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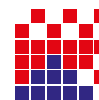
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## ORIGINAL SCIENTIFIC PAPER

# Interpopulations variability of *Teucrium arduini* L. (Lamiaceae) essential oil

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Analiziran je kemijski sastav eteričnih ulja šest populacija endemske biljke dubčac arduini (*Teucrium arduini* L.) koje rastu na području Hrvatske plinskom kromatografijom-spektrometrijom masa radi dodatnog istraživanja varijabilnosti unutar populacije. Identificirano je ukupno 43 komponenta a glavnu skupinu čine seskviterpenski ugljikovodici (66,00-89,69%). Germakren D je glavni sastojak ulja dobivenog destilacijom biljaka ubranih na lokaciji Učka (30,51%) i na Biokovu (lokacije Vošac - 34,47% i Sveti Jure - 49,14%) dok je  $\beta$ -kariofilen najprisutnija komponenta eteričnog ulja dobivenog destilacijom biljaka ubranih na Velebitu (Šušanj - 32,09% i Veliki Vaganac - 40,13%) i Sniježnici (36,81%). Od ostalih komponenta najzastupljeniji su bili kariofilen oksid (1,17-13,42%),  $\alpha$ -humulen (2,54-7,70%) i spatulenol (1,78-5,00%).

**Ključne riječi:** dubčac, eterično ulje, fitokemija, *Teucrium arduini*

**Summary**

Chemical composition of the essential oil (analysed by GC-MS) was conducted on six populations of endemic *Teucrium arduini* L. growing in Croatia to obtain additional knowledge on interpopulations variability of oil. Totally 43 components, in all, were identified and the main class were sesquiterpene hydrocarbons (66.00–89.69%). Germacren D was the main constituent in essential oil from Mt Učka (30.51%) and Mt Biokovo (Vošac– 34.47%, Sveti Jure – 49.14%), while  $\beta$ -caryophyllene was identified as a major compound in the oil from Mt Velebit (Šušanj– 32.09%, Veliki Vaganac – 40.13%) and Mt Sniježnica (36.81%). Among the other more represented components were caryophyllene oxide (1.17–13.42%),  $\alpha$ -humulene (2.54–7.70%), and spathulenol (1.78–5.00%).

**Keywords:** essential oil, phytochemistry, *Teucrium arduini*

**Introduction**

The genus *Teucrium* L. (family Lamiaceae) comprises about 200 herbs and shrubs distributed worldwide, especially in the Mediterranean region with about 140 species. A few species are found in South America, in the mountainous, tropical areas of northeast and southern Africa, and in Australia (Wielgorskaya, 1995). Forty nine *Teucrium* genus have been described in Europe (Tutin and Wood, 1972), and eleven in Croatia (Domac, 1994). *Teucrium arduini* L. (syn. *T. arduinoi* L.) is an endemic Illyric-Balcanic species with restricted range in the Western Balkans, distributed mainly on the mountains along the Adriatic Coast from the Istria Peninsula in Croatia in the north to northern Albania in the south. It is a semi-woody, branchy, erect or ascending dwarf shrub up to 60 cm high with whitish flowers which form simple, dense, up to 16 cm long inflorescences. *Teucrium arduini* grows on calcareous rocks, on rocky outcrops, and in ravines at altitudes between 0 and 1600 m (Lakušić et al., 2006; Tutin and Wood, 1972).

Many *Teucrium* species have been used for centuries in cooking as spices and in folk medicine as cholagoga, carminative, flavouring, stimulants, antipyretics, anthelmintic, antiseptic, diuretic, antispasmodic, antidiabetic, antirheumatic, and antiphlogistic agents (Gharaibeh et al., 1988, 1989; Sundaresan

et al., 2006). *Teucrium arduini* is used in folk medicine in Bosnia and Herzegovina in the form of an infusion for stomach ailments (Redžić, 2007) so it is not surprising either use as a spice. Recent investigations showed that *T. arduini* possesses considerable antimicrobial and antioxidant activity (Šamec et al., 2010; Vuković et al., 2010; Kremer et al., 2013). Moreover, chemical investigations of *T. arduini* revealed the presence of volatile oil (Bezić et al., 2011; Dunkić et al., 2011), flavonoids (Harborne et al., 1986; Valant-Vetschera, 2003), phenolic acids (Šamec et al., 2010; Kremer et al., 2013), tannins, phytosterols and bitter principles (Jurišić Grubešić et al., 2012), macroelements and micronutrients (Kremer et al., 2012).

The aim of the presented study is to obtain additional knowledge on the interpopulation variability of essential oil content of *T. arduini* growing in Croatia.

**Material and methods***Herbal material*

The above ground parts of several dozen randomly selected samples (200 g per sample) of wild growing plants *T. arduini* were collected during the blooming period in June and July of 2011 at six localities in Croatia (Table 1). Voucher

specimens were deposited in the Herbarium “Fran Kušan” of the Department of Pharmaceutical Botany with “Fran Kušan” Pharmaceutical Botanical Garden in the Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia. Plants were harvested from mature plants on a dry day, mixed to obtain randomly selected sample, and air-dried protected from direct

sunlight and single-layered for 15 days in a well-ventilated room at room temperature (22 °C) and 60% air humidity. Dried aerial parts (100 g) were subjected to hydro distillation for 3 h in Clevenger type apparatus, and obtained essential oil was dried over anhydrous sodium sulphate.

**Table 1.** Details on origin and collection data of investigated *Teucrium arduini* samples.

Locality	Voucher no.	Latitude	Longitude	Altitude a.s.l. (m)
Učka (Croatia)	HFK-HR-36-2011	45°17'32" N	14°12'28" E	1187
Šušanj (Mt Velebit, Croatia)	HFK-HR-12-2011	44°31'33" N	15°06'45" E	605
Veliki Vaganac (Mt Velebit, Croatia)	HFK-HR-14-2011	44°19'46" N	15°26'45" E	667
Vošac (Mt Biokovo, Croatia)	HFK-HR-21-2011	43°18'46" N	17°03'07" E	1297
Sv Jure (Mt Biokovo, Croatia)	HFK-HR-22-2011	43°19'08" N	17°03'15" E	1361
Mt Sniježnica (Croatia)	HFK-HR-33-2011	42°34'08" N	18°21'27" E	1148

#### Gas chromatography and mass spectrometry (GC, GC/MS)

GC/MS analyses were performed on Trace GC Ultra (Thermo Scientific, Palo Alto, USA) using DSQ II MSD. Capillary column ZB-5ms 30 m x 0.25 mm, film thickness 0.25 µm, was used (Phenomenex, Torrance, USA) and the temperature program was 60°C (1 min) rising to 250°C at rate of 4°C/min. Helium was used as carrier gas at a flow rate of 1 mL/min. The temperature of split/splitless injector was 260°C, split ratio was 1:50 and transfer line temperature was set to 270°C for GC/MS analyses. Samples were injected manually (0.1 µL). Mass spectra were recorded at 70eV (EI), the mass range was 45-350 m/z and Xcalibur version 2.0.7. was used for results processing and quantification. The identity of the components was assigned by obtained GC/MS spectra and retention indices (RI) relative to C<sub>8</sub>-C<sub>20</sub> n-alkanes. AMDIS program version 2.62 was used for GC/MS data processing using NIST library version 2.0 (both National Institute of Standards and Technology, U.S. Department of Commerce, Gaithersburg, MD, USA). Spectra and obtained retention indices were compared with literature (Adams, 2001 and in-house library).

## Results and discussion

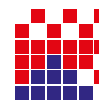
The results of phytochemical composition and percentage of each component of different *Teucrium arduini* essential oil

are presented in Table 2 and the percentage of total identified compounds and major groups are presented in Table 3. Based on dry mass of samples, the total yield of essential oil ranged from traces (Mt Sniježnica) to 0.11% (Sveti Jure). Totally, 43 compounds were identified in all six investigated essential oil samples, represented from 98.45% to 99.23% of the total oil. Sesquiterpene hydrocarbons (66.00–89.69%) were the main class of components of all investigated *T. arduini* populations, while sesquiterpene oxides (6.59–25.74%) were the second class.

Germacren D was the main constituent in essential oil from Mt Učka, Mt Biokovo Vošac, and Mt Biokovo Sveti Jure with 30.51, 34.47 and 49.14% of oil, respectively. According to Kremer et al. (2012, 2013) germacren D was one of the most dominant components in *T. arduini* collected on Mt Biokovo with contents of 16.4 and 18.7%, respectively. Variation in the essential oil content of *T. arduini* from Mt Biokovo could be attributed to different ecological conditions between micro locations. Additionally, germacrene D was the major component in the oil of *T. arduini* from Montenegro and *T. chamaedrys* from Turkey (Vuković et al., 2010; Bageci et al., 2011). It was also identified in oil of *T. flavum* L. (2.8%), *T. montbretii* Benth. ssp. *heliotropiifolium* (Barbey) P. H. Davis (2.4%) and *T. polium* L. (3.1%) (Menichini et al., 2009), but not in oil of *T. brevifolium* Schreb.

**Table 2.** Phytochemical composition, and identification (%) *Teucrium arduini* essential oil.

Component	Apex RT	RI	Učka	Šušanj	Veliki Vaganac	Vošac	Sveti Jure	Sniježnica
<b>Yield (%)</b>			0.03	0.02	0.08	0.10	0.11	Tr
1-Octen-3-ol	7.83	980	0.77	0.48	0.31	0.82	0.30	1.08
cis-β-Ocimen	9.47	1039	–	0.27	0.32	–	0.12	0.79
Linalool	11.66	1100	1.53	2.16	1.74	1.72	1.95	2.42
1-Octen-3-ol acetate	11.78	1104	–	0.60	0.77	–	0.35	–



3-Octanol acetate	12.16	1116	–	–	0.26	–	–	–
$\alpha$ -Terpineol	15.17	1200	–	–	0.26	–	–	–
Hexyl isovalerate	16.20	1234	–	–	–	–	0.17	–
trans- Linalyl oxide acetate (pyraniod)	17.80	1280	–	0.64	1.06	–	–	1.28
Unknown monoterpene oxide	18.40	1297	0.22	0.24	0.30	–	–	0.62
$\delta$ -Elemene	19.49	1335	–	–	–	–	0.16	–
$\alpha$ -Copaene	21.02	1377	–	–	–	–	0.12	–
$\beta$ -Bourbonene	21.31	1386	2.20	1.66	1.06	3.91	2.41	2.67
$\beta$ -Elemene	21.40	1388	0.30	–	0.20	0.44	0.09	–
$\alpha$ -Gurjunene	22.03	1407	–	–	–	0.21	0.19	–
$\beta$ -Caryophyllene	22.52	1421	29.90	32.09	40.13	26.40	23.30	36.81
$\beta$ -Copaene	22.83	1431	0.47	0.46	0.31	0.65	0.48	0.67
Aromadendrene	23.27	1443	–	–	–	0.25	0.13	–
$\beta$ -Farnesene	23.36	1450	0.39	0.87	0.51	0.24	–	0.59
$\alpha$ -Humulene	23.68	1457	5.48	6.41	7.70	4.76	2.54	7.34
allo-Aromadendrene	23.81	1462	2.17	2.37	1.70	2.54	2.36	2.21
$\gamma$ -Gurjunene	24.09	1472	–	–	–	0.25	0.18	–
$\gamma$ -Muurolole	24.23	1475	0.42	0.53	0.38	0.42	0.30	0.47
Germacrene D	24.51	1485	30.51	23.96	20.31	34.47	49.14	14.00
$\beta$ -Selinene	24.73	1491	0.35	–	0.20	0.43	0.18	–
Bicyclogermacrene	24.93	1497	2.10	1.41	1.13	3.25	6.92	–
$\gamma$ -Cadinene	25.45	1518	0.36	0.41	0.25	0.36	0.23	0.35
$\delta$ -Cadinene	25.54	1522	1.05	1.17	0.88	1.06	0.96	0.89
Salvial-4(14)-en-1-ol	26.70	1553	0.35	–	0.19	–	–	–
Unknown sesquiterpene oxide	26.93	1560	–	0.27	0.33	–	–	0.39
Unknown sesquiterpene oxide	27.24	1570	0.23	–	–	–	0.28	–
Germacrene D-4-ol	27.44	1577	–	–	–	–	0.21	–
Spathulenol	27.50	1578	4.76	5.00	3.79	4.35	1.78	4.19
Caryophyllene oxide	27.67	1583	5.32	7.25	7.50	4.72	1.17	13.42
Salvial-4(14)-en-1-on	28.00	1592	1.49	3.44	3.22	1.11	0.59	3.92
Globulol	28.34	1602	0.41	0.58	0.44	0.63	0.52	0.51
Humulene epoxide II	28.52	1609	0.57	1.02	0.81	0.59	0.19	1.48
Humulene-1,6-dien-3-ol	28.80	1619	–	0.39	0.34	–	–	–
Isospathulenol	29.10	1631	0.64	0.47	0.27	0.37	0.42	–
allo-Aromadendrene oxide (1) + $\tau$ -Cadinol	29.35	1638	3.56	2.03	0.95	2.14	0.30	1.36
$\tau$ -Muurolole	29.44	1641	0.73	0.31	0.32	0.56	0.20	–
$\alpha$ -Cadinol	29.79	1653	1.23	1.23	1.03	1.19	1.04	0.86
Unknown sesquiterpene oxide	30.34	1672	0.74	0.36	0.25	0.61	0.39	–
allo-Aromadendrene oxide (2)	30.65	1683	0.30	–	–	0.45	0.17	–
Unknown sesquiterpene oxide	30.90	1693	–	–	–	0.20	–	–
Hexadecanon	33.27	1780	–	–	–	–	–	0.36
Hexahydrofarnesyl acetone	34.44	1826	1.13	1.91	0.75	0.62	0.05	0.89
Unknown	34.75	1838	0.32	–	–	0.28	0.11	–
Octadecanon	38.32	1980	–	–	–	–	–	0.42

Note: Apex RT - Retention time of compound at peak apex; RI - Retention indices on ZB-5ms column

**Table 3.** Percentage of total identified chemical compounds and major groups (%) of *Teucrium arduini* essential oil.

Component	Locality					
	Učka	Šušanj	Veliki Vaganac	Vošac	Sveti Jure	Sniježnica
<b>Total identified (%)</b>	98.49	99.14	99.11	98.91	99.23	98.99
Monoterpene hydrocarbons (%)	-	0.27	0.32	-	0.12	0.79
Oxygenated monoterpenes (%)	1.53	2.79	3.07	1.72	1.95	3.70
Sesquiterpene hydrocarbons (%)	75.70	71.35	74.75	79.62	89.69	66.00
Sesquiterpene oxides (%)	19.36	21.73	18.87	16.12	6.59	25.74
Other compounds (%)	1.91	2.99	2.10	1.45	0.87	2.75

On the other hand,  $\beta$ -caryophyllene was identified as a major compound in the oil from Šušanj (32.09%), Sniježnica (36.81%), and Veliki Vaganac (40.13%). In our earlier investigations  $\beta$ -caryophyllene was the main component in *T. arduini* oil from Mt Biokovo with content of 32.9% and 35.2% (Kremer et al., 2012, 2013).  $\beta$ -caryophyllene was also identified as a major compound in the oil of *Teucrium chamaedrys* ssp. *lydium* (19.7%), *T. orientale* L. var. *puberulens* (21.7%) (Küçük et al., 2006), *T. scordium* L. (22.8%) (Morteza-Semnani et al., 2007), *T. royleanum* Wall. ex Benth. (23.6%), and *T. quadrifarium* Buch.-Ham. ex D. Don (38.3%) (Mohan et al., 2010).

The third significant component was caryophyllene oxide with contents from 1.17% (Sveti Jure) to 13.42% (Sniježnica). According to Bezić et al. (2011) caryophyllene oxide was identified as a major component (14.6%) in *T. arduini* essential oil from Mt Biokovo. Caryophyllene oxide was also identified as the main compound in the essential oil of *Teucrium stocksianum* Boiss. (5.7%) and *T. montbretii* ssp. *heliotropiifolium* (8.8% and 12.7%, respectively) (Abdulkhader et al., 2006; Menichini et al., 2009). On the other hand, caryophyllene oxide was present only in small quantity (3.20%) in *T. marum* L. ssp. *marum* from Sardinia (Ricci et al., 2005).

Among the other components it is worth to mention  $\alpha$ -humulene (2.54–7.70%) and spathulenol (1.78–5.00%). Both components were also identified by Kremer et al. (2012, 2013) in *T. arduini* from Mt Biokovo.  $\alpha$ -humulene was present in *Teucrium flavum* (6.0%), *T. montbretii* ssp. *heliotropiifolium* (2.4%) and *T. polium* (3.8%) from Greece (Menichini et al., 2009). On the other hand, spathulenol was a major compound (9.0%) in *Teucrium brevifolium* from Greece (Menichini et al., 2009).

## Conclusions

Totally, 43 components were identified in essential oil of *Teucrium arduini* growing in Croatia. The main compounds were germacren D (14.00–49.14%),  $\beta$ -caryophyllene (23.30–40.13%), and caryophyllene oxide (1.17–13.42%). Germacren D was the main constituent in essential oil from Mt Učka and Mt Velebit, while  $\beta$ -caryophyllene was the most dominant in oil from Mt Velebit and Mt Sniježnica. Sesquiterpene hydrocarbons (66.00–89.69%) were the main class of components of all investigated *T. arduini* populations, while sesquiterpene ox-

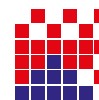
ides (6.59–25.74%) were the second class. This study confirms the differences in the composition of essential oils caused by microclimate conditions during the growth of the plant.

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