Screening of yarrow (*Achillea millefolium* Agg.) populations in Serbia for yield components and essential oil composition

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Yarrow (*Achillea millefolium* Agg.) is well-known medicinal plant, with a wide spectrum of applications and it is one of the most frequently used plant drug in Serbia. In this study, we have observed cultivation and essential oil chemical properties of 28 *A. millefolium* populations collected from Serbian sites. In the second vegetation, the yield of useful part (upper 15 cm) ranged from 925 – 3630 kg/ha, while the yield of essential oil ranged from 0.40 – 0.82%. The most dominant compounds in monoterpene fraction were β-pinene (max. 36.3%), sabinene (max. 35.7%), 1,8-cineol (max. 26.6%) and borneol (max. 20.2%), while in the sesquiterpene fraction the most abundant compounds were *trans*-caryophyllene (max. 18.6%) and lavandulyl acetate (max. 18.1%). Among aromatic compounds, the most abundant was chamazulene (max. 29.1%). This screening has shown that only 10 populations out of 28 satisfied official quality requirement of 0.02% of chamazulene in the dried drug. Four populations had higher yield than commercial variety ProA, while one of them had even higher level of chamazulene.

Key words: *Achillea millefolium, essential oil, chemistry, GC, GC-MS, yield*

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1. INTRODUCTION

Genus *Achillea* includes about 120 perennial species, which are mostly present in Euro-Asian region. Plants from this genus are, in wide diversity, spread in Serbian territory, where 19 species have been recognized (Gajić, 1975). Regarding pharmacological properties, the most important species belong to the *Millefolium* group, which is characterized by wide morphological, cytological and chemical diversity, and it has been botanically systemized in few subspecies whose chromosome numbers are ranging from diploid (2n=18) to octaploid (8n = 72) forms (Nemeth, 2005). Therefore *A. millefolium* L. *sensu lato* is the developing evolutionary taxon currently considered as an aggregate of 12 species (Saukel and Langer, 1992). Since this group consists of several species difficult to distinguish which again are polymorphic, the raw material from wild collecting often represents a mixture of several species. According to European Pharmacopoeia (Ph.Eur.8.0., 2013), the main quality parameter is the essential oil content (minimum 2 ml/kg), with not less than 0.02% of proazulenes, expressed as chamazulene. Diploid species (*A. asplenifolia* Vent. and *A. setacea* Waldst. et Kit.) and tetraploid species (*A. collina* Becker ex Reichenb.) are considered pharmaceutically acceptable, while hexaploid species (*A. millefolium* L. *sensu stricto* and *A. distans* Waldst. et Kit. and octaploid species (*A. pannonica* Scheele.) are considered as chamazulene-free and therefore should be avoided (Chandler et al., 1982).

Yarrow (*Achillea millefolium* Agg.) is well-known medicinal plant, with a wide spectrum of applications. It is one of the most frequently used plant drug in Serbia (Tucakov, 1984). Upper parts of the plant collected during the blooming are recognized as an anti-inflammatory, antinociceptive, spasmolytic, antimicrobial and holagog drug (Nadim et al., 2011; Cavalcanti et al., 2006; Benedek et al., 2008; Ali et al., 2017). Yarrow preparations in the form of infusions, decoctions or fresh juices have been applied against various indications such as anorexia, stomach cramps, flatulence, gastritis, enteritis, internal and external bleeding (Willuhn, 2002; Wichtl, 2004).

In this study, we have observed cultivation and chemical properties of 28 *A. millefolium* populations collected from Serbian sites. The main objective of this research was to evaluate local yarrow populations for yield components and chemical constituents of the essential oil regardless their explicit botanical taxonomy. Furthermore, we tried to explore what would be the yield and quality of only upper 15 cm of plant considering that this part represents "flowering tops" as it is suggested by official quality standard (Ph.Eur.8.0., 2013).

Table 1. The origin of yarrow (*A. millefolium* Agg.) populations

Population	Locality	Latitude	Longitude	Altitude [m]
1	Sokobanja - Ozren	43° 38′ 13.12" N	21° 52′ 27.02" E	472
3	Sokobanja - Ripaljka	43° 38′ 01.69" N	21° 52′ 51.20" E	573
4	Sokobanja - Očno	43° 37′ 52.92" N	21° 52′ 22.19" E	634
5	Sokobanja - Moravica	43° 38′ 38.80" N	21° 53′ 01.55" E	330
8	Sokobanja - Bovan lake	43° 39′ 36.44" N	21° 48′ 16.44" E	266
11	Aleksinac	43° 33′ 08.53" N	21° 42′ 17.98" E	218
12	Aleksinac - Žitkovac	43° 31′ 09.14" N	21° 41′ 28.71" E	161
13	Ražanj - Deligrad	43° 39′ 47.48" N	21° 32′ 58.65" E	250
14	Busilovac	43° 47′ 57.09" N	21° 26′ 01.60" E	150
15	Ćuprija	43° 57′ 04.93" N	21° 22′ 13.22" E	116
16	Vojska	44° 03′ 48.88" N	21° 12′ 09.15" E	119
18	Velika Plana	44° 22′ 55.25" N	21° 04′ 12.76" E	102
19	Begaljica	44° 38′ 11.94" N	20° 38′ 14.82" E	255
20	Padej	45° 49′ 00.15" N	20° 09′ 51.96" E	72
23	Ravna Gora - monument	44° 06′ 31.15" N	20° 09 ′21.19" E	731
24	Topli Do – Mijina pojata	43° 20′ 25.55" N	22° 42′ 31.44" E	833
25	Dobro polje	43° 53′ 32.00″ N	19° 29′ 55.00" E	1140
26	Divčibare - viewpoint	44° 05′ 48.18" N	19° 58′ 12.64" E	1012
27	Maljen	44° 06′ 59.45" N	20° 03′ 45.63" E	898
28	Rajac (top)	44° 08′ 17.65" N	20° 13′ 18.06" E	840
29	Medvednik	44° 12′ 44.00" N	19° 40′ 50.00" E	834
30	Rajac - Dobre Vode	44° 08′ 10.00" N	20° 11′ 10.00" E	620
31	Suvobor - Vučje trkalište	44° 07′ 11.38" N	20° 11′ 38.38" E	710
32	Divčibare - Kraljev sto	44° 07′ 20.00" N	20° 01′ 45.00" E	1030
35	Ovča - Rugby field	44° 53′ 00.39" N	20° 28′ 50.90" E	69
36	Hohenheim 1[a]			
38	Hohenheim 2[a]			
39	ProA[b]			

[a]Botanical garden seed exchange, Hohenheimer Gärten
[b]Commercial variety, Pharmaplant GmbH, Germany

2. MATERIALS AND METHODS

1. Plant material

1.1. Population origin

Seeds of 35 local yarrow populations were collected during the August 2004 (1-35). Each collecting site was at least 5 km distant (air distance) from the nearest one. Additionally, seeds of three populations (36-38) were purchased from seed exchange programme (Hohenheimer Gärten) and one commercial variety (ProA, Pharmaplant) has been included (39). All collected populations were sown in the glass-house for seedlings production, but only 28 among them had seeds viable enough to form a sufficient number of seedlings to be included in field cultivation experiment. Details of included populations are listed in Table 1.

1.2. Field trial

Field trial has been conducted on April 20th, 2005 at Pančevo (South Banat, Serbia). Soil type was Calcic Gleysol (pH 6.8) at altitude 81 m. Average rainfall for 2005 and 2006 was 560 mm and 620 mm, respectively, while min-max vegetation (Mar-Sep.) temperatures were 7-33°C and 5-32°C, respectively. The experimental design was completely randomized design where each population has been represented with 40 plants separated in four repetitions. Planting scheme was 70×50 cm representing approximate plant density of 20000 plants/ha. All plants were in the rosette phenophase during the first year of the trial. Samples for yield quality estimation has been taken from flowering plants during June and July 2006. Plants were harvested to the ground level for the purpose of yield components estimation. Harvested plants have been tied in bundles and dried in shade. Dried plants were measured for total yield (whole plant), useful part yield (upper 15 cm of the plant). Useful part of the plant has been separated with manual steel sheet cutter. Count of flowering stems and plant height were also recorded on dried plants, while width of plant habitus was measured *in situ*. Useful part of plants has been considered as main cultivation property of populations. Since yarrow plants are very polymorphic, each measurement has been done in ten replications.
Collected samples from field trial were determined and classified taxonomically as *A. millefolium* Agg. by Prof. Dr. Zora Dajić Stevanović. Voucher specimens have been deposited at the Herbarium of Institute for medicinal plants research „Dr. Josif Pančić“, Belgrade, Serbia.

2. Gas chromatography and Mass spectroscopy

2.1. Essential oil extraction

The air-dried yarrow plants (useful parts) were grounded, and the volatile oils were obtained by hydrodistillation using a Clevenger-type apparatus according to Procedure I of the Yugoslav Pharmacopoeia IV (Ph.Jug.IV, 1951). The essential oil yield, expressed as a percentage, was calculated on a moisture-free basis. Samples (20 µL) were dissolved in EtOH 96% (2 mL) and kept in the refrigerator until further analysis. A single plant from population 31 was morphologically very distinct from rest of the population. Thus, that plant was analyzed separately and marked as 31a.

2.2. GC-FID analysis

The GC-FID analyses were carried out with HP-5890 Series II apparatus (Hewlett-Packard, Waldbronn, Germany) equipped with a split-splitless injector, a flame-ionization detector (FID), and HP-5 capillary column (25 m×0.32 mm i.d., film thickness 0.52 µm). The oven temperature was programmed rising from 40 to 260°C at 4°C/min; injector temperature 250°C; detector temperature 300°C; carrier gas, H_2 (1.0 mL/min). Samples were injected in the amount of 1 µL. Split ratio was 1:5. The relative contents expressed as percentages were obtained from electronic integration of the peak areas measured using FID.

2.3. GC/MS analysis

The GC/MS analyses were performed under almost the same analytical conditions as the GC-FID analyses, with HP G 1800C Series II GCD analytical system (Hewlett-Packard, Palo Alto, CA, USA) equipped with HP-5MS capillary column (30 m×0.25 mm i.d., film thickness 0.25 µm). Helium (1.0 mL/min) was used as carrier gas, and the transfer-line temperature (MSD) was heated to 260°C. Mass spectra were acquired in the EI mode (70 eV) over the *m/z* range 40–450 amu. Samples (1 µL) were injected in split mode (1:30). The identification of

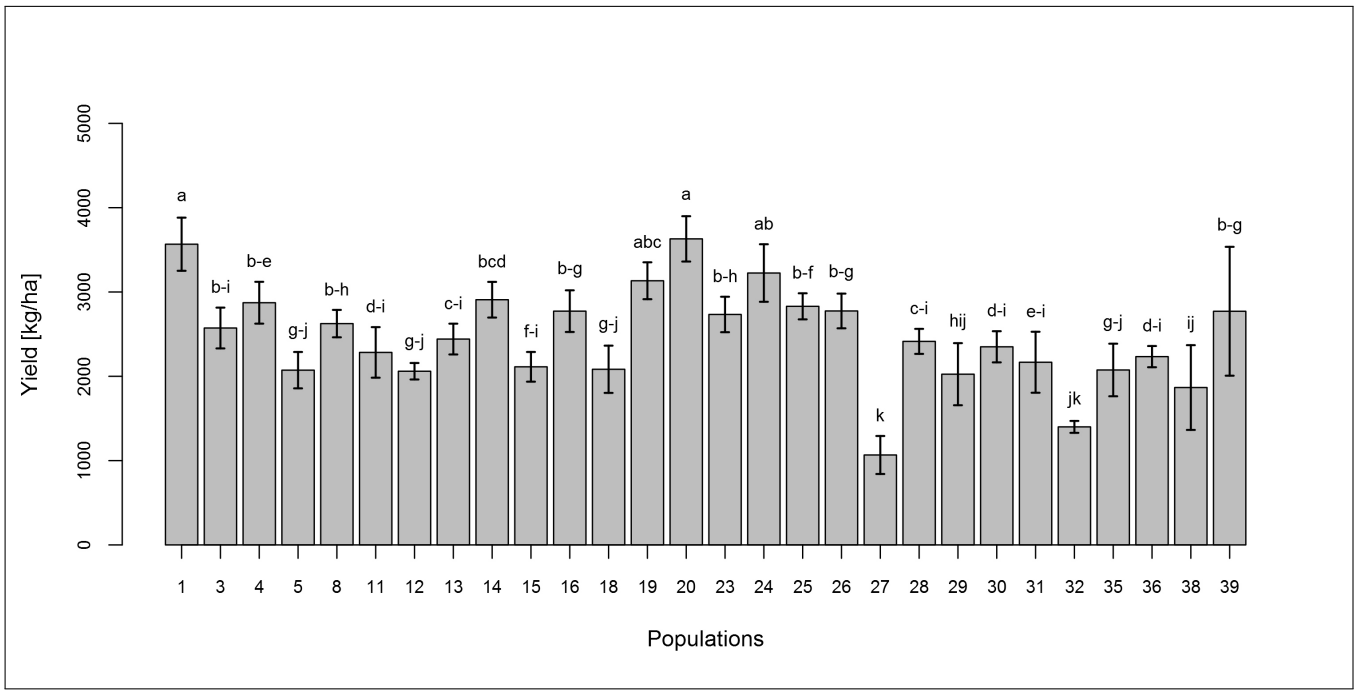

Fig. 1. Yields of cultivated yarrow populations (upper 15 cm of the plant).

the constituents was performed by comparing their mass spectra and retention indices (RIs) with those obtained from authentic samples and/or listed in the NIST/Wiley mass-spectra libraries, using different types of search (PBM/NIST/AMDIS) and available literature data (Hochmuth, 2006; Adams, 2007).

3. Statistical analysis

Since measurements of a large number of replications in agronomical assay produced data with very wide variance in populations, estimation of differences between groups was inaccessible. Therefore for each population quantile reduction of data has been applied, where only second and third quartiles have been taken for further statistical analysis. The hypothesis that all populations yield useful parts equally has been evaluated with one-way ANOVA and differences among mean values has been tested with *post-hoc* Duncan's multiple range test. Production differences among populations have been presented graphically (bar plot with standard deviations). The strength of yield components relationships has been estimated through Pearson's correlation coefficients. Essential oil compositions of observed populations have been presented in the table, while their similarity has been accessed through cluster analysis with Euclidian single linkage distance and presented graphically as an unrooted dendrogram. All statistical computing and graphs production was made by R software packages (The R Project for Statistical Computing).

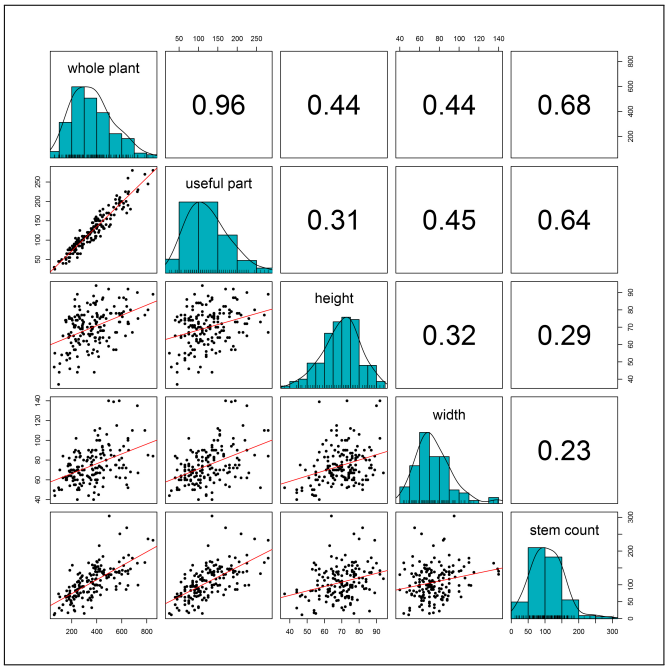

Fig. 2. Correlation coefficients among yield components of *A. millefolium* Agg. populations.

3. RESULTS AND DISCUSSION

1. Yield components

In the second vegetation, yarrow populations' yield ranged from 925 – 3630 kg/ha (Figure 1). Our findings differ from previously published papers most probably because we have observed yields of only upper 15 cm of the plants. Giorgi et al. (2005) reported yield in range of 5-12 t/ha, while Dachler and Pelzmann (1999) reported ca. 4.5 t/ha. Since these papers lack in cutting height information, we believe that discrepancies in reported yields and the yield range of our research is result of our intention to estimate dry mass of the "flowering tops". Population 20 had the highest yield, while population 27 had the smallest, 3630 kg/ha and 925 kg/ha, respectively. Most of the populations, accurately 25 of them, yielded more than 2000 kg/ha. Populations 1, 19, 20 and 25 showed the highest yield potential and no statistically significant differences among their mean values has been observed. The smallest yield potential has been recorded in populations 27 and 32. Yield components have been observed through correlation matrix (Figure 2). Whole plant yield ranged from 20 – 300 g/plant and showed the strongest correlation to useful part yield (r = 0.96). This was expected since robustness of the whole plant was inherited by the useful part of the plant trait. In other words useful part of the plant was only the part of the whole plant with exactly the same number of stems, which is strongly correlated with both traits (r = 0.64 and r = 0.68, respectively). On the other hand, plant height and width traits

showed weaker correlation to the useful part of the plant (r = 0.31 and r = 0.45, respectively). This is observations clearly showed that only number of stems, among morphological traits, contributes to useful part yield.

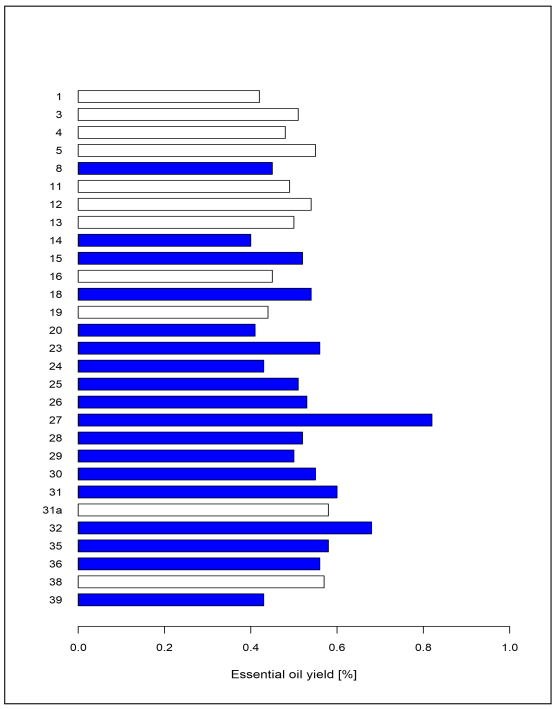

Fig. 3. Essential oil yield of cultivated yarrow populations (upper 15 cm of the plant); white bars represent chamazulene-free populations.

2. Essential oil yield

The yield of essential oil of the cultivated yarrow populations in our experiment ranged from 0.40 – 0.82% (Figure 3). Chamazulene-free populations have been represented in the figure with white bars. Total count of chamazulene-free populations was 11 and at this level we can distinguish which population is not suitable for pharmaceutical purposes according to official standards (Ph.Eur.8.0., 2013). The highest oil yield was observed in population 27 (0.82%), while the lowest yield was recorded in population 14 (0.40%). Majority of oil yield (59% of populations) have been observed in amounts more than 0.5%. These data are accordance than previously reported, where yarrow oil yield ranged from 0.11 to 1.03% (Verma et al., 2017; Spinarova and Petrikova, 2003; Raal et al., 2012; Ghasemi Pirbalouti, 2017). Dachler and Pelzmann (1999) reported yarrow yields up to 0.5%. According to mean values of reported yields, oil content in our samples was above average. Again, this is most probably the result of plant cutting height in our experiment.

3. Essential oil composition

Chromatogram integration of yarrow essential oils recognized 113 components out of which we were able to identify 96 (Table 2). One of these identifications was tentative (RI = 1679.4). In all samples number of total compounds varied from 45 to 74. Compositions of essential oils were very diverse, but in general the most dominant compounds in monoterpene fraction were β-pinene (max. 36.3%) and sabinene (max. 35.7%), followed by 1,8-cineol (max. 26.6%), borneol (max. 20.2%), trans-β-ocimene (max. 16.1%), camphor (max. 11.3%), *cis*-chrysanthemol (max. 11.3%) and *trans*-verbenol (max. 10.1%). In the sesquiterpene fraction the most abundant compounds were , *trans*-caryophyllene (max. 18.6%) and lavandulyl acetate (max. 18.1%), followed by elemol (max. 15.5%), α-bisabolol (max. 14.9%), terpinen-4-ol (max. 12.9%). Among aromatic compounds, the most abundant was chamazulene (max. 29.1%).

Our results are in accordance with previously reported chemical compositions of yarrow essential oils. Shawl et al. (2002) reported camphor (28%), 1,8-cineole (12%), germacrene-D (12%) and cischrysanthenyl acetate (8%) as the major components of essential oils of *A. millefolium* from Iran. Furthermore, Mockute and Judzentiene (2003) partially confirmed our findings with reporting chamazulene (max. 23.2%), β-pinene (max. 26.5%), borneol (max. 13.2%) and camphor (max. 13.1%), but also with addition of *trans*-nerolidol (max. 13.5%), which has been found in our samples in very low amount (0.1-0.9%). Camphor was identified as main component in amount of about 40% in *A. sieheana* and *A. clavennae*, while borneol was most abundant compound in *A. holosericea* (Tabanca et al., 2004; Stojanović et al., 2005). The high amount of 1,8-cineole (34.2%) has been reported *A. eriophora* (Weyerstahl et al., 1997). The high amount of sabinene, as we found in our populations 36, 38 and 39 (19.3-35.7%), has been also previously reported(Verma et al., 2017; Nadim et al., 2011; Conti et al., 2010; Boskovic et al., 2005).

Serbian populations were lower in sabinene content, while introduced populations (Hohenheimer Gärten and commercial variety ProA) had very high content. One morphologically different plant from population 31, which was separately analyzed, gave completely different chemical profile than the population from which it has been drawn (sample 31a). Furthermore, this plant was chamazulene-free, while chamazulene has been identified in a population sample. Regarding chamazulene content, among all analyzed populations only 10 of them satisfied official quality requirement of 0.02% of chamazulene in dried drug (Ph.Eur.8.0., 2013). These populations were 20, 23, 24, 26, 27, 29, 31, 32, 36 and 39. The level of chamazulene in these populations ranged from 0.02% (pop. 31) to 0.24% (pop. 27).

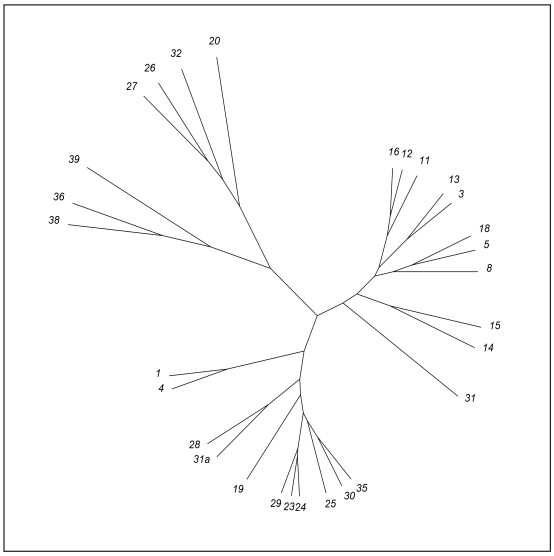

Fig. 4. Cluster analysis based on chemical composition of yarrow essential oils.

Table 2. Essential oil profiles of *Achillea millefolium* populations

			Populations																												
			1	3	4	5	8	11	12	13	14	15	16	18	19	20	23	24	25	26	27	28	29	30	31	31a	32	35	36	38	39
	Constituents[a]	RI[b]	% m/m	% m/m	% m/m	% m/m	% m/m	% m/m	% m/m	% m/m	% m/m	% m/m	% m/m	% m/m	% m/m	% m/m	% m/m	% m/m	% m/m	% m/m	% m/m	% m/m	% m/m	% m/m	% m/m	% m/m	% m/m	% m/m	% m/m	% m/m	% m/m
1	1-nonene	889.4	-	0.11	-	0.29	-	-	-	-	-	-	0.23	-	0.10	-	-	-	-	-	-	0.07	-	-	-	-	-	-	0.14	-
2	santolinatriene	904.3	-	0.74	-	0.60	0.26	1.60	-	1.88	1.14	1.17	0.49	-	-	0.15	0.10	0.22	-	-	-	0.06	0.05	0.28	0.06	-	0.09	0.53	-	-	0.41
3	tricyclene	915.3	0.08	0.15	0.08	-	0.13	0.83	0.52	0.08	0.57	0.16	1.09	0.12	0.28	0.13	0.12	-	-	-	-	0.23	0.13	0.19	0.13	0.19	-	0.28	0.46	0.84	-
4	α-thujene	921.2	0.21	0.06	0.20	0.79	-	0.12	0.05	0.16	0.07	0.17	-	-	-	-	-	0.16	0.20	-	0.22	-	-	-	-	-	0.13	-	-	-	0.41
5	α-pinene	926.8	4.84	1.24	5.48	10.64	4.03	1.64	1.31	2.17	1.76	1.41	4.67	0.84	3.21	0.30	6.22	5.55	8.46	0.95	1.71	12.36	6.62	6.70	7.18	3.01	6.37	8.36	1.95	2.24	0.92
6	camphene	940.9	1.16	0.48	1.23	0.42	0.32	0.32	0.29	0.13	0.45	0.61	0.28	0.38	0.52	-	0.07	0.17	0.11	-	-	0.09	0.76	0.78	0.08	0.12	0.20	0.52	0.91	1.33	0.09
7	verbenene	947.2	-	-	-	0.20	-	-	-	0.10	-	-	0.07	-	-	-	-	-	0.09	-	-	0.10	-	0.12	-	-	-	-	-	-	-
8	sabinene	967.0	1.10	0.93	1.89	6.08	2.57	7.96	12.17	1.97	16.86	5.56	10.17	6.12	6.88	6.26	4.61	4.45	3.65	0.63	8.89	5.68	4.88	3.04	1.23	2.90	3.09	1.98	26.09	35.67	19.28
9	β-pinene	969.9	21.29	13.17	15.39	8.07	12.57	3.57	2.87	16.34	5.10	5.64	0.84	3.93	19.57	0.91	23.59	26.68	36.28	10.95	22.21	18.74	24.77	28.57	10.12	28.74	10.24	28.21	2.57	1.72	4.07
10	β-myrcene	987.4	0.21	0.25	0.13	0.16	0.37	0.37	0.24	0.08	0.44	0.18	-	0.28	0.51	0.18	0.53	-	-	0.10	-	0.43	0.42	0.50	0.54	-	-	0.83	2.87	0.15	-
11	dehydrocineole	987.4	-	-	0.11	0.17	-	-	-	-	-	-	0.52	-	0.18	-	-	0.45	0.38	-	0.64	-	-	0.09	0.21	-	0.43	-	-	0.10	0.48
12	α-phellandrene	999.5	0.13	0.17	0.16	-	0.07	0.41	0.11	0.31	0.59	0.35	0.18	-	-	0.50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.75
13	α-terpinene	1011.3	0.17	0.22	0.35	0.86	0.20	0.76	0.52	0.11	1.21	0.25	1.67	0.33	0.33	0.16	0.14	0.12	0.24	0.09	0.40	0.20	0.35	1.04	0.43	0.13	0.20	0.29	0.98	0.97	0.70
14	*p*-cymene	1019.3	0.15	0.21	0.20	0.88	0.46	0.48	0.36	0.17	0.45	0.09	2.59	1.16	0.15	-	0.13	0.24	0.24	-	-	0.26	0.20	0.53	0.09	0.67	0.31	0.12	0.26	1.09	0.22
15	β-phellandrene	1022.9	0.51	0.20	0.27	0.28	0.54	0.31	0.20	-	0.71	0.52	-	0.38	1.07	0.10	0.66	1.04	-	-	0.63	0.34	0.99	0.75	0.64	-	0.28	0.98	0.43	0.35	0.82
16	1,8-cineol	1026.0	7.25	11.06	14.32	12.91	14.51	10.44	17.32	7.84	10.07	2.06	14.34	13.60	26.60	1.68	16.90	17.07	18.35	4.48	1.93	10.79	10.50	11.71	1.96	10.30	18.13	11.67	8.59	3.42	3.03
17	*cis*-β-ocimene	1034.1	0.12	0.09	0.25	0.18	0.17	0.47	0.27	0.58	0.14	-	0.13	0.20	-	-	0.07	0.40	-	-	0.40	0.11	0.19	0.26	0.65	0.21	0.27	0.47	0.20	0.27	0.07
18	*trans*-β-ocimene	1044.2	0.29	0.42	0.53	1.85	0.31	1.46	0.96	1.38	1.77	0.46	3.08	1.50	0.60	0.34	0.43	0.33	0.67	0.09	0.64	0.44	0.36	0.63	0.83	1.12	0.47	0.56	1.64	1.80	16.05
19	γ-terpinene	1053.3	0.26	0.52	0.29	0.54	0.31	0.61	0.92	0.63	0.34	0.18	0.83	0.36	0.20	0.20	0.33	0.30	0.34	0.22	0.15	0.27	0.27	0.20	-	0.20	0.24	0.19	0.38	0.79	0.61
20	n.i.	1059.4	-	-	-	-	-	0.11	-	0.14	0.25	0.14	0.09	-	-	4.56	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.06
21	*cis*-sabinene hydrate	1064.0	0.12	0.13	0.20	0.42	0.13	0.28	0.26	0.06	0.45	0.11	0.73	0.19	0.17	0.10	0.10	0.09	0.13	-	0.17	0.11	0.10	0.32	0.21	0.11	0.12	0.18	0.39	0.41	0.28
22	α-terpinolene	1082.7	0.23	0.37	0.47	0.43	0.55	0.49	0.86	0.22	0.52	0.41	0.95	0.61	0.33	0.33	0.33	0.31	0.30	0.22	0.17	0.34	0.27	0.47	0.19	0.50	0.69	0.32	0.43	0.73	0.44
23	n.i.	1101.5	-	-	0.15	-	-	-	-	0.05	-	0.12	-	-	-	-	-	-	-	-	-	-	-	0.81	-	-	-	-	-	-	-
24	n.i.	1109.1	-	-	-	-	-	-	-	-	0.17	-	0.07	-	-	0.23	-	-	-	-	-	-	-	0.11	-	-	-	-	1.02	-	-
25	β-thujone	1112.3	-	0.08	0.11	0.18	-	0.32	0.12	-	0.25	0.06	0.66	0.10	0.09	-	-	-	-	-	-	-	-	0.06	-	-	-	0.06	0.20	0.29	0.11
26	α-campholenal	1121.7	0.09	0.05	0.09	0.36	0.08	-	-	-	0.10	-	0.23	0.08	0.06	-	0.11	-	0.07	0.07	-	0.16	0.06	0.07	-	-	-	-	0.09	-	-
27	1,3,8-*p*-menthatriene	1129.6	-	-	-	-	-	-	-	-	-	-	0.16	-	-	-	-	0.10	-	-	-	-	-	-	-	-	-	-	-	0.10	-
28	*trans*-pinocarveol	1133.5	0.83	0.95	0.56	1.30	0.66	0.39	-	0.51	0.45	0.42	1.19	0.33	1.57	0.11	0.76	1.90	0.42	0.60	0.24	0.43	0.26	0.53	0.11	0.26	0.19	0.67	0.22	0.37	0.25
29	camphor	1138.6	3.71	0.49	0.88	3.04	3.78	1.11	1.11	1.83	7.06	9.41	1.14	1.37	2.62	0.25	0.42	0.97	1.14	0.28	0.11	1.29	3.85	11.32	0.23	0.09	0.90	1.84	8.87	4.84	0.31
30	*trans*-verbenol	1141.6	0.34	4.34	-	2.94	1.15	10.09	3.76	4.82	3.59	4.33	0.40	2.36	-	0.57	0.28	2.57	0.29	0.86	-	0.31	0.30	0.49	0.19	0.51	0.81	1.00	-	-	0.49
31	sabina ketone	1154.1	-	-	-	-	-	-	-	-	-	-	1.20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.36	1.09
32	pinocarvone	1157.4	0.75	0.83	1.25	1.03	0.07	0.80	-	0.12	2.23	5.92	0.85	-	-	-	-	-	0.10	-	-	3.27	0.15	0.49	-	-	0.13	-	0.09	-	-
33	*cis*-chrysanthemol	1159.6	-	-	-	-	1.12	1.07	-	0.52	-	-	0.76	0.69	-	11.34	2.23	-	1.40	4.88	0.48	2.70	0.48	-	0.75	6.10	-	0.68	-	-	0.35
34	borneol	1162.8	19.54	6.70	20.23	6.59	0.91	-	1.33	0.64	1.55	3.07	1.66	13.50	9.05	-	-	2.82	-	-	0.14	-	1.71	5.11	-	-	0.77	2.21	3.79	10.40	7.84
35	terpinen-4-ol	1173.5	0.81	1.12	1.23	3.54	0.54	3.01	2.55	0.47	12.89	1.07	6.55	1.91	1.18	0.59	0.61	0.45	0.89	0.39	1.28	0.78	0.55	1.20	0.80	0.73	0.75	0.90	3.05	7.24	0.67
36	β-pinene epoxide (isomer)	1176.9	0.13	-	-	1.07	-	1.44	0.20	-	0.19	3.96	0.52	-	-	-	-	-	-	-	-	-	1.70	0.14	-	-	-	-	-	0.19	-
37	α-terpineol	1186.8	2.83	1.74	2.25	1.80	2.03	2.45	3.79	1.50	2.96	1.33	1.67	3.25	5.10	0.57	2.93	2.77	3.51	2.81	0.52	3.05	2.29	2.92	5.19	1.90	3.16	4.07	1.53	0.72	-
38	myrtenol	1192.2	0.82	0.62	0.56	1.31	0.72	0.58	-	0.64	0.41	0.73	0.71	0.25	0.77	-	0.42	0.58	0.52	0.82	0.17	0.56	0.41	0.65	0.45	0.66	0.23	0.45	0.23	-	-
39	*trans*-carveol	1206.9	3.25	1.80	1.05	1.15	0.07	0.66	-	-	-	1.29	0.09	0.28	-	-	-	-	-	0.40	-	0.15	0.59	0.92	-	-	-	-	-	-	-
40	*cis*-carveol	1216.4	0.55	-	0.21	0.41	-	-	-	-	-	-	0.12	0.18	-	-	-	-	-	-	-	0.09	-	0.26	-	-	-	-	0.19	0.24	-
41	neral	1226.8	0.10	0.16	0.09	0.20	2.70	2.94	0.98	-	0.39	0.84	0.08	5.43	-	-	-	-	-	0.16	-	0.08	0.78	0.29	0.10	0.60	-	1.07	-	0.09	-
42	*trans*-chrysanthenyl acetate	1231.4	-	-	-	0.09	-	-	-	-	2.99	-	-	-	-	0.76	-	-	-	-	-	-	-	-	-	-	-	-	4.08	-	-
43	cuminaldehyde	1235.6	-	-	-	0.11	-	-	-	-	-	-	0.43	0.06	-	-	-	-	-	-	-	-	-	0.18	-	-	-	-	-	0.10	-
44	*cis*-chrysanthenyl acetate	1257.4	0.32	-	0.81	0.14	-	-	-	0.06	0.07	-	-	0.09	-	-	-	-	-	-	-	0.96	0.17	0.14	0.06	-	-	-	0.24	0.18	-
45	*trans*-sabinyl acetate	1271.3	-	-	0.09	-	-	-	0.53	0.22	-	-	-	0.23	-	-	-	-	0.34	-	-	1.08	-	-	-	-	0.17	-	-	-	-
46	bornyl acetate	1282.2	0.07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	terpinen-4-ol acetate	1286.4	-	0.76	0.39	0.19	0.93	1.64	-	1.70	3.78	2.02	0.13	0.11	-	0.09	0.11	0.45	-	0.12	-	0.08	0.09	0.12	0.24	-	-	0.52	-	-	-
48	lavandulyl acetate	1287.1	-	0.16	0.11	0.10	-	0.25	-	0.31	3.40	11.00	0.32	0.76	-	18.14	-	-	-	8.17	1.59	-	-	-	-	-	-	0.06	-	-	0.24
49	n.i.	1288.0	0.18	-	-	0.39	-	-	-	-	-	-	0.13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.12	1.02	-
50	thymol	1293.4	-	0.06	-	-	-	0.15	-	-	-	-	0.39	0.27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
51	*trans*-verbenyl acetate	1304.4	0.86	-	0.53	-	-	-	-	-	-	-	-	0.14	-	-	-	-	-	-	-	-	0.15	0.14	0.14	-	-	-	-	-	-
52	n.i.	1311.2	-	-	-	0.09	-	0.04	-	-	-	-	0.59	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
53	*trans*-carvyl acetate	1333.9	0.41	-	0.36	0.05	-	-	-	-	-	-	-	0.33	-	-	-	-	-	-	-	-	-	0.23	-	-	-	-	0.08	0.14	-
54	n.i.	1342.7	-	-	-	-	-	-	-	-	0.74	-	-	-	-	-	-	-	-	0.43	-	-	-	-	-	-	-	-	-	-	-
55	*cis*-carvyl acetate	1359.3	0.19	-	0.41	0.23	2.13	1.18	1.33	0.10	-	0.97	0.93	0.85	0.28	0.11	0.06	-	-	-	-	0.26	0.22	0.23	0.19	-	0.24	1.18	0.10	0.08	-
56	neryl acetate	1361.3	-	0.21	-	-	0.09	-	0.20	-	-	-	0.15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
57	α-copaene	1369.6	-	0.11	-	0.07	0.09	-	-	0.05	-	0.07	0.12	0.11	0.14	-	-	-	-	0.06	-	-	0.08	-	0.08	-	-	0.07	-	-	-

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			Populations																													
			1	3	4	5	8	11	12	13	14	15	16	18	19	20	23	24	25	26	27	28	29	30	31	31a	32	35	36	38	39
	Constituents[a]	**RI[b]**	**% m/m**	**% m/m**	**% m/m**	**% m/m**	**% m/m**	**% m/m**	**% m/m**	**% m/m**	**% m/m**	**% m/m**	**% m/m**	**% m/m**	**% m/m**	**% m/m**	**% m/m**	**% m/m**	**% m/m**	**% m/m**	**% m/m**	**% m/m**	**% m/m**	**% m/m**	**% m/m**	**% m/m**	**% m/m**	**% m/m**	**% m/m**	**% m/m**	**% m/m**	**% m/m**	**% m/m**
58	*β*-bourbonene	1378.2	0.32	0.50	0.15	0.14	0.35	0.18	0.11	0.13	0.38	0.12	-	0.62	-	0.09	0.36	0.32	0.16	0.68	0.24	0.32	0.48	0.24	0.61	0.21	0.83	0.26	0.19	-	0.13
59	*β*-elemene	1386.2	-	-	0.05	0.21	0.25	-	-	0.41	0.07	0.17	-	0.07	0.15	1.95	-	0.11	-	0.17	-	-	-	-	0.09	-	-	0.62	-	0.07	-
60	*cis*-jasmone	1396.2	-	-	-	-	-	-	-	-	-	-	0.11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.21	0.29
61	*trans*-caryophyllene	1412.3	2.76	2.51	2.51	1.87	8.30	3.97	2.53	3.39	3.25	5.34	0.75	3.25	7.39	3.16	8.33	9.44	5.86	15.80	13.91	15.95	5.70	3.13	12.13	18.65	5.10	7.92	2.51	1.19	8.40
62	*β*-copaene	1422.2	0.11	0.24	-	-	0.10	0.05	-	0.06	0.07	0.07	-	0.10	-	0.05	0.07	-	-	0.14	0.14	0.06	0.09	0.06	0.13	-	0.12	-	-	-	-
63	isogermacrene D	1437.7	0.13	0.28	0.07	0.11	0.16	0.26	0.28	-	0.05	0.08	-	0.15	-	0.18	0.11	-	-	0.09	0.08	-	0.09	0.08	0.17	-	0.11	0.16	-	0.09	-
64	*α*-humulene	1446.9	0.25	0.37	0.33	0.24	0.88	0.49	0.34	0.44	0.42	0.61	0.10	0.40	0.75	0.51	1.05	1.26	0.79	1.97	1.56	1.78	0.74	0.42	1.72	1.84	0.68	1.21	0.40	0.37	0.91
65	*allo*-aromadendrene	1454.1	-	0.09	-	-	-	-	-	-	-	-	-	0.05	-	-	-	-	0.21	0.11	-	-	0.12	-	0.09	0.27	0.12	-	0.08	0.11	-
66	*γ*-muurolene	1470.5	1.09	1.18	0.29	0.42	0.27	0.32	0.45	0.68	0.26	0.35	0.07	0.83	0.18	1.54	0.16	-	0.14	0.14	0.41	0.09	1.48	0.75	0.30	0.08	0.19	0.67	0.13	0.72	0.12
67	germacrene D	1474.6	2.65	4.72	1.23	1.26	5.59	1.55	1.94	1.84	2.05	4.04	0.73	3.65	0.64	3.78	6.93	1.78	1.06	5.22	4.04	2.27	3.75	1.84	9.76	1.74	4.60	2.93	1.66	0.76	1.87
68	*ar*-curcumene	1477.3	0.44	0.31	-	0.17	-	0.38	-	0.53	-	-	-	0.16	0.20	0.64	0.33	-	0.15	-	1.98	0.26	0.27	0.19	-	-	-	0.77	0.40	0.25	0.78
69	*α*-zingiberene	1490.1	0.34	1.88	0.05	0.31	0.30	0.46	0.14	0.14	0.22	1.05	0.06	0.18	0.19	1.66	0.50	0.23	0.15	1.17	0.25	0.15	0.33	0.09	1.03	0.33	0.30	1.05	0.50	0.11	0.17
70	*α*-muurolene	1494.0	0.20	-	-	0.11	-	0.11	-	0.11	0.10	0.22	0.07	-	-	-	-	-	-	-	-	-	-	-	0.06	-	-	-	0.06	-	-
71	sesquicineol	1508.2	-	-	-	-	-	-	-	-	-	0.14	-	1.77	-	-	0.47	-	-	0.25	-	-	-	-	-	-	0.17	0.11	-	-	-
72	*cis*-calamenene	1514.6	-	-	-	-	-	-	-	-	-	-	-	-	0.27	-	0.14	-	-	-	-	0.22	-	-	-	0.09	-	-	-	0.11	-
73	*δ*-cadinene	1517.1	0.66	0.65	0.24	0.54	0.26	0.40	0.34	1.62	0.23	0.43	0.22	0.44	0.20	0.31	0.17	0.28	0.15	0.22	0.56	-	0.47	0.14	0.76	0.07	0.25	0.36	0.50	0.23	0.23
74	*β*-cadinene	1531.4	-	0.11	-	-	0.29	-	-	-	-	0.17	-	0.12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.15	-
75	n.i.	1536.5	-	-	0.14	-	4.99	0.47	0.48	-	-	-	0.27	-	-	-	-	-	-	-	-	-	0.12	-	-	-	-	-	-	-	-
76	elemol	1544.3	0.62	8.85	8.44	3.92	-	7.64	15.49	14.66	-	1.12	12.49	4.81	0.13	-	-	-	-	0.08	-	-	1.12	1.05	-	-	-	0.12	-	0.13	0.15
77	salviadienol	1548.2	0.18	0.40	0.09	0.61	0.28	0.94	-	-	0.23	0.45	0.27	0.32	-	0.84	0.08	-	0.11	0.08	-	0.49	0.20	0.13	0.48	-	0.24	0.18	0.80	2.47	0.08
78	n.i.	1550.4	-	-	-	-	-	-	-	-	-	-	-	0.22	-	-	-	-	-	0.09	0.07	-	-	-	-	0.16	-	-	-	-	-
79	longipin-3-ene-10-ol	1554.0	-	-	-	-	0.25	-	-	-	-	-	1.10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
80	*trans*-nerolidol	1559.5	0.17	-	-	0.81	-	-	0.23	-	-	0.24	-	0.96	-	0.55	-	-	0.25	-	-	-	-	-	0.09	-	-	-	0.35	0.22	-
81	palustrol	1560.4	0.19	2.96	-	-	-	-	-	-	-	-	-	-	-	-	0.14	0.13	-	0.28	-	0.12	0.27	-	-	0.19	-	0.19	-	-	-
82	spathulenol	1571.1	0.61	1.55	2.63	1.24	4.19	4.71	8.39	5.62	0.41	0.87	6.07	1.14	0.38	0.21	0.26	0.27	0.20	0.83	0.16	0.24	0.41	0.35	1.05	0.38	0.53	0.54	0.23	0.39	0.23
83	caryophyllene oxide	1576.1	3.18	3.07	2.04	4.03	5.27	3.00	2.00	6.35	2.07	4.81	1.91	3.09	1.64	1.20	2.38	3.00	1.54	7.66	2.57	3.49	1.98	1.53	3.06	3.40	1.88	2.51	1.28	1.04	1.77
84	globulol	1584.1	0.81	1.82	0.74	0.70	0.25	0.66	0.41	-	0.05	0.19	0.37	1.12	-	0.25	0.42	0.23	2.94	1.54	-	0.72	1.33	0.19	0.71	4.47	-	0.38	0.67	1.74	-
85	viridiflorol	1595.3	0.24	0.66	0.25	0.33	0.22	0.41	0.14	0.71	-	0.10	-	0.37	0.25	-	-	0.13	0.36	0.19	-	0.31	0.20	0.13	0.77	0.58	-	0.09	0.09	-	-
86	humulene epoxide II	1600.7	0.58	0.98	0.34	0.64	0.83	0.78	0.41	0.83	0.27	0.75	1.05	0.63	0.22	0.10	-	0.32	0.27	0.59	0.17	0.42	0.19	0.21	0.86	0.40	0.29	0.34	0.10	1.73	0.17
87	torilenol	1604.2	0.40	-	-	-	-	-	-	-	-	-	-	-	-	-	1.36	-	-	0.55	-	-	0.19	-	-	-	0.35	-	-	-	-
88	humulane-1,6-dien-3-ol	1611.8	-	0.60	0.34	0.27	0.55	0.93	1.23	-	0.10	-	1.45	0.36	0.09	0.06	-	-	0.12	-	0.14	0.12	0.16	0.09	0.60	0.20	-	0.10	0.11	4.56	-
89	*γ*-eudesmol	1624.3	0.27	3.22	1.49	1.87	-	1.27	1.38	5.93	-	0.28	3.67	2.85	0.11	-	1.11	0.76	-	0.67	0.12	-	0.52	0.61	0.41	-	0.41	-	-	2.40	0.47
90	*allo*-aromadendrene epoxide	1628.9	0.68	0.49	0.28	0.86	1.63	0.69	0.53	0.50	0.41	1.26	0.70	0.50	0.76	0.20	0.31	-	0.69	0.91	0.23	0.82	0.24	0.19	2.13	0.84	0.44	0.48	0.59	0.57	0.14
91	copaene-15-ol	1646.2	0.13	0.24	-	-	-	-	-	-	-	1.45	-	0.93	-	0.27	-	-	-	-	0.10	0.20	0.79	-	0.63	0.25	-	-	-	-	-
92	8-hydroxybicyclogermacrene	1650.7	0.83	3.75	2.87	2.23	0.62	2.10	2.31	5.78	-	0.97	2.44	1.79	-	8.19	1.16	0.80	0.87	1.06	0.35	0.84	0.87	0.83	1.64	1.13	1.12	0.65	0.70	0.39	0.57
93	*ar*-curcumen-15-al	1662.6	2.17	1.25	0.50	1.58	0.74	0.49	0.32	0.46	0.84	0.41	-	0.70	0.67	-	-	2.29	3.38	-	0.32	-	0.22	0.62	3.10	-	-	-	-	0.27	-
94	*α*-bisabolol	1677.1	0.50	0.44	0.15	0.33	-	0.49	0.59	0.30	-	-	-	-	1.93	-	0.29	-	-	-	-	3.32	0.90	0.59	14.92	-	9.00	2.42	-	0.12	-
95	amorpha-4,9-diene-2-ol*	1679.4	0.61	0.84	0.08	0.13	1.63	0.29	0.21	0.24	0.11	1.44	0.27	1.71	1.79	0.30	-	0.26	0.53	0.68	0.43	-	0.27	-	-	4.62	-	-	0.28	0.20	-
96	*α*-cuparenone	1684.6	3.12	1.18	0.52	2.20	1.83	1.69	1.49	0.61	1.43	2.45	0.21	0.40	-	0.17	-	0.30	-	0.29	-	-	1.17	0.99	0.61	-	0.42	-	-	0.15	-
97	n.i.	1687.2	-	1.78	0.70	-	1.13	0.65	0.39	0.48	-	1.92	1.12	2.17	0.27	0.35	0.74	0.23	-	0.47	-	0.41	-	-	1.56	0.51	0.60	1.24	0.30	-	-
98	n.i.	1692.4	-	-	-	-	-	1.12	1.73	-	-	-	-	-	-	-	-	-	-	0.12	-	-	0.15	-	0.29	-	0.22	-	0.23	0.15	-
99	*cis,trans*-farnesol	1698.1	0.20	0.20	0.10	-	0.82	0.61	-	-	0.06	0.11	-	-	0.14	0.11	0.24	0.28	0.28	0.38	0.22	0.33	0.14	0.12	1.36	0.26	0.36	0.11	-	0.16	0.11
100	n.i.	1714.5	0.24	0.11	-	-	-	0.38	0.12	-	-	0.09	-	-	-	0.16	-	-	-	-	-	-	-	-	0.87	-	-	-	-	-	-
101	italicen-4-one	1719.4	-	-	0.16	-	-	0.15	0.29	-	-	-	0.14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
102	chamazulene	1722.0	-	-	-	-	2.23	-	-	-	0.38	0.17	-	0.94	-	23.10	10.54	7.38	1.67	18.34	29.13	1.28	10.13	2.10	3.36	-	22.22	1.72	15.77	-	22.38
103	albicanol	1740.9	0.50	0.72	0.21	0.48	-	0.44	0.23	0.19	-	-	-	0.28	-	-	-	-	-	-	-	-	-	0.65	-	-	-	-	-	0.17	-
104	n.i.	1755.2	-	-	-	-	-	-	-	-	-	-	-	-	-	0.62	-	-	-	-	-	-	-	-	0.15	-	-	-	-	0.14	-
105	*α*-santalolacetate	1760.0	0.45	0.26	-	-	-	0.47	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.18	-	-	-	-
106	n.i.	1794.2	-	0.32	-	-	0.24	0.24	-	-	-	-	-	0.18	-	0.28	-	-	-	0.22	-	-	-	-	0.48	0.16	-	0.30	-	-	-
107	n.i.	1794.9	-	-	-	-	-	-	0.08	-	0.07	0.51	-	-	-	-	-	-	-	0.14	0.14	-	-	-	-	-	0.18	0.34	-	-	-
108	n.i.	1795.1	-	-	-	0.22	0.43	0.63	0.29	0.06	-	-	0.18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
109	brachilaenalone A	1796.8	0.31	0.06	-	-	0.13	0.20	-	-	0.40	1.48	-	-	-	-	-	-	-	-	-	-	-	-	0.26	0.08	-	1.65	-	-	-
110	n.i.	1801.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.13	-	-	-	-	0.17	-	-	0.06	-	-	-
111	n.i.	1922.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.38	-	0.11	-	-	-	-
112	*m*-camphorene	1944.3	-	0.68	0.09	-	0.40	-	-	0.10	-	0.06	-	0.06	0.10	-	0.07	-	-	0.77	-	-	0.14	0.11	0.14	-	-	0.25	-	0.26	-
113	*p*-camphorene	1977.1	0.15	-	-	-	-	-	-	-	-	-	-	-	-	0.48	-	-	-	0.19	-	-	-	-	-	-	0.34	-	-	-	-
	Sum of identified compounds		99.58	97.79	99.01	99.29	93.21	96.35	96.91	99.27	98.76	97.21	97.56	97.42	99.73	93.80	99.26	99.77	100.00	98.40	99.80	99.59	99.74	99.08	96.10	99.17	98.90	98.06	98.34	98.68	98.94
	Total number of compounds		71	73	68	72	65	73	59	62	62	70	71	74	49	56	52	46	47	59	45	57	69	68	67	46	55	61	55	66	46

[a] Asterisk sign (*) denote tentative identification; n.i.- not identified.

[b] RI, retention indices as determined on HP-5 column using homologous series of C_8-C_{30} alkanes.

Cluster analysis of yarrow essential oil chemical profiles in our experiment revealed branching showed on unrooted dendrogram in Figure 4. Population grouping based on single linkage Euclidian distances has distinguished sub-branch with populations rich in chamazulene (18.3-29.1% of essential oil or over 0.09% in the dry drug). Those populations are 20, 26, 27 and 32, and all of them could be included in the future selection process. On the same branch, sabinene rich populations (pop. 38, 36 and 39) formed sub-branch. Commercial variety ProA (pop. 39) has been grouped on the same branch with chamazulene rich populations, but thanks to its high level of sabinene (19.3%) it has been grouped in sub-branch with sabinene rich populations.

CONCLUSION

Screening of yarrow populations in Serbia revealed valuable information regarding yield of raw material and quality of the essential oil. The yield has been observed in the range of 925 – 3630 kg/ha, while essential oil content ranged from 0.40 – 0.82%. The most dominant compounds in monoterpene fraction were β-pinene (max. 36.3%), sabinene (max. 35.7%), 1,8-cineol (max. 26.6%) and borneol (max. 20.2%), while in the sesquiterpene fraction the most abundant compounds were trans-caryophyllene (max. 18.6%) and lavandulyl acetate (max. 18.1%). Among aromatic compounds, the most abundant was chamazulene (max. 29.1%). Moreover, this screening has shown that only 10 populations out of 28 satisfied official quality requirement of 0.02% of chamazulene in the dried drug. Four populations (1, 19, 20, 24) had higher yield than commercial variety ProA (39), while one of them (20) had even higher level of chamazulene (23.1%). These results favors cultivation of species with known chemical profile versus wildcrafting of morphologically very similar yarrow plants which do not satisfy quality requirements.

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