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CHEMICAL COMPOSITION OF ESSENTIAL OILS OF BERRIES OF *JUNIPERUS MACROCARPA* SİBTH. & SM. FROM TURKEY

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Abstract

The chemical compositions of the hydrodistilled essential oils of ripen and unripen berries of *Juniperus macrocarpa* Sibth. & Sm. (Jom) were analysed by GC-MS. Thirty eight compounds were identified from unripe berries, accounting for approximately 0.03% oil yield (v/w dried weight) and 16 compounds from ripe berries with 0.003% oil yield (v/w dried weight). While the major compounds in the essential oils of unripe berries were α-cedrol (30.04%), α-pinene (18.81%), germacrene-D (14.58%), δ-cadinene (3.80%), γ-cadinene (2.29%), α-humulene (2.29%), γ-muurolene (2.27%), epi-bicyclosesquiphellandrene (1.69%), trans-caryophyllene (1.68%), valencen (1.53 %) and α-cedrol (49.87%), β- caryophyllene (29.57%), α-cedrene (4.75%), γ-muurolene (2.54%), carvacrol methyl ether (1.4%), trans-pinocarveol (1.2%), α-pinene (1.13%), α-muurolene (1.04%) were identified from ripe berries and their mean percentage varied according to their phenological stage. Both qualitative and quantitative differences between ripe and unripe berries of the plant were observed.

Introduction

The genus *Juniperus* L. has a total of 52 species, with the majority in the temperate zone of the northern hemisphere of the world and 11 taxa including 8 species and three subspecies in Turkey. *Juniperus oxycedrus* L. (Cupressaceae) (prickly juniper, plum juniper, cade juniper, red-berry juniper, cada) is a shrub or small tree native across to the Mediterranean region from Morocco and Portugal east to western Caucasus, growing on a variety of rocky sites from sea level up to 1600 m altitude (Orhan *et al.* 2011). Three subspecies: *oxycedrus*, *macrocarpa* (Sm.) Ball and *badia* (H. Gay) Debeaux of *J. oxycedrus* are mentioned in the Flora Europea; in the Flora of Turkey two subspecies, *oxycedrus* (Joo) and *macrocarpa* (Jom) were found (Amaral-Franco 1993, Farjon 2000). After the revision studies made in Turkey, the subspecies known as *macrocarpa* has been changed to species epithet (Kandemir 2018). *Juniperus* false fruits, female cones - improperly called "berries" - are used as spice, mainly in European cuisine; they are used in Northern European and particularly Scandinavian cuisine to impart a sharp, clear flavour to meat dishes (Loizzo *et al.* 2007). Taviano *et al.* (2013) indicated the phenolic profile and some biological properties of the ripe "berries" methanol extracts of Joo and Jom from Turkey as three-fold higher in Jom (17.89 ± 0.23 mg GAE/g extract) than in Joo (5.14 ± 0.06 mg GAE/g extract). In folk medicine *J. oxycedrus* berries have widely been used in the treatment of gastrointestinal disorders, common colds, as expectorant in cough, to treat calcinosis in joints and as diuretic to pass kidney stone, against urinary inflammations, hemorrhoids, and as hypoglycemic; leaves and berries are applied externally for parasitic disease (Sezik *et al.* 1997, Loizzo *et al.* 2007, Akkol *et al.* 2009). Leaves, resin, bark and berry extracts of *J. oxycedrus* were found to inhibit the growth of numerous microorganisms (Karaman *et al.* 2003). At the same time, *J. oxycedrus* is commonly used for the preparation of traditional medicinal brandy in Dalmatia (Öztürk *et al.* 2011). In

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Turkey, Joo berries are consumed for treatment of diabetes; while powdered berries and leaves are used internally as tea (Orhan *et al.* 2012).

Although Jom has been reported to have a variety of uses in studies conducted in different countries, the essential oil composition of fruits harvested from Turkey is not found in any study. The essential oil composition of leaf samples harvested from different months of plants collected from the Ciftlik Village in Cesme - Izmir, Turkey, which is very close to the area fruit samples, were collected and it was revealed in a study conducted by Sezik *et al.* (2005). Because the berries of Jom are eaten in Turkey (Öztürk *et al.* 2011), and in folk medicine the ripe berries of this species were used as diuretic to pass kidney stones. In the present experiment the essential oils of the ripen and unripen berries of Jom growing in Turkey were focused and their chemical competitions were determined.

Materials and Methods

The ripe and unripe berries of Jom were collected in Izmir (Çeşme, around Ilıca-Altınyunus). The taxonomic identification of the plant materials was confirmed by Prof. Aykut Güvensen at the Department of Botany, Ege University, Turkey. Voucher specimens of Jom are deposited in the Herbarium (AEF 23799 and AEF 23855) of the Faculty of Science, Ege University.

The ripe berries without seeds were dried at room temperature (8 days, 200 g) and steam-distilled for 8 hrs using a circulatory Clevenger-type apparatus (Adams 1991). The essential oil was recovered with diethyl ether, dried over anhydrous sodium sulfate, concentrated under a gentle stream of nitrogen and stored under nitrogen at –20°C until analyzed (0.006% yield). Air-dried unripe berries (8 days, 100 g) were grounded and distilled for 8 hrs. The essential oil was recovered in the same manner as above (0.03% yield).

Qualitative data were determined by gas chromatography and mass spectrometry. Gas chromatography analyses were carried out on Hewlett Packard 6890 gas chromatograph coupled with HP 5973 MS dedector capillary column (HP 1909İZ-102) (25 m × 200/µm), film thickness 0.33/µm. Oven temperature program of 50/300°C at 10°C/min, carrier gas, helium, 2 ml/min, autoinjection (Agilent 6890) temperature: 150°C, FID detector temperature: 250°C, 'split mode' ratio of 1 : 30 with hexane with volüme/µl. The identification of components was done with the use of the volatile oil LIBR (TP) library coupled with retention indices of reference compounds (Adams 1995) and built-in Wiley 275 library search systems comparing with standard essential oils data in ARGEFAR essential oil library of Ege University. Quantitative data (percentage composition) were determined from the GC peak areas without correction factors.

Results and Discussion

Table 1 shows sum up the yields (v/w, dried weight) of essential oil and chemical content (% w/w) of the different samples of *Juniperus*. The yield obtained from the unripen berries of *J. macrocarpa* was 0.03% (100 g berry), while ripe berries exhibited a lower yield with 0.006% (200 g berry) by Clavenger method. In the results of present study, the content of monoterpenes was lower in the ripe berries (5.15%) than in unripe berries (20.9%) with higher (1.2%) oxygenated monoterpene content in ripe berries (1.11%). Generally, the maximum yields were obtained by distilling ripe berries of *J. phoenicea* ssp. *turbinata* and *J. communis* except for *J. oxycedrus* ssp. *oxycedrus* (Joo), whose maximum yield was obtained by distilling unripe berries. The content of monoterpenes was lower in the ripe and unripe berries (81.88 and 83.51%, respectively) than in the leaves (95.58%) and sesquiterpenes were higher in ripe and unripe berries, (14.79 and 13.89%, respectively) than in the leaves 1.00% (Angioni *et al.* 2003). Sesquiterpenes was lower (65.23%) content in unripe berries, while (90.66%) were present in ripe berries of Jom. Also in this study,

Table 1. Percentage composition of essential oil of dry ripen and unripen fruits of *J. macrocarpa*.

Compounds	RI_a		% content		Identification method
	Ripen	Unripen	Ripen	Unripen	
Monoterpenes					
α-pinen	1048	1017	1.13	18.81	GC-MS
Carvacrol methyl ether	1522		1.4		"
α-campholene aldehyde	1081	1592	0.96	0.22	"
α- phellandrene	1071		0.93		"
Camphene	1055	1049	0.43	0.09	"
(1R)-(-)-myrtenal	1178		0.30		"
3-carene		1034		1.01	"
β- pinen		1080		0.55	"
α-terpinolene		1525		0.17	"
Sabinene		1019		0.05	"
Oxygenated monoterpenes					
Trans-pinocarveol	1027	1554	1.2	0.54	GC-MS
3-thujen 2-ol		1538		0.23	GC-MS
Caryophyllene oxide		1573		0.34	GC-MS
Sesquiterpenes					
α- cedrol	1583	1563	49.87	30.04	GC-MS
β-caryophyllene	1505		29.57		"
α-cedrene	1087		4.75		"
γ- muurolene	1535		2.54		"
α- cubabene	1580	1082	0.98	0.17	"
α- muurolene	1025		1.04		"
D-germacrene	1510	1568	0.90	14.58	"
Cuparene	1543		0.58		"
Calacorene	1551		0.43		"
δ- Cadinene		1549		3.80	"
β- Cubenene		1062		0.66	"
Copaene		1567		0.54	"
(-)-Thujopsen		1583		0.49	"
β-silinene		1575		0.45	"
α-humulene		1548		2.29	"
Cadına-1,4 diene		1518		0.39	"
α-longipinene		1525		0.37	"
γ-muurolene		1568		2.27	"
epi-bicyclosesquiphellandrene		4520		1.69	"
trans-caryophyllene		1544		1.68	"
Valencen		1542		1.53	"
α-amorphene		1528		1.12	"
Bicyclo germacrene		1557		1.04	"
α-ylangene		1538		0.66	"
trans-α-bergamotene		1575		0.29	"
+calerene		1029		0.26	"
Salvial-4(14)-en-1-one		1837		0.22	"
α-copaene		1508		0.21	"
Limonene		1077		0.19	"
Germacrene		1518		0.12	"
β-phellandrene		1015		0.09	"
γ-elemene		1538		0.08	"

a Retention indices in elution order from DB-5 colum.

sesquiterpenes were higher in ripe and unripe berries (90.66 and 65.23%, respectively) than monoterpenes (5.15 and 20.9%, respectively). In all samples, α-cedrol (49.87%) was the main component in ripe berries, and 30.04% in unripe berries while α-pinene was the main component (85.95% in leaves, 70.64% in ripe berries and 62.26% in unripe berries) in Joo (Angioni *et al.* 2003). In the present results α-pinene, α-campholene aldehyde, trans-pinocarveol, camphene, α-cubabene, germacrene-d, α-cedrol and γ-muurolene were similar in all berries. α-cedrol was higher in the ripe berries than unripe ones (49.87 versus 30.04%). The content of α-pinen was higher in unripe berries than ripe berries (18.81% versus 1.13%) of *J. macrocarpa.* D-germacrene was higher in the unripen berries than ripen ones (14.58 versus 0.90%). Trans-pinocarveol was higher in ripe berries than unripe berries (1.2 versus 0,54%) of Jom. In the present study β-pinen, sabinene, 3-carene, limonene, β-phellandrene, α-terpinolene, α-copaene, trans-α-bergamotene, germacrene, trans-caryophyllene, (-)-thujopsen, +calerene, β-cubanene, 3-thujen 2-ol, α-humulene, α-amorphene, bicyclo germacrene, γ-elemene, δ-cadinene, γ-cadinene, cadina-1,4 diene, copaene, valencene, caryophyllene oxide, salvial-4(14)-en-1-one, α-ylangene, limonene, β-silinene, α-longipinene, epi-bicyclosesquiphellandrene, trans-caryophyllene, solanesol were only found in unripe berries of *J. macrocarpa*, while α-cedrene, carvacrol methyl ether, (1R)-(-)-myrtenal, α-phellandrene, α-muurolene, cuparene, calacorene, β-caryophyllene were found in ripe berries of *J. macrocarpa.*

The seasonal differences of oils of Jom leaves were 84.6%, in May, 95.2% in August, and 90.1% in October samples rich in manoyl oxide (7.7 - 21.9%), α-pinene (7.2 - 11.1%), α-cedrol (2.3 - 9.7%), widdrene (2.1 - 5.7%), α-muurolene (4.1 - 4.8%), trans-verbenol (1.7 - 4.3%), germacrene D (1.5 - 4.1%), δ-cadinene (3.2 - 3.8 %), α-campholene aldehyde (1.7 - 3.2%), trans-pinocarveol (1.5 - 3.0%), cubebol (1.4 - 2.4%), caryophyllene oxide (1.5 - 1.9%), δ-cadinene (1.0 - 1.8%), β-caryophyllene (0.7 - 1.8%), and epi-cubebol (1.0 - 1.4%). Main component of ripe and unripe berries was α-cedrol (49.87 and 30.04%, respectively), although it was present in August samples of leaves of the same species (Sezik *et al.* 2005). α-cedrol ratio was; in May (2.3%), in August (9.7%) and in October (3.4%) in the leaves of Jom likely as the berries of *J. macrocarpa.* Similarly in ripe berries α-pinene content was higher than unripe berries of Tunusian Jom while myricene, β-phellandrene, β-pinene, respectively in unripe berries (Medini *et al.* 2010).

According to the present results, unripe berries were rich in α-cedrol (30.04%), α-pinene (18.81%), germacrene-D (14.58%); α-cedrol (49.87%), β-caryophyllene (29.57%), α-cedrene (4.75%) were identified from ripe berries. It was concluded that the yield and the composition of the *J. oxycedrus* essential oils depend on the origin of the plant; e.g. while the leaves from Elaphonios (Greece) were rich in α-pinene (26.94%) and α-cedrole (13.88%) (Stassi *et al.* 1995), α-cedrol was shown only in berries of *J. macrocarpa.* β-Myrcene, α-pinene, and DL-limonene and germacrene D were indicated in berries of different countries (Guerra-Hernandez *et al.* 1987, Cavaleiro 2001, Koukos *et al.* 2002, Salido *et al.* 2002, Valentini *et al.* 2003, Asllani 2004, Hajdari *et al.* 2014). The variations were related to genetic and climatic factors, soil conditions, phase of growth (vegetative or flowering stage), and part of the plant (Nemeth 2005).

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