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CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF ESSENTIAL OILS OF THREE ENDEMIC MEDICINAL PLANTS OF IRAN

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Abstract

Hydro-distillated essential oils (EO) antioxidant activity of three endemic medicinal plants of Iran namely, *Heracleum lasiopetalum* Boiss., *Kelussia odoratissima* Mozaff., and *Ferulago angulata* (Schlecht.) Boiss were studied. The results indicated that the major components of the EOs were 2-ethylhexyl acetate (34.5%), *n*-octanol (6.5%), and hexanol (5.1%) for *H. lasiopetalum* fruits; *Z*-β-ocimene (20.5), α-pinene (11.6%), and α-phellandrene (9.3) for *F. angulata* aerial parts; α-pinene (20.1%), 1,8-cineole (18.2%), and *Z*-ligustilide (15.5%) for *K. odoratissima* aerial parts. The three EOs having high antioxidant activity could be used as an alternative preservative instead of synthetic ones in food industry.

Introduction

The essential oil (EO) and extracts from medicinal and aromatics plans may contain a wide variety of free radical scavenging molecules, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids, and some other endogenous metabolites, which are rich in antioxidant activity (Shahidi and Naczk 1995, Velioglu *et al*. 1998, Cai *et al*. 2004). Studies demonstrate the medicinal activities, in particular antioxidant activities, of natural plant materials against various diseases such as atherosclerosis, cancer, diabetes, Alzheimer's, HIV, Parkinson's, and cataracts (Moon and Shibamoto 2009). In addition, interest has increased considerably in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their carcinogenicity (Velioglu *et al*. 1998).

The family Apiaceae has more than 300 genera and 3000 species of the aromatics. Plants of this family can produce monoterpenes, sesquiterpenes, and phenyl components and related resins in their secretary ducts, roots, stem, leaf, flowers, seeds, and fruits (Sodeifian and Ansari 2011). In the present study, the antioxidant activity of EOs from three species of Iranian medicinal herbs (Apiaceae), namely *Heracleum lasiopetalum* Boiss., *Kelussia odoratissima* Mozaff., and *Ferulago angulata* (Schlecht) Boiss. were measured for natural antioxidants.

All these three species are endemic to Iran and their description, distribution and ethnobotanical properties having been described elsewhere (Ghasemi Pirbalouti 2009, 2010, Javidnia *et al*. 2006, Mozaffarian 2008; Rabbani *et al*. 2011).

So far, a few reports on chemical composition and antioxidant activity of the EOs of *H. lasiopetalum* fruits, *F. angulata* aerial parts and *K. odoratissima* aerial parts from Bakhtiari Zagros Mountains are available. The objective of the present study was to evaluate the antioxidant activity, and chemical composition of the EOs from three medicinal and aromatic plants of Iran.

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Materials and Methods

The fruits of *H. lasiopetalum* and the aerial parts (leaves and stem) of *F. angulata* and *K. odoratissima* were collected from mountains of Zagros, southwestern Iran, during May-June, 2012. Collected specimens were properly processed. Identifications were consequently confirmed with the help of the authentic specimens deposited at the Herbarium of Research Center of Agriculture and Natural Resources of Chaharmahal va Bakhtiari (CHB) and I.A.U. Shahrekord Branch (IAUSHK), Iran. Voucher number of *F. angulata*, *K. odoratissima* and *H. lasiopetalum* are CHB-1712, IAUSHK-150, IAUSHK-149, respectively.

Harvested parts were dried at room temperature for two weeks. Dried plant materials were powdered (100 g) and subjected to hydro-distillation (1000 ml distillated water) for 3 hrs using a Clevenger-type apparatus.

The antioxidant capacity of the essential oils was evaluated by the method of Wang *et al.* (1998). The EOs at concentrations of 8 - 500 µg/ml were mixed with an equal volume of 0.2 mM ethanol solution of DPPH. The absorbance was measured using a Perkin–Elmer Lambda UV/Vis spectrophotometer at 515 nm against a blank, i.e. without DPPH. All tests were run in triplicate and an average was used. Decreasing of DPPH solution absorbance indicates an increase of DPPH radical scavenging activity. The amount of sample necessary to decrease the absorbance of DPPH by 50% (IC50) was calculated graphically and the percentage inhibition was calculated according to the equation:

$$\% \text{ inhibition} = \frac{AC(0) - AA(t)}{AC(0)} \times 100$$

where $A_{C(0)}$ is the absorbance of the control at t = 0 min and $A_{A(t)}$ is the absorbance of the antioxidant at t = 30 min. The food preservative butylhydroxyanisole (BHA) was used as positive control.

Gas chromatography/mass spectrometry (GC/MS) analysis: The EOs were analyzed using an Agilent 7890 A gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with a HP-5MS 5% phenylmethylsiloxane capillary column (30.00 m × 0.25 mm, 0.25 µm film thickness). Oven temperature was kept at 60°C for 4 min initially, and then raised at the rate of 4°C/min to 260°C. Injector and detector temperatures were set at 290 and 300°C, respectively. Helium was used as carrier gas at a flow rate of 2 ml/min, and 0.1 µl samples were injected manually in the split mode. Peaks area per cents were used for obtaining quantitative data. The gas chromatograph was coupled to an Agilent 5975 C (Agilent Technologies, Palo Alto, CA, USA) mass selective detector. The EI-MS operating parameters were as follows: Ionization voltage, 70 eV; Ion source temperature, 200°C. Retention indices were calculated for all components using a homologous series of *n*-alkanes (C_5 - C_{24}) injected in conditions equal to samples ones. Identification of oil components was accomplished based on comparison of their retention times with those of authentic standards and by comparison of their mass spectral fragmentation patterns (WILLEY/ChemStation data system) (Adams 2007).

The data was statistically analyzed using one-way ANOVA by the program SPSS $_{(19.0)}$, and comparison of the means of the main constituents of essential oils evaluated by Duncan's multiple range test at $p \leq 0.05$ level.

Results and Discussion

The yellow oil of *F. angulata* and *K. oderatissma*, and opaque-yellow color oil of *H. lasipetalum* were obtained in the yields of 0.81, 0.53, and 0.35% (v/w) based on dry matter, respectively. The EO yield and its chemical composition were expected as they are affected by

several factors, including species and genotype (Ghasemi Pirbalouti *et al.* 2013a,b), ecological conditions (Ghasemi Pirbalouti and Moalem 2013), growth stage, and extraction methods (Sodeifian and Ansari 2011). Sodeifian and Ansari (2011) reported the oil yield of *F. angulata* under various factors in supercritical extraction method is highly variable (0.05 to 0.82%). Results of other study (Aklaghi 2008) indicated the flower, stem, and leaves of *F. angulata* (collected from east-north, Iran) yielded 0.66, 0.54 and 0.43% (w/w), respectively. In agreement with previously published reports, Rabbani *et al.* (2011) reported that the aerial parts of *K. odoratissima* yielded a 0.4% (v/w). The results of a study (Sonboli *et al.* 2006) indicated the EO yield of the aerial parts (leaves and stem) of *T. lasiopetalum* (synonym: *H. lasiopetalum*) collected from west Iran was 0.03% (w/w) based on the dry weight of plant.

The chemical constituents identified by GC and GC/MS, the major components of the essential oils are presented in the Table 1. In total, 42 compounds in the EO from *F. angulata* were identified. The major components were *Z*-β-Ocimene (20.54%), α-pinene (11.59%) and α-phellandrene (9.33%) that they were dominant among monoterpenes components (Table 1). The following components (%) have been identified in EO of *F. angulata* two ecotypes: *E*-β-ocimene (22.6-27.9), α-pinene (25.7-27.1%), bornyl acetate (3.9-8.5%) (Ghasempour *et al*. 2007). The aerial parts of *F. angulata* in which *(Z)*-β-ocimene (35.5%), terpinolene (5.7%), and α-phellandrene (5.4%) have been the main components (Javidnia *et al*. 2006). Aklaghi (2008) reported that *α*-phellandrene and *α*-pinene are main compositions in flowers, stems, and leaves of *F. angulata*.

Table 1. Chemical compositions of the essential oils of three Iranian medicinal and aromatic plants.

Components	RI	KI	Percentage		
			F. angulata	*H. lasipetalum*	*K. oderatissma*
α-Thujan	930	931	0.67	0.11	0.16
α-Pinene	939	939	**11.59**	4.82	**20.09**
Camphene	951	953	1.06	-	0.13
Verbenene	967	967	0.69	-	-
Sabinene	981	976	1.37	0.56	-
β-Pinene	985	980	1.33	0.48	0.44
β-Myrcene	995	991	3.42	0.22	0.31
α-Pphellandrene	1008	1005	**9.33**	0.57	0.36
Δ-3-Carene	1012	1011	1.24	0.09	0.26
α-Terpinene	1017	1018	0.29	-	0.10
p-Cymene	1025	1026	2.02	0.22	0.70
β-Phellandrene	1030	1031	**8.11**	0.89	-
1,8-Cineole	1030	1033	-	2.18	**18.17**
Z-β-Ocimene	1041	1040	**20.54**	2.10	1.03
E-β-Ocimene	1048	1050	3.23	0.25	0.32
□-Terpinene	1058	1062	0.65	0.08	0.36
n-Octanol	1072	1070	-	**6.50**	-
E-Linalool oxide	1072	1074	0.05	-	0.15
Terpinolene	1088	1088	2.24	0.26	0.59
Camphenone	1096	1093	0.29	-	-

(Contd.)

Linalool	1104	1098	2.98	1.33	10.66
Alloocimene	1126	1123	1.25	0.09	-
Z-Pinocarveol	1134	1139	-	-	0.37
E-Verbenol	1138	1140	0.95	-	-
Z-Verbenol	1143	1144	2.79	-	-
Citronella	1150	1153	0.18	-	-
Borneol	1065	1165	-	-	0.12
Terpinene-4-ol	1175	1166	0.78	-	0.38
α-Terpineol	1187	1189	0.67	1.03	5.28
Hexanol	1197	1203	-	5.12	-
Z-Carveol	1215	1217	-	-	0.21
Octanol acetate	1216	1211	-	**34.48**	-
Citronellol	1224	1228	1.32	-	-
Nerol	1225	1228	-	-	0.24
Pulegone	1235	1237	-	-	0.39
Geraniol	1250	1255	0.31	0.45	-
E-Anethole	1280	1282	-	-	0.22
Bornyl acetate	1280	1285	2.39	-	-
Thymol	1286	1290	0.15	0.34	0.99
Carvacrol	1295	1298	1.98	0.60	1.51
α-Terpinyl acetate	1344	1350	-	0.31	-
Citronellyl acetate	1348	1354	tr	-	-
Neryl acetate	1369	1365	-	0.22	-
α-Copaene	1369	1376	0.14	0.21	0.55
β-Elemene	1386	1391	0.05	-	-
E-Jasmone	1393	1394	0.45	-	-
Methyleugenol	1399	1402	0.41	-	-
β-Caryophyllene	1412	1418	0.81	0.38	0.52
α-Humulene	1446	1440	0.09	0.29	0.46
Z-β-Farnesene	1452	1443	-	0.08	-
□-Curcumene	1473	1480	0.40	-	-
Germacrene D	1474	1480	-	0.52	0.32
α-Curcumene	1477	1483	-	0.39	-
α-Zingibirene	1489	1495	-	0.70	-
Bicyclogermacrene	1490	1494	4.03	-	-
β-Himachalene	1492	1499	-	-	0.41
Cuparene	1498	1502	-	0.11	0.22
β-Bisabolene	1502	1509	0.12	0.47	0.10
E-□-Bisabolene	1508	1515	-	0.21	0.18
δ-Cadinene	1516	1524	0.46	-	0.40
β-Sesquiphellandrene	1516	1524	-	0.53	-
Z-□-Bisabolene	1524	1533	-	0.11	-
Spathulenol	1569	1576	0.45	-	-
Caryophyllene oxide	1573	1581	-	0.17	0.37
Z-3-Butylidene phthalide	1662	1668	-	1.01	0.87
Z-Ligustilide	1727	1726	-	-	**15.52**
Total			91.32	68.48	83.46
Oil yield v/w (%)			0.81	0.35	0.53

tr: (< 0.05%). RI: Retention indices determined on HP-5MS capillary column. KI: Kovats index. %: Calculated from TIC data.

In total, 38 compounds in the EO from *K. odoratissima* were identified; the major compounds were α-pinene (20.1%), 1,8-cineole (18.2%), and *Z*-ligustilide (15.5%), while the results of other reports indicated that the main components of the EO of *K. odoratissima* were 3-*n* butyl phtalide and *Z*-ligustilide (Rabbani *et al.* 2011; Ghasemi Pirbalouti *et al.* 2012; Sajjadi *et al.* 2013). Various factors, including ecotype, harvesting stage, drying, and extraction methods caused on this variation.

Results of current study indicated that 39 compounds were identified in the EO of *H. lasiopetalum*. As a determined by GC and GC–MS analyses, *H. lasiopetalum* contained 2-ethylhexyl acetate (34.5%), *n*-octanol (6.5%) and hexanol (5.1%) as the major compounds. Sesquiterpene hydrocarbons were found as the major group of compounds in *H. lasiopetalum. (E)*-anethole has been characterized as the main constituent of the leaves, stem and flower of *H. persicum* Desf. ex Fisch from Iran (Sefidkon *et al*. 2002, 2004).

Antioxidant properties are very important in counteracting the deleterious role of free radicals in foods or biological systems. Excessive formation of free radicals accelerates the oxidation of lipids in foods, impairs food quality and consumer acceptance (Cheung *et al.* 2007). The DPPH is a stable free radical, which has been widely accepted as a tool for estimating the free radical scavenging activities of antioxidants (Hu *et al.* 2004). The lower IC_{50} value indicates a stronger ability of the extract to act as a DPPH scavenger while the higher IC_{50} value indicates a lower scavenging activity of the scavengers as more scavengers were required to achieve 50% scavenging reaction. The IC_{50} values were found to be 0.035, 0.027, 0.028, and 0.065 mg/ml for *K. odoratissima*, *H. lasiopetalum*, *F. angulata*, and L-Ascorbic acid, respectively. The results indicated no significant difference between activities of the EOs. An important characteristic of EO and their components is their hydrophobicity, which enables them to partition in the lipids of the bacterial cell membrane and mitochondria, disturbing the structures and rendering them more permeable. Leakage of ions and other cell contents can then occur. Free radicals cause auto oxidation of unsaturated lipids in food, and the antioxidant activity of the EOs could be attributed to their hydrogen donating ability (Kaur and Perkins 1991).

The results of this study suggest the possibility of using the EOs as natural food preservatives, because the EOs possesses high antioxidant activities. This study can be considered as the first report on the *in vitro* antioxidant activity of the EOs prepared from the aerial parts of *K. odoratissima* and *F. angulata* and fruits of *H. lasiopetalum*, collected from Southwestern Iran. We hope that our results introduce a unique natural source which possesses strong antioxidant substances.

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