±4.24	94.00 ±3.52	60.66±1.63
O.E.O.	100	21.17±1.17** (47.30)	35.83±1.94*** (55.67)	50.00±2.89*** (56.70)	53.16±3.25*** (57.12)	45.50±2.07*** (51.59)	30.50±1.87*** (49.72)
O.E.O.	200	18.50±1.64*** (53.94)	32.67±1.86** (59.58)	43.50±2.42*** (62.33)	45.00±3.34*** (63.70)	44.00±1.41*** (53.19)	31.17±1.16*** (48.62)
O.E.O.	300	17.33±1.03* (56.84)	31.67±1.75*** (60.82)	37.67±2.33*** (67.38)	42.33±3.88*** (65.86)	36.83±1.83*** (60.81)	27.83±2.78*** (54.12)
ASL	300	16.50±0.54*** (58.92)	19.66±0.81** (75.67)	21.66±1.21*** (81.24)	25.50±1.37*** (79.43)	26.50±1.64*** (71.80)	34.83±3.31*** (42.58)

Values are expressed as mean ± S.E.M (N = 8); *p≤0.05, **≤0.01, ***≤0.001 significant from control; ns: not significant from the control; ASL Acetyl salicylate of lysine.

Table 4. Scavenging of DPPH radicals by Tunisian *Olea europaea* L. cv. Chemlali oil (%).

Concentration (mg/ml)	Scavenging activity (%) O.E.O.	BHT
0.5	14.47 ± 0.23	90
1	45.96 ± 0.38	95
2	68.88 ± 1.89	97
5	74.44 ± 0.79	99

dimethyloctane were reported to be the major components (respectively 52.70% and 16. 57%). EOL showed anti-oxidant, antinoceptive and anti-inflammatory activities.

The major compound of EOL, α-pinene, was shown to display insecticidal, spasmolytic, antilisterial and anticholinesterase effects[37-40]. This compound was also found to possess antistress potency, exerting an alleviative effect on stress-induced hyperthermia in rats [41].

A well-known anti-oxidant, α-pinene (in both DPPH and β carotene systems), exhibited remarkable antioxidant activities [42]. Other studies [43, 44] showed that α-pinene tested individually didn't exhibit strong antioxidative activity in all methods employed (DPPH and β carotene tests). In this study, essential oil showed a dose dependent anti-oxidant activity which confirmed the antioxidant effect previously reported for olive leaf extracts [10-13]. We think that there is a synergism between α-pinene (the major component at a level of 52.70%) and the minor substances of the oil.

Experimental results demonstrated that intraperitoneal administration of the EOL (100-300 mg/ml) significantly inhibited the paw edema formation induced by carrageenan, although the essential oil was less effective than the reference drug ASL.

The time course of edema development in carrageenan-induced paw edema model in rats is generally represented by a biphasic curve [32]. The first phase (90-180 min) is partly due to the trauma of injection and also to different chemical mediators as histamine and serotonin. The second phase (270-360 min) is associated with the release of other mediators, for example prostaglandins, leucotrienes and proteases [45].

EOL showed a dose-depending anti-inflammatory activity. It inhibited the both phases of the carrageenan-induced edema by reducing the release of histamine and serotonin and also the kinin-like substances and prostaglandins. This pharmacological property may be attributed to a possible molecular mechanism by effectively decreasing the production of the pro-inflammatory cytokines of IL-6 and IL-1 and the expression of COX-2 and simultaneously elevating the level of anti-inflammatory cytokine IL-4 in the carrageenan-injected rat paw tissues [46].

The major constituent of the essential oil, α-pinene, is known to possess anti-inflammatory activity [47] and may account for the anti-inflammatory activity of the extract, but it is not clear whether it is the only contributing component or not.

EOL also exhibited analgesic activity in mice. It was found that this extract significantly inhibited the acetic acid induced writhing response in a dose dependent manner. This method is not only simple and reliable but also affords rapid evaluation of peripheral type of analgesic action. The abdominal constriction is related to the sensitization of nociceptive receptors to prostaglandins, so substances that exhibit activity against carrageenan also inhibit this algogenic process. It is therefore possible that EOL exerts an analgesic effect probably by inhibiting synthesis or action of prostaglandins. It appears that the inhibitory effect of the EOL is due mainly to the interaction of all constituents and not only to the major compound, since previous work has demonstrated that pure α-pinene has insignificant antinociceptive effects [48]. This suggested that the compounds present in the greatest proportions are not necessarily responsible for the greatest share of the total activity. Thus, the involvement of the less abundant constituents should be considered and then, the activity could be attributed to the presence of minor components.

Conclusions

For the first time, the chemical composition of the essential oil of Tunisian *Olea europaea* L. cv. Chemlali leaves (EOL) was carried out using gas chromatography and mass spectrometry (GC-MS). These results clearly show *in vitro* antioxidant activity and significant anti-inflammatory and analgesic effects suggesting a rational basis for folk and traditional uses of this plant in Tunisia for some inflammatory ailments. Future studies are needed to better evaluate these activities and the potential of the plant essential oil.

Acknowledgements

Thanks to Prof. Dalila Mosbahi Saidane and Dr. Wahida Borgi for their precious help.

References

[1]Khlif, M., Ayadi, M., Grati-Kammoun, N., Arous, M. N., Rekik, H., Hamdi, M. T. and Rekik-Fakhfakh, B. 2002. Identifying Chemlali olive variety in its traditional area. Acta Hortic. **586**:117-120.

[2]Ciafardini, G. and Zullo, B. A. 2002. Microbiological activity in stored olive oil. Int. J. Food Microbiol. **75**:111-118.

[3]Fernandez-Escobar, R., Moreno, R. and Garcia-Creus, M. 1999. Seasonal changes of mineral nutrients in olive leaves during the alternate-bearing cycle. Sci. Hortic. **82**:25-45.

[4]Gucci, R., Lombardini, L. and Tattini, M. 1997. Analysis of leaf water relations in leaves of two olive (*Olea europaea*) cultivars differing in tolerance to salinity. Tree Physiol. **17**:13-21.

[5]Boukef, M. K. 1986. Médecine traditionnelle et pharmacopée. Les plantes dans la médecine traditionnelle tunisienne. Agence de coopération culturelle et technique. Paris, 165 p.

[6]Bisignano, G., Tomaino, A., Cascio, R. L., Crisafi, G., Uccella, N. and Saija, A. 1999. On the *in vitro* antimicrobial activity of oleuropein and hydroxytyrosol. J. Pharm. Pharmaco. **51**:971-974.

[7]Pereira, A. P., Ferreira, I. C., Marcelino, F., Valentao, P., Andrade, P. B., Seabra, R., Estevinho, L., Bento, A and Pereira, J. A. 2007. Phenolic compounds and antimicrobial activity of olive (*Olea europaea* L.cv. Cobrancosa) leaves. Molecules **12**:1153-1162.

[8]Markin, D., Duek, L. and Berdicevsky, I. 2003. *In vitro* antimicrobial activity of olive leaves. Mycoses **46**:132-136.

[9]Dekanski, D., Janicijevic-Hudomal, S., Tadic, V., Markovic, G., Arsic, I. and Mestrovic, D. M. 2009. Photochemical analysis and gastroprotective activity of an olive leaf extract. J. Serb. Chem. Soc. **74**:367-377.

[10]Benavente-Garcia, O., Castillo, J., Lorente, J., Ortuno, A. and Del Rio, J. A. 2000. Antioxidant activity of phenolics extracted from *Olea europaea* L. leaves. Food Chem. **68**:457-462.

[11]Briante, R., Febbraio, F. and Nucci, R. 2003. Antioxidant properties of low molecular weight phenols present in the Mediterranean diet. J. Agric. Food Chem. **51**:6975-6981.

[12]Skrget, M., Kotnik, P., Hadolin, M., Hra, A. R., Simoni, M. and Knez, Z. 2005. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. Food Chem. **89**:191-198.

[13]Somova, L. I., Shode, F. O., Ramnanan, P. and Nadar, A. 2003. Antihypertensive, antiatherosclerotic and antioxidant activity of triterpenoids isolated from *Olea europaea*, subspecies Africana leaves. J. Ethnopharmacol. **84**:299-30.

[14]Khayyal, M. T., El-Ghazaly, M. A., Abdallah, D. M., Nassar, N. N., Okpanyi, S. N. and Kreuter, M. H. 2002. Blood pressure lowering effect of an olive leaf extract (*Olea europaea*) in L-NAME induced hypertension in rats. Arzneimittel-Forsch. **52**:797-802.

[15]Gonzalez, M., Zarzuelo, A., Gamez, M. J., Utrilla, M. P., Jimenez, J. and Osuna, I. 1992. Hypoglycemic activity of olive leaf. Planta Med. **58**:513-515.

[16]Al-Azzawie, H. and Alhamdani, M. S. S. 2006. Hypoglycemic and

antioxidant effect of oleuropein in alloxan-diabetic rabbits. Life Sci. **78**:1371-1377.

[17]Somova, L. I., Shode, F. O. and Mipando, M. 2004. Cardiotonic and antidysrhythmic effects of oleanolic and ursolic acids, methyl maslinate and uvaol. Phytomedicine **11**:121-129.

[18]Wang, L., Geng, C., Jiang, L., Gong, G., Liu, D., Yoshimura, H. and Zhong, L. 2008. The anti-atherosclerotic effect of olive leaf extract is related to suppressed inflammatory response in rabbits with experimental atherosclerosis. Eur. J. Nut. **47**:235-243.

[19]Lee-Huang, S., Zhang, L., Huang, P. L. and Chang, Y. T. 2003. Anti-HIV activity of olive leaf extract (OLE) and modulation of host cell gene expression by HIV-1 infection and OLE treatment. Biochem. Biophys. Res. Commun. **307**:1029-1037.

[20]Micol, V., Caturla, N., Pérez-Fons, L., Más, V., Pérez, L. and Estepa, A. 2005. The olive leaf extract exhibits antiviral activity against viral haemorrhagic septicaemia rhabdovirus (VHSV). Antiviral Res. **66**:129-136.

[21]Hamdi, H. K. and Castellon, R. 2005. Oleuropein, a non-toxic olive iridoid, is an anti-tumor agent and cytoskeleton disruptor. Biochem. Biophys. Res. Commun. **334**:769-778.

[22]Abaza, L., Talorete, T. P., Yamada, P., Kurita, Y., Zarrouk, M. and Isoda, H. 2007. Induction of growth inhibition and differentiation of human leukemia HL-60 cells by a Tunisian gerboui olive leaf extract. Biosci. Biotechnol. Biochem.**71**:1306-1312.

[23]Pieroni, A. Heimler, D. Pieters, L. van Poel, B. and Vlietinck, A. J. 1996. *In vitro* anti-complementary activity of flavonoids from olive (*Olea europaea* L.) leaves. Pharmazie **51**:765.

[24]Campeol, E., Flamini, G., Chericoni, S., Catalano, S. and Cremonini, R. 2001. Volatile compounds from three cultivars of *Olea europaea* from Italy. J. Agric. Food Chem. **49**:5409-5411.

[25]Campeol, E., Flamini, G., Cioni, P. L., Morelli, I., Cremonini, R. and Ceccarini, L. 2003.Volatile fractions from three cultivars of *Olea europaea* L. collected in two different seasons. J. Agric. Food Chem. **51**:1994-1999.

[26]Flamini, G., Cioni, P. L. and Morelli, P. L. 2003. Volatiles from leaves, fruits and virgin oil from *Olea europaea* cv. Olivastra Seggianese from Italy. J. Agric. Food Chem. **51**:1382-1386.

[27]Pottier-Alapetite, G. 1981. Flore de Tunisie: Angiospermes-Dicotylédones: Gamopétales. Imprimerie officielle de la République Tunisienne.

[28]Adams, R. P. 2001. Identification of Essential Oils Components by Gas Chromatography/Mass Spectroscopy. Allured,Carol Stream, IL, USA.

[29]Wiley 1998. Registry of Mass Spectral Data. 7th edn Database. NIST Spectra Data CD-ROM. Wiley and Sons, New York, NY.

[30]Miller, L. C. and Tainter, M. L. 1944. Estimation of the ED_{50} and its error by means of log-probit graphic paper. Proceedings of the Society of Experimental Biology and Medicine **57**:261-264.

[31]Koster, R., Anderson, M. and De Beer, E. J. 1959. Acetic acid for analgesic screening. Federation Proceedings **18**:418-420.

[32]Winter, C. A., Risley, E. A. and Nuss, G. W. 1962. Carrageenan induced edema hind paw of the rat as an essay for anti-inflammatory drugs. Proceedings of the Society for Experimental Biology and Medicine **3**:544-547.

[33]Cuendet, M., Hostettmann, K. and Potterat, O. 1997. Iridoid glucosides with free radical scavenging properties from *Fragraea blumei*. Helvetica Chim. Acta **80**:1144-1152.

[34]Burits, M. and Bucar, F. 2000. Antioxidant activity of *Nigella sativa* essential oil. Phytother. Res. **14**:323-328.

[35]Tepe, B., Daferera, D., Sokmen, A., Sokmen, M. and Polissiou, M. 2005. Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae). Food Chem. **90**:333-340.

[36]Lee, S., Umano, K., Shibamoto and Tand Lee, K. 2005. Identification of volatile components in basil (*Ocimum basilicum* L.) and thyme leaves (*Thymus vulgaris* L.) and their antioxidant properties. Food Chem. **91**:131-137.

[37]Lee, B. H., Choi, W. S., Lee, S. E and Park, B. S. 2001. Fumigant toxicity of essential oils and their constituent compounds towards the rice weevil, *Sitophilus oryzae* (L.). Crop Protection **20**:317-320.

[38]Sadraei, H., Ashgari, G. R., Hajhashemi, V., Kolagar, A. and Ebrahimi, M. 2001. Spasmolytic activity of essential oil and various extracts of *Ferula gummosa* Boiss. on ileum contractions. Phytomedicine **8**:370-376.

[39]Mourey, A. and Canillac, N. 2002. Anti-*Listeria monocytogenes* activity of essential oils components of conifers. Food Contam. **13**:289-292.

[40]Savelev, S., Okello, E., Perry, N. S. L., Wilkins, R. M and Perry, E. K. 2003. Synergistic and antagonistic interactions of ticholinesterase terpenoids in *Salvia lavandulaefolia* essential oil. Pharmacol. Biochem. Be. **75**:661-668.

[41]Akutsu, H., Kikusui, T., Takeuchi, Y., Sano, K., Hatanaka, A. and Mori, Y. 2000. Alleviating effects of plant-derived fragrances on stress-induced hyperthermia in rats. Physiol Behav. **75**:355-360.

[42]Wang, W., Wu, N., Zu, Y. G. and Fu,Y. J. 2008. Antioxidative activity of *Rosmarinus officinalis* L. essential oil compared to its main components. Food Chem. **108**:1019-1022.

[43]Kelen, M. and Tepe, B. 2008. Chemical composition, antioxidant and antimicrobial properties of the essential oils of three *Salvia* species from Turkish flora. Bioresource Technol. **99**:4096-4104.

[44]Burits, M., Asres, K. and Bucar, F. 2001. The antioxidant activity of the essential oils of *Artemisia abyssinica* and *Juniperus procera*. Phytoter. Res. **15**:103-108.

[45]Olajide, O. A., Makinde, M. J. and Awe, S. O. 1999. Effects of the aqueous extract of *Bridelia ferruginea* stem bark on carrageenan-induced oedema and granuloma tissue formation in rats and mice. J. Ethnopharmacol. **66**:113-117.

[46]Moulin, M. and Coquerel, A. 2002. Pharmacologie, connaissance et pratique. 2ème édn. Masson, Paris, 845 p.

[47]El Tantaway, M. E., El Shakhawy, F. S., El Sohly, M. A and Ross, S. A. 1999. Chemical composition and biological activity of the essential oil of the fruit of *Taxodium distichum* L. Rich, growing in Egypt. J. Essent. Oil Res. **11**:386-392.

[48]Orhan, I., Küpeli, E., Aslan, M., Kartal, M. and Yesilada, E. 2006. Bioassay-guided evaluation of anti-inflammatory and antinociceptive activities of pistachio, *Pistacia vera* L. J. Ethnopharmacol. **105**:235-240.