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Composition and Antioxidant Effect of the Essential Oils of the Flowers and Fruits of *Ducrosia assadii* Alava., a Unique Endemic Plant from Iran

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Abstract: Essential oils are a kind of secondary metabolites with a variety of valuable biological activities. In the present study, the essential oils of the flowering and fruiting tops of *Ducrosia assadii* Alava. (Apiaceae), were isolated separately by steam distillation and studied by Gas Chromatography (GC) and Gas Chromatography Mass Spectrometry (GC/MS). Antioxidant effect of the essential oils was studied using diphenylpicrylhydrazil (DPPH) scavenging assay. The results indicated some quantitative but not qualitative differences between the analyzed oils. The major components of the oil were found to be n-decanal 46.68 and 42.21%, (n-decanol) 13.79, 13.27% and (dodecanal) 10.01 and 8.8%, respectively in flowers and fruits. In both studied oils, oxygenated aliphatic compounds comprise the highest percentage of the oil (76.88, 77.71% in flower and fruit oil, respectively) while the sesquiterpenes were at low percentage in both oils. Both these oils exhibited relative antioxidant effect in comparison to butylated hydroxyanisole (BHA). The composition of the essential oil of *D. assadii* was completely different from the other studied species of the plant such as *D. anethifolia* and *D. esmaeli* which could be accounted for their biologic activities.

Key words: *Ducrosia assadii*, essential oil, flowers, fruits, DPPH assay

INTRODUCTION

Ducrosia belongs to the family of Apiaceae (Umbelliferae) and comprises about only 4 known species which occur largely in Africa and Asia especially in Iran, Afghanistan, Pakistan, Syria, Lebanon and Iraq (Aynehchi, 1990; Zargari, 1996). This genus comprises about 3 habitat species: *Ducrosia anethifolia* Boiss., *Ducrosia flabellifolia* Boiss. and *Ducrosia assadii* Alava in Iran (Rechinger, 1987). The plant of *D. assadii* is described by Mozaffarian for a vast region in Kerman province between Dehbakri and Jiroft, Lalehzar and Baghin regions and known in Persian as “Moshgake bakraei” (Mozaffarian, 1998). Phytochemical studies have demonstrated the presence of some coumarins such as pangelin in *D. anethifolia* (Starvi *et al.*, 2003). The flowers and fruits of *D. assadii* Alava have been used as a flavoring agent, antispasmodic and carminative in folk medicine. Janssen *et al.* (1984) reported α -pinene (59.2%), myrcene (11.6%) and limonene (7.5%) as the

major compounds of the essential oil of *D. anethifolia* (Janssen *et al.*, 1984). In another report, the major compounds of the oil of *D. anethifolia* were reported to be α -pinene, terpineolene and ocimene (Mostafavi *et al.*, 2008). Hagi *et al.* (2004) reported the presence of α -pinene, citronellal, limonene, linalool and myrcene in this oil (Hagi *et al.*, 2004) while in the another report, n-decanal constitutes more than 70% of the essential oil which has exhibited antianxiety effect in mice (Hajhashemi *et al.*, 2010). There are lesser studies for *D. assadii* in the literature. A report shows n-decanal (36.4%) as the main component among the 29 constituents characterized in the oil of *D. assadii* (Rustaiyan *et al.*, 2006), meanwhile Mostafavi *et al.* (2008) reported that the essential oil of the aerial parts of the plant is composed of n-decanal (74%), dodecanal (7.2%) and α -pinene (4.0%) (Mostafavi *et al.*, 2010).

Several studies suggest that free radicals can damage cellular macromolecules (Bergendi *et al.*, 1999).

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Some of plant secondary metabolites especially essential oils exhibit antioxidant effect (Bensky *et al.*, 2004; Sharififar *et al.*, 2003, 2007a, b, 2009, 2011; Yassa *et al.*, 2005). As a part of a project on the volatile composition of *Ducrosia* species endemic to Iran, we have studied the oil composition of the aerial parts of *D. assadii* in two separate stages of flowering and fruiting times by GC and GC/MS. We think that the knowledge of the essential oils may be useful to clarify the systematic status of *D. assadii*. On the other hand the antioxidant effect of these essential oils has been studied using DPPH free radical scavenging assay.

METHODS AND MATERIALS

Plant materials: The air-dried tops of *D. assadii* were gathered at flowering and fruiting stages (late June and August, 2011, respectively) from Dehbakri, Kerman, Iran (at altitude of 1900 m above sea level). Voucher specimen was deposited at the Herbarium of department of Pharmacognosy of Faculty of Pharmacy in Kerman University of Medical Sciences, Kerman, Iran (KF1387).

Isolation of essential oil: The gathered parts of the plant were left to dry at room temperature and 100 g of flowering and fruiting tops of the plant were coarsely minced and placed in a flask containing 500 mL of water and steam distilled in a Clevenger apparatus for 4 h. The obtained essential oils were dried over anhydrous sodium sulfate and stored at 4°C in the darkness. Essential oil yield was 0.71% and 0.49% based on dried weight of sample for flower and fruit tops, respectively.

Gas chromatography: The essential oils were analyzed using a Shimadzu QP 5000 gas chromatograph equipped with a FID detector and HP-5 MS capillary column (30 m*, 0.25 mm, film thickness 0.25 m). Injector and detector temperatures were set at 220 and 290°C, respectively. Oven temperature was kept at 50°C for 3 min, then gradually raised to 160 at 3°C min⁻¹, held for 10 min and finally raised to 240 at 3°C min⁻¹. Helium was the carrier gas, at a flow rate of 1 mL min⁻¹. Diluted samples (1/100 in acetone, v/v) of 1,0 L were injected manually in the splitless mode. Quantitative data were obtained electronically from area percent data without the use of correction factors.

Gas chromatography/mass spectrometry: GC-MS analysis of the essential oils was performed under the same conditions with GC (column, oven temperature, flow rate of the carrier gas) using a Shimadzu QP 5000 gas chromatograph equipped with a Shimadzu QP 5050 mass selective detector in the electron impact mode (70 eV). Injector and MS transfer line temperatures were set at 220 and 290°C, respectively.

Qualitative and quantitative analysis: The components of the oils were identified based on the comparison of their relative retention times and mass spectra with those of standards. The individual compounds were identified by MS and their identity was confirmed by comparing their retention indices relatives to n-alkanes and by comparing their mass spectra and retention times with those of authentic samples or with data already available in the NIST and Wiley library and literature (Adams, 1995). GC and GC/MS analysis results are given in Table 1.

Table 1: GC/MS analysis of the essential oil of *Ducrosia assadii* in two stage of flowering (FLD) and fruiting (FRD)

Compound	RI	FLD essential oil (%)	FRD essential oil (%)
n-hexanal	804	-	0.10
n-hexanol	867	0.2	-
n-nonane	898	0.63	0.15
α-pinene	938	6.14	2.30
Sabinene	976	0.92	0.39
beta-pinene	982	0.24	0.21
beta-myrcene	989	4.4	2.72
Dehydro,2,8-cineol	993	-	0.25
Para-cymene	1025	-	0.81
Limonene	1032	1.55	0.76
1,8-cineol	1037	0.39	0.12
e-beta ocimene	1052	-	0.58
Gamma terpinene	1062	0.14	1.09
n-octanol	1071	0.21	0.23
Alpha terpineolene	1088	1.32	1.54
Nonanone	1090	0.23	-
Linalool	1098	2.17	2.51
Nonanal	1105	-	1.42
Trans verbenol	1145	-	0.25
Isopulegone	1156	0.22	-
n-nonanol	1171	0.41	0.56
z-4-decenal	1185	0.22	0.49
e-4-decenol	0.34	0.25	-
n-decanal	1206	46.68	42.21
6-nonenal	1211	0.34	-
Carvacrol	1231	-	1.23
n-decanol	1276	13.79	13.27
Decanoic acid	1279	2.27	5.53
n-undecanol	1369	-	1.18
1-tetradecene	1394	0.64	-
Dodecanal	1407	10.01	8.87
Dodecanol	1411	0.28	-
β-farensene	1426	0.6	-
n-dodecanol	1461	0.45	0.92
e-2-dodecanal	1469	-	0.35
Dodecanoic acid	1567	-	1.10
β-caryophyllene	1585	0.45	2.80
Tetradecanal	1611	0.99	-
bate-eudesmol	1638	0.2	0.59
n-tetradecanol	1674	0.46	1.23
1-nonadecane	1882	-	0.63
Incensol	2158	0.78	-
Total		97.67	96.64
Monoterpenes		17.49	14.76
Oxygenated monoterpenes		2.78	4.63
Sesquiterpenes		1.05	2.8
Naphthalene compounds		0.98	0.59
Aliphatic compounds		78.15	77.71
Oxygenated aliphatics		76.88	77.25

DPPH free radical scavenging assay: The antioxidant activity of FLD and FRD was determined in terms of hydrogen donating ability using the DPPH free radical scavenging assay as modified by Sanchez-Moreno *et al.* (1998). At first a serial dilution of the essential oils was prepared. A volume of 0.1 mL of each sample was added to 2.9 mL of DPPH solution (60 μ M). The absorbance was read for 30 min at 5 min intervals using a spectrophotometer. The remaining concentration of DPPH in the reaction medium was calculated through the linear regression. The percentage of remaining DPPH was expressed as following formula:

$$\text{Percentage of remaining DPPH} = \frac{\text{DPPH}_{30}}{\text{DPPH}_0} \times 100$$

where, DPPH_{30} is DPPH concentration after 30 min and DPPH_0 is the initial concentration. The percentage of DPPH inhibition of different concentration of essential oils was determined as follow equation:

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

where, A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. Each experiment was performed in triplicate and data was reported as Mean \pm SD.

RESULTS AND DISCUSSION

The components of the oils from the flowering and fruiting tops of *D. assadii*, their retention indices and percentage composition are summarized in Table 1, where all the components are listed in order of elution on the DB-5 column. The major constituents of the essential oil of the flowers of *D. assadii* (FLD) were found to be n-decanal (46.68%), n-decanol (13.79%), dodecanal (10.01%) and alpha-pinene (6.14%). Other representative components of the oil were identified as beta-myrcene (4.4%), decanoic acid (2.27%), linalool (2.17%), limonene (1.55%) and alpha terpineolene (1.32%). The total amount of aliphatic compounds was 78.15%, one of monoterpenes was 17.49%, whereas the one of sesquiterpenes was 1.05%. The major constituents of the oil from the fruiting tops of *D. assadii* (FRD) were found to be n-decanal (42.21%), n-decanol (13.27%) and dodecanal (8.8%). Other characteristic components of this oil were decanoic acid (5.53%), beta-myrcene (2.72%), linalool (2.51%), alpha-pinene (2.30%), beta-caryophyllene (2.8%) and alpha-terpineolene (1.54%). The amount of aliphatic

compounds was 77.77% which 77.25% of them were oxygenated. The amount of monoterpenes was 14.76% and one of sesquiterpenes was about 2.8%. These results show that the composition of essential oils from FLD and FRD are similar qualitatively but not quantitatively. n-decanal, n-decanol and dodecanal have been identified as main constituents in both oils. The components such as n-decanal and dodecanal are currently obtained from synthetic sources and the high percentage of them in plant sources would be a good source for them. The composition of essential oils of *D. assadii* are completely different from the others species of *Ducrosia*. As the report of Janssen *et al.* (1984) alpha-pinene, citronellal, limonene, linalool and myrcene (Janssen *et al.*, 1984; Mostafavi *et al.*, 2008) were presented as main components and n-decanal, n-dodecanal, n-decanol, trans-2-dodecenal and cis-chrysanthenyl acetate were lesser while as in *D. ismaelis*, α -pinene, n-butylbenzene, gamma-cadinene, 3,5-dimethylstyrene, 5-methylindan have been identified and reported as major compounds (Al-Meshal *et al.*, 1985). Our results also show some differences with previous studies for this plant. Rustaiyan *et al.* (2006) reported n-decanal (36.4%) as the main compound in the oil of aerial parts of *D. assadii* and Mostafavi *et al.* (2008, 2010) indicated the amount of n-decanal about 70 and 36.4% in the oil of aerial parts of the plant collected from Lalehzar and Dehbakri, respectively. These differences might be due to the environmental factors such as humidity, temperature and even altitude of natural habitat where this species grows. In DPPH assay, the degree of DPPH discoloration is attributed to reducing potency of test compounds. The results have given in Fig. 1. As shown in this figure, both FLD and FRD essential oils could inhibit DPPH radical in a concentration-dependent manner. The highest inhibition was at $67.4 \pm 3.6\%$ and $68.5 \pm 2.9\%$ for FLD and FRD,

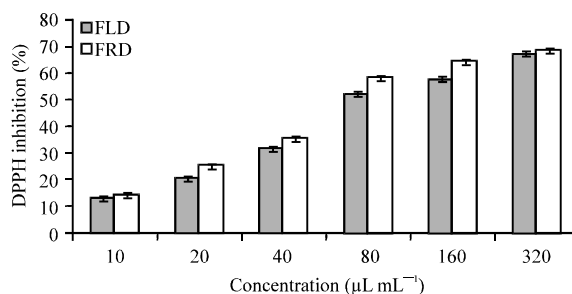


Fig. 1: Antioxidant effect of the essential oil of *Ducrosia assadii* in two stage of flowering (FLD) and fruiting (FRD), FLD: Flower essential oil, FRD: Fruit essential oil experiment was carried out in triplicate and results was reported as Mean \pm SD

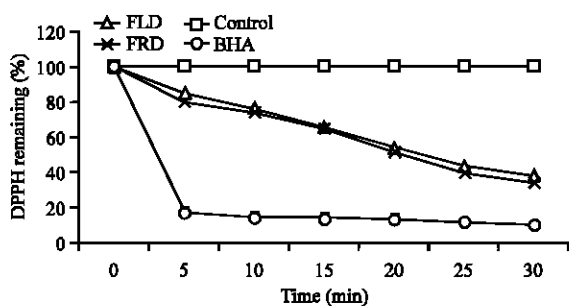


Fig. 2: Time-remained DPPH radical study of the essential oil of *Ducrosia assadii* in two stage of flowering (FLD) and fruiting (FRD) in comparison to BHA, FLD: Flower essential oil, FRD: Fruit essential oil experiment was carried out in triplicate and results was reported as Mean±SD

respectively. These results demonstrated the relative effectiveness of FLD and FRD essential oils in comparison to BHA, however this activity was weaker than BHA. The highest DPPH inhibition effect of both essential oils was seen at 320 $\mu\text{L mL}^{-1}$, so, this concentration was used for time- activity studies. The time- remaining DPPH kinetic study shows that the concentration of DPPH decreases with time in the presence of all concentrations of both essential oils (Fig. 2). DPPH concentration was about $38.1 \pm 2.6\%$ and $34.3 \pm 2.4\%$ for FLD and FRD at highest used concentration. No significant difference was observed in antioxidant effects of tested essential oils. Analysis of FLD and FRD oils indicated the presence 20.27% and 19.39% monoterpenes in these oils, respectively (Table 1). Monoterpenes with methylene group exhibit potent antioxidant effect which attributed to the presence of methylene groups in monoterpene hydrocarbons (Tepe *et al.*, 2005). These oils contain a low percentages of monoterpenes and so exhibited a moderate antioxidant effect. Furthermore, it is expected, in the plants exposed to dry climate, the compounds with high molecular weight impedes it to low humidity, although more studies are needed for this hypothesis.

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