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Chemical Characterization of the Volatile Oil Isolated from Leaves of *Schinus molle* L. and its Antioxidant Activity

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In the present study the volatile oil obtained by hydro-distillation from leaves of *Schinus molle* was analyzed by GC/MS and measured for its antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl radical-scavenging assay (DPPH). Fifteen components were identified in the volatile oil, representing 99.99% of the total oil. The identified components in the volatile oil were α-pinene (2.53%), cis-ocimene (5.65%), α-phellandrene (27.67%), sabinene (17.41%), adamantane (0.67), cis-caryophyllene (1.98%), α-muurolene (1.76%), valencene (5.27%), calarene (13.06%), β-cubebene (0.70%), β-selinene (1.13%), α-selinene (2.81%), (+)-cycloisosativene (3.99%), Δ-cadinene (9.22%), and γ-selinene (6.23%). The total antioxidant activity of the tested volatile oil was 40±1.23% and this value is lower that the TAA% observed by Tannic acid (90±5.12%).

Key words: Antioxidant activity; volatile oil; Schinus molle, DPPH method, α-phellandrene.

The aromatic plants have been reported as sources rich in secondary chemical and their derivatives usefully as drugs. Today, about 80% of the world's population relies predominantly on plants and plant extracts for health care and source for clinically useful drugs (Balandrin *et al.*, 1985).

The pepper tree (*Schinus molle* L.) is a pepper tree belonging to the family Anacardiaceae. It originates from South America and a tree native to the Peruvian Andes (Huerta *et al.*, 2010), but has been introduced to most of the tropical and subtropical areas of the world (Taylor, 2005; Wimalaratne *et al.*, 1996). In popular medicine, the leaves are the most sources for isolation of the essential oil used and as a repellent and bioinsecticide (Ferrero *et al.*, 2006 and 2007; Huerta *et al.*, 2010). Traditionally, the volatile compounds from *S. molle* are isolated by hydro-distillation (Santos *et al*, 2007; Zahed *et al.*, 2010) or by steam distillation (Bendaoud *et al.*, 2010) in a Clevenger-type apparatus and the isolated oil is dried over anhydrous Na_2SO_4.

The results of some studies have revealed the antimicrobial and antioxidant properties of volatile oils and extracts of *S. molle* (Dikshit *et al.*, 1986, Hayouni *et al.*, 2008, Murray *et al.*, 2009 and Salazar-Aranda *et al.*, 2011), as well as to have antispasmodic, antipyretic, antifungal and cicatrizing properties (Marongiu *et al.*, 2004; Ferrero *et al.*, 2006). The Brazilian pepper tree have

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different biological properties such as insecticidal activity, repellent, fumigant activity, nutritional indices, and feeding deterrent action on *S. oryzae* adults (Machado *et al.,* 2007). Pharmacologically, the extracts of *S. molle* showed several biological effects, such as: hypotensive, antitumoral, anti-inflammatory, antifungal and antispasmodic (Schmourlo *et al.,* 2005; Yueqin *et al.,* 2003; Ruffa *et al.,* 2002; Quiroga *et al.,* 2001; Bello *et al.,* 1998). The goal of this investigation is to analysis the volatile oil of *Schinus molle* leaves and to measure and evaluate the free radical scavenging activity of the volatile oil by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method comparing these activities with the value from Tannic acid as a standard antioxidant activity.

MATERIALS AND METHODS

Plant material and Isolation of the Essential Oil

Fresh leaves of *S. molle* were collected from the garden of the Faculty of Agriculture, Alexandria University, Egypt, in the middle of September, 2013 and cut to small pieces (100g) and then subjected to 3 h of hydro-distillation in a Clevenger apparatus. The resulting oils were separated from the aqueous phase, dried over anhydrous Na_2SO_4, weighed and the reported yield was calculated with respect to the mass of fresh weight of the leaves (mL/100 g fresh weight). The oil kept dry in sealed brown bottles and stored at 4°C before chemical analysis.

Chemical analysis of Volatile oil by Gas Chromatography-Mass Spectrometry (GC-MS)

Detection of the volatile oil composition made using (Hewlett Packard) HP (High performance) 5890 gas liquid chromatography (GLC) coupled with coupled with 5989 B series mass spectrometer (MS) at Central Lab. Unit in High Institute of Public Health, Alexandria, Egypt. The gas liquid chromatography was equipped with a split less injector at 280ºC, and a flame ionization detector (FID) held at 300°C using helium as a carrier gas. Samples were separated on a capillary column (30m long, and 0.25mm internal diameter) HP-5 (Avondale, PA, USA) of 0.25ìm film thickness. The temperature of the gas chromatograph column was programmed from 80°C to 100°C at a heating rate of 15°C/minute, and then increased to a maximum final temperature of 310°C at a heating rate of 5ºC/minute, holding the maximum final temperature for a residence time of 10 minutes. The temperature of ion source in the mass spectrometer was held at 200°C. All mass spectra were recorded in the electron impact ionization (EI) at 70 electron volts. The mass spectrometer was scanned from m/z 40 to 410 at a rate of two scans per second. An integrator automatically calculated peaks areas. Neither internal nor external chemical standers were used in this chromatographic analysis. Identification of the components of the oils were identified the basis of MS library search (NIST and Wiley), and by comparing with the MS literature data (Adams, ESO, 1999, 2000). The relative amounts of individual components were calculated based on the GC peak area (FID response) without using correction factors.

DPPH radical-scavenging assay

Free radical scavenging activity of the methanolic crude extracts from different parts of the selected trees was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Elansary *et al.,* 2012) with some modifications. An aliquot of 2 mL of stock solution of 0.1 mM DPPH (Sigma-Aldrich) reagent dissolved in pure methanol was added to a test tube with 2 mL of the sample solution in methanol (200 µg/L). The reaction mixture was mixed for 10 s and left to stand in fibber box at room temperature in the dark for 30 min. The absorbance was measured at 517 nm, using a UV scanning spectrophotometer (Unico® 1200). Pure methanol (Sigma-Aldrich) was used to calibrate the spectrophotometer. The decrease in the absorbance of the DPPH solution indicates an increase in DPPH radical scavenging activity. Total antioxidant activity (TAA%) was expressed as the percentage inhibition of the DPPH radical and was determined by the following equation; TAA (%) = $(A_0–A_s/A_0)\times100$; where TAA is the total antioxidant activity, A_0 is the absorbance of DPPH solution in methanol and A_s is the absorbance of a DPPH solution with a tested fraction solution (test) or Gallic acid (positive control) solution. The control contained 2 mL of DPPH solution and 2 mL of methanol. It should be noted here that the TAA% value was taken from the previous work (Abdel-Megeed *et al.,* 2013). The average values of total antioxidant activity (TAA %) were carried out for three replicates. The results are expressed as mean values ± standard deviation (SD).

RESULTS AND DISCUSSION

Essential oil yield was 0.40ml/100g fresh weight and this value was nearly similar to those obtained by Zahed *et al.*, (2010). Fig. 1 is shows the chromatogram of the injected oil sample in GC/MS apparatus. The identified fifteen components representing 100% in the volatile oil (Table 1) were α-pinene (2.53%), cis-ocimene (5.65%), α-phellandrene (27.67%), sabinene (17.41%), adamantane (0.67), cis-caryophyllene (1.98%), α-muurolene (1.76%), valencene (5.27%), calarene (13.06%), β-cubebene (0.70%), β-selinene (1.13%), α-selinene (2.81%), (+)-cycloisosativene (3.99%), Δ-cadinene (9.22%), and γ-selinene (6.23%). Fig. 1 presents chemical structures of the volatile oil components found in *S. molle* leaves.

In the present study the main chemicals composition in the volatile oil from *S. molle* leaves were α-phellandrene (27.67%), sabinene (17.41%), calarene (13.06%) and Δ-cadinene (9.22%). The chemical composition of the essential oil extracted from aromatic and higher plants depends on several factors such as: geographic location, phenologic state, ecologic factors (habitat), genetic variability (chemotype), different climatic and soil-growth conditions, the effect of intra-specific differences, and the extraction process (Bandoni, 2000). Sequently, the chemical composition of the *S. molle* leaves volatile oil presented in Table 1 differs from results published by Barroso *et al.,* (2011) and Marongiu *et al.,* (2004), but is similar to results obtained by Ennigrou *et al.*, (2011). According to the region and continent, some studies of leaf volatile oil of *S. molle* showed different chemical composition. Results from the present study were shown that chemical composition of volatile oils of *S. molle* was nearly similar with those obtained from plants collected in North Africa and Europe, than with southern American plants (Bernhard *et al.,* 1983, Lawrence, 1984, Maffei and Chialva, 1990, Baser *et al.*, 1997; Marongiu *et al.*, 2004). However, studies with Italian leaves oil (Maffei and Chialva, 1990 and Marongiu *et al.,* 2004) and Tunisian leaf oil (Bendaoud *et al.,* 2010; Zahed *et al.,* 2010) referred that the main components may be different either in the same region or in leaf and in percent of the component. Additionally, Rossini *et al.,* (1996) differences on chemical composition suggest the presence of different chemotypes of *Schinus molle*.

This similarity can be attributed to the geographical factor and the chemotype of the plant studied like in Tunisia (Ennigrou *et al.,* 2011). Also, Martins *et al.,* (2013) reported that the leaf essential oil was characterized mainly by α-phellandrene (25.9%), limonene (11.7%), β-myrcene (11.1%), β-phellandrene (10.5%) and elemol (9.0%) and the plant was grown in autumn in the Évora region, in

Table 1. Volatile oil composition of *Schinus molle* leaves

Constituent	RT (min)*	Oil%†	Class
α-pinene	3.55	2.53	monoterpene
cis-ocimene	4.56	5.56	monoterpene
α-phellandrene	5.01	27.67	monoterpene
sabinene	5.48	17.41	monoterpene
adamantane	13.24	0.67	cycloalkane
cis-caryophyllene	13.87	1.98	sesquiterpene
α-muurolene	15.51	1.76	sesquiterpene
valencene	16.07	5.27	sesquiterpene
calarene	16.74	13.06	sesquiterpene
β-cubebene	17.39	0.70	sesquiterpene
β-selinene	17.59	1.13	sesquiterpene
α-selinene	18.18	2.81	sesquiterpene
(+)-cycloisosativene	18.35	3.99	sesquiterpene
Δ-cadinene	18.76	9.22	sesquiterpene
γ-selinene	19.43	6.23	sesquiterpene
Total		99.99%	

* RT: Retention time (min).
†Percentage of total FID area obtained on HP-5 capillary column.

southeast Portugal. Leaf oil of *S. molle* grown in Italy also contained α- and β-phellandrene and limonene (30.2%, 9.6% and 9.3% respectively) (Maffei and Chialva, 1990).

The volatile oil of *S. molle* grown in Costa Rica was characterized and had 42 constituents and the major constituents of the oil were α-pinene and β-pinene (Díaz *et al.*, 2008) and *p*-cymene was identified as a major component in the oil (Abdel-Sattar *et al.*, 2010) and the high yield and efficacy of *S. molle* essential oil against *Trogoderma granarium* and *Tribolium castaneum* suggest that it may provide leads for active insecticidal agents. α-phellandrene (46.64%) followed by β-phellandrene (28.53%) were found the main constituents in the volatile oil of *S. molle* from Tunisia (Ennigrou *et al.*, 2011). In Uruguayan Origin the bicyclo-germacrene (29.20%) was the main component (Rossini *et al.*, 1996). In Turkey, the α-phellandrene (45.7%), β-phellandrene (13.6%) and hmonene (13.4%) were the main components in the leaf oil (Baser *et al.*, 1997). While the main compounds obtained by supercritical fluid extraction from *S. molle* from Rio Grande do Sul in southern Brazil were sabinene (5.85%), limonene (41.37%), (E)-caryophyllene (15.60%), bicyclogermacrene (11.59%), germacrene D (8.86%), and spathulenol (4.03%) (Barroso *et al*., 2011). In the voaltile oil of *S. molle* from Mexico, the main component was α-phellandrene with 32.8% (Guerra-Boone *et al.*, 2013). The berries of *S. molle* had α-phellandrene (35.86%), β-phellandrene (29.3%), β-pinene (15.68%), p-cymene (5.43%) and α-pinene (5.22%) (Hayouni el *et al.*, 2008).

The total antioxidant activity was weak of leaves volatile oil (Díaz *et al*., 2008) and observed

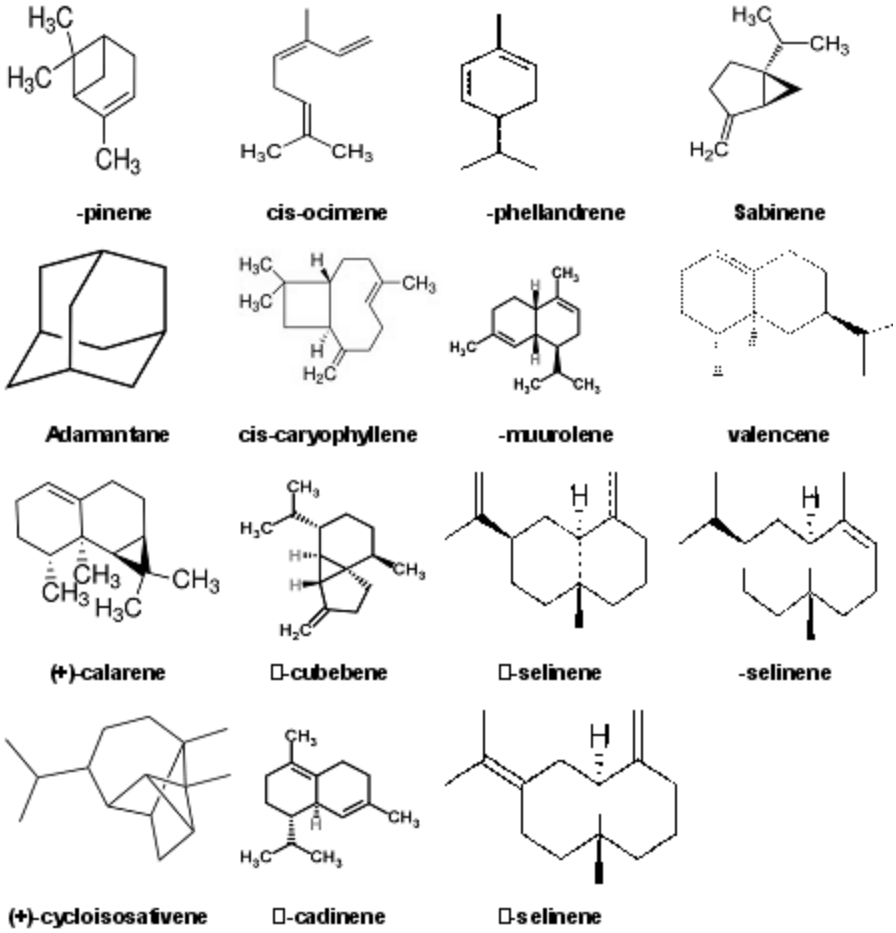

Fig. 1. Chemical structures of the volatile oil components presented in *Schinus molle* leaves

a TAA value of 40±1.23% and this value is lower that than the observed by Tannic acid (90±5.12%). On the other study the berries volatile oil found to have a good antioxidant activity (Bendaoud *et al.*, 2010; Hayouni el *et al.*, 2008). However, the antioxidant activities of all oils were small (half maximal effective concentration values >250 microg/mL) (Guerra-Boone *et al.*, 2013). Results for antioxidant properties are reported that the leaf essential oil (16 mg/ml) promote a free radical scavenging effect of 4.8% , however this is much lower than that which is observed for ascorbic acid standard (Martins *et al.*, 2013).

CONCLUSION

In the present study fifteen components were identified in the volatile oil obtained by hydro-distillation from leaves of *Schinus molle* and analyzed by GC/MS representing 99.99% of the total oil. The main constituents were α-phellandrene (27.67%), sabinene (17.41%), calarene (13.06%), Δ-cadinene (9.22%), γ-selinene (6.23%), cis-ocimene (5.65%), and valencene (5.27%).The total antioxidant activity as measured by 2,2-diphenyl-1-picrylhydrazyl radical-scavenging assay (DPPH)of the tested volatile oil was 40±1.23% and this value is lower that the TAA% observed by Tannic acid (90±5.12%). Essential oil of leaves of *Schinus molle* showed moderate antioxidant properties. This suggests they have a moderate potential for use in biotechnology, food and/or pharmaceutical industries.

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