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Free Radical Scavenging Potential and Essential Oil Composition of the *Dorema glabrum* Fisch. C.A. Mey Roots from Iran

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ABSTRACT

Introduction: *Dorema glabrum* is an endangered species that grow in Transcaucasia and North West of Iran. The plant has extensive uses e.g. as an herbal remedy or food additive in these regions. The chemical composition of hydrodistilled oil of *D. glabrum* growing in Iran was analyzed by GC-MS for the first time. ***Methods:*** The essential oil of air-dried roots was obtained by hydrodistillation using a Clevenger type apparatus. The oil was subjected to GC-MS analysis and its free radical scavenging properties were determined by DPPH method. ***Results:*** Thirty four constituents were identified that represented 81.6% of the total oil. The main compounds were delta-Cadinene (12.77%), beta-bisabolene (7.48%), alpha-Fenchyl acetate (6.32%), Copaene (5.68%) and Cubenol (5.42%). The essential oil had weak free radical scavenging properties with the RC50 value of 2.24 mg/mL. ***Conclusion:*** Present work is the first report on chemical composition of the essential oil obtained from *D. glabrum* roots. GC-MS Analysis showed that the oil was rich in sesquiterpenes. It deems that weak free radical scavenging activity of the oil is due to absence of potent antioxidant compounds.

Introduction

The genus Dorema (Apiaceae) is represented by seven species in Iranian flora, among them *Dorema glabrum* Fisch. C.A. Mey, *D. aucheri* Boiss and *D. ammonicum* D. Don are endemic (Mozaffarian 2003). *Dorema glabrum* is a perennial herb that grows in loamy or rocky slopes of Nakhichevan, Autonomous Republic-Azerbaijan, Armenia and Iran. Though, according to Rechinger, distribution of *Dorema glabrum* is restricted to Transcaucasia region (Nakhichevan and Armenia zone) (Rechinger 1987), recent works show that this plant is growing in some locations in North-West of Iran (Mozaffarian 2007, Ajani *et al* 2008).

Members of this genus possess antispasmodic, expectorant, carminative, diaphoretic, mild diuretic, emmenagogue, stimulant, vasodilator (Ghollassi Mood 2008, Yousefzadi *et al* 2011a), antimicrobial and antifungal (Shahidi *et al* 2002, Kumar *et al* 2006, Yousefzadi *et al* 2011a) and hepatoprotector (Govind 2011) properties and are intensively used as a green vegetable or as a folk medicine for treatment of many diseases (Ibadullayeva *et al* 2011). According to the common folk believes of Armenian and Azeri people, *D. glabrum* can cure many anomalies especially different kinds of cancer. It seems that extensive use of the plant for medicinal and domestic purposes is the major cause of dramatic reduction in the natural resources of *D. glabrum* (Ibadullayeva *et al* 2011, Gabrielian 1981).

In a preliminary work, the crude extract of the plant demonstrated antioxidant activity and anti-lipidemic effects (Dehghan *et al* 2009). To the best of our knowledge, there is no report on the chemical composition and pharmacological properties of *D. glabrum* and this paper is the first report on GC/MS analyses of the essential oils obtained from the roots of the plant.

Materials and methods

Plant material

Underground parts of *Dorema glabrum* Fisch. C.A. Mey were collected during the fruiting stage from rocky slopes of Aras River bank; Jolfa, Eastern Azerbaijan (38 30' 9.2", 45 27'36.2"; 1590 m, 15 km from Jolfa to St. Stephanus Church), Iran in July 2008. A voucher speci-

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men (TUM-FPh-541) has been deposited at the Herbarium of the Faculty of Pharmacy, Tabriz University of Medical Sciences.

Oil extraction

Air-dried and finely powdered roots were subjected to hydrodistillation for 3 h using a Clevenger type apparatus and the yielded oil was subsequently dried over anhydrous sodium sulphate.

GC-MS analysis

The GC-MS analysis was carried out on a Shimadzu GCMS-QP5050A gas chromatograph- mass spectrometer fitted with a fused silicon capillary DB-1 column (60 m x 0.25 mm i.d., 0.25 µm film thickness). Helium was used as the carrier gas at a flow rate of 1.3 mL/min). The oven temperature was kept at 50° C for 2 min and programmed to 260° C at a rate of 3° C/min. The injector temperature was 230 ºC and split ratio was adjusted at 1:63. The MS was taken at the following condition: ionization potential, 70 ev, ion source temperature 200 ºC; quadrupole 100 ºC; solvent delay 8 min; mass range, em voltage 3000 volts. Identification of compounds was based on direct comparison of the retention times and mass spectral data with those for standard compounds, and computer matching with the NIST 21, NIST 107 and WILEY229 library, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature (Adams 2004).

Free radical scavenging activity: the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The free radical scavenging effect of the essential oil was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Nazemiyeh *et al* 2008). DPPH was obtained from Fluka Chemie AG, Bucks and a solution of DPPH (0.08 mg/mL) in chloroform (CHCl3) was used. The essential oil was dissolved in CHCl3 to obtain the stock concentration of 1 mg/mL. Dilutions were made to obtain concentrations of 5×10-1, 2.5×10-1, 1.25×10-1, 6.25×10-2, 3.13×10-2 and 1.56×10-2 mg/mL. Diluted solutions (5 mL each) were mixed with DPPH solution (5 mL) and allowed to stand for 30 min for any reaction to occur. The UV absorbance was recorded at 517 nm. The experiment was done in triplicate and the mean absorption was measured for each concentration. The same manner was followed for the positive control, quercetin.

Results and discussion

Different Dorema species have been in use in orient medicine specially in Middle East countries as folk remedies in asthma, bronchitis, diabetes, infections or mummifying agent (Ghollassi Mood 2008, Yousefzadi *et al* 2011, Lev *et al* 2000, Lev *et al* 2002, Ram *et al* 2011). Existing documents show that Iranian people were familiar with applications of *Dorema ammoniacum* and collection of its resin-gum ammoniacum was started nearly 4,000 years ago in Persia (Duthie 1956).

Dorema glabrum is an endangered species (Ibadullayeva *et al* 2011, Gabrielian 1981) which has limited distribution and was formerly believed that it was restricted to Caucasia and Transcaucasia region. Recent researches carried out by our team and also other groups obviously show that this plant is growing wildly in different areas of Eastern Azerbaijan (Nazemiyeh *et al* 2008, Aras River bank, Jolfa, TUM-FPh-541; Talebpour *et al* 2010, Miyaneh, Zanjan Road, 40 21’ 37”, 38 48’ 47”) (unpublished data) and Western Azerbaijan (Mozaffarian 2007) provinces, Iran. In an old literature, the plant was reported from Afghanistan (Aitchison 1887). Considering its collection area, the issue could not be correct and it seems that the discussed plant should be another species, probably *Dorema hyrcanum* Kos (Syn.: *D. glabrum* sensu Aitch.) which grows in North East of Iran and Afghanistan (Mozaffarian 2007). Reviewing the published data shows secondary metabolites of *D. glabrum* was not investigated, yet and the present work is the first report on the chemical composition of the essential oils of *Dorema glabrum*. The air-dried roots of *Dorema glabrum* yielded 0.98% (w/w) colorless essential oils. The GC-MS analysis of the essential oils led to the identification of 34 terpenoidal compounds accounting for over 81.6% of the total oils. The majority of components present in the oils (Table 1) were sesquiterpens (non oxygenated 42.60%, oxygenated 14.18%) and monoterpenes (oxygenated 12.59%).

Among the monoterpenes, alpha- fenchyl acetate (6.32) and carvacrol methyl ether (3.13) were the major component while delta- Cadinene (12.78 %), beta-bisabolene (7.48 %), Copaene (5.68 %), Cubenol (5.42 %) and Calamenene (4.77 %) assumed as major sesquiterpens (Fig. 1).

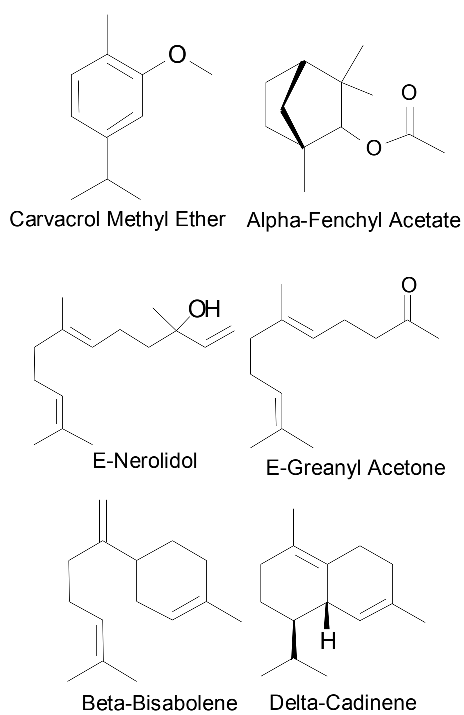
Previous studies on the other Iranian species, *Dorema ammoniacum* D. Don., revealed the presence of (*Z*)- and (*E*)-ocimenone, β-cyclocitral and *ar*-curcumene in fruits (Yousefzadi *et al* 2011a, Yousefzadi *et al* 2011b) and α-gurjunene (49.5 %), β-gurjunene (19.0 %) and α-selinene (4.6 %) in the leaves (Sajjadi *et al* 2007) as the main components. The findings of the present study are also different from those reported *for Dorema aucheri* Boiss which mainly comprised of α-Eudesmol (31.2%) and δ-cadinene (10.9%) (Masoudi *et al* 2006).

The essential oil had weak free radical scavenging properties with the RC50 value of 2.237 mg/mL which was because of the absence of free phenolic or other susceptible scavenging groups in the oil composition.

Table 1. Essential oil composition of *Dorema glabrum* roots

Compounds	RI*	Content (%)
Alpha.- Fenchyl acetate	1205	06.32
Carvacrol methyl ether	1226	03.13
Bornyl acetate	1270	02.11
Alpha- Cubebene	1355	00.77
Alpha- Copaene	1383	05.70
Trans Caryophyllene	1432	00.52
E- Geranyl acetone	1447	01.03
Alpha- Humulene	1460	00.51
Alloaromadendrene	1466	01.16
Bicyclosesquiphellandrene	1487	00.38
Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.alpha.,8a.alpha.)-	1496	01.29
Alpha.-Muurolene	1496	01.29
Beta.-Bisabolene	1503	07.48
Gamma.-Cadinene	1507	00.77
Calamenene	1517	04.77
Delta.-Cadinene	1520	12.77
Beta.-Cadinene	1526	01.03
Alpha.-Calacorene	1527	01.55
Trans-.psi.-Ionone	1550	02.19
Germacrene B	1552	01.03
Nerolidol E	1553	02.84
Tetradecanal	1592	00.90
Hexadecane	1600	00.65
Cubenol	1602	05.42
Alpha.-Cadinol	1610	00.65
Delta- Cadinol	1627	02.19
Cadalin	1630	00.90
Tetradecanoic acid	1740	00.39
Hexadecanal	1795	01.68
Heptadecanal	1898	04.26
Hexadecanoic acid	1940	00.65
Eicosane	2000	03.74
Docosane	2200	00.90
Tricosane	2300	00.52
Terpenes		
Monoterpenes: oxygenated		12.69
Sesquiterpenes: non oxygenated		42.60
Sesquiterpenes: oxygenated		14.18
Others		12.14
Total identified		81.60

*Retention indices relative to C_6 –C_{24} *n*-alkanes on DB-1 column



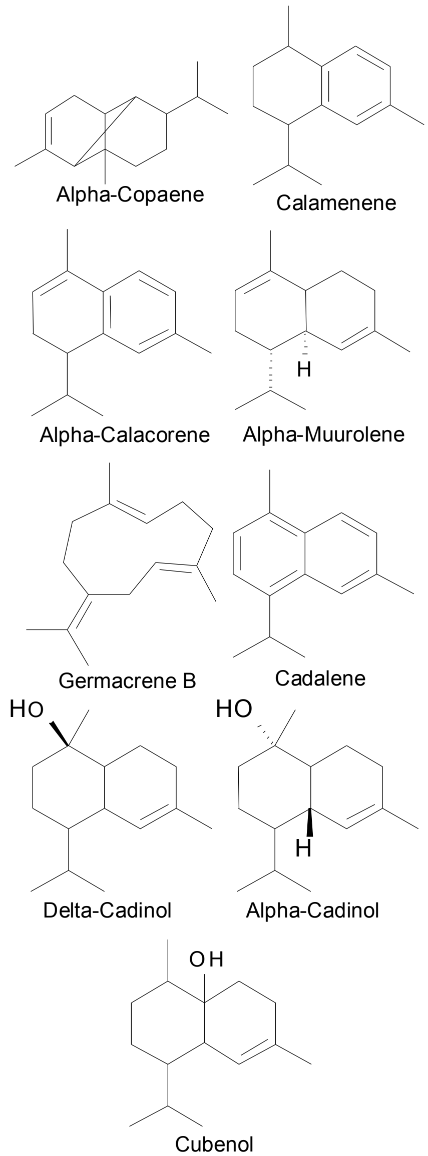


Fig. 1. The major monoterpenes and sesquiterpenes present in the essential oil of *D. glabrum* roots.

Conclusion

D. glabrum is a perennial medicinal plant growing commonly in Armenia, Nakhichevan and Iran. The plant is currently used as a remedy for treating cancerous diseases in folk medicine and also as a green vegetable in domestic use. Unfortunately there is no literature and research works on *D. glabrum* and we believe that extensive works are needed to reveal its medicinal values and protection as well.

Ethical issues

Not applicable in this research.

Conflict of interests

Authors declared no conflicts of interests.

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