

Composition of Essential Oil of Costmary [*Balsamita major* (L.) Desf.] at Different Growth Phases

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The essential oils from leaves and flowers of costmary, *Balsamita major* (L.) Desf. (syn. *Chrysanthemum balsamita* L.), were analyzed at various phases of plant growth. The highest contents of oil both in leaves and in flowers were determined before full blooming, 1.15 and 1.34% (w/w), respectively. Seventy-eight volatile compounds have been identified in the oils of *Balsamita major*, of which 58 (19 tentatively, 39 positively) have not been reported in this plant previously. Carvone and α -thujone were found to be dominating compounds constituting from 51.8 to 68.0% and from 9.0 to 16.1% in the total oil, respectively. Seasonal variations in the oil compositions were not considerable except for the starting phase (May 25, 1995), when the content of carvone was lower and the content of α -thujone and sesquiterpenes higher. The content of sesquiterpenes was ~2 times higher in flowers than in the leaves. Absolute amount of most components was highest at the bud formation period.

Keywords: *Balsamita major* (L.) Desf.; *Chrysanthemum balsamita* L.; Asteraceae; costmary; essential oil; harvesting time; carvone

INTRODUCTION

Costmary, *Balsamita major* (L.) Desf. (syn. *Chrysanthemum balsamita* L.) Asteraceae, is a large perennial plant of Asian origin with yellow flowers grown in Europe and Asia from the Middle Ages. Fresh and dried leaves of costmary possess a strong mint-like aroma and an astringent taste. On a domestic scale they have been used to flavor different dishes including fatty minced meat, game, poultry, lamb, fish, curd, potato, soups, sauces, beverages, and cakes. The essential oil of costmary together with some other volatile products was successfully used in the creation of flavoring compositions for confectionery (Dapkevičius et al., 1992). However, to our knowledge, the commercial cultivation, processing, and application of costmary are not widely developed. Publications on the essential oil composition of this plant are rather scarce.

Göckeritz (1968) defined two chemotypes of *B. major* according to the dominant terpene in the essential oil: camphor-type, *Balsamita major* (L.) Desf. subsp. *majus* (Desf.) Asch.; and carvone-type, *Balsamita major* (L.) Desf. var. *tanacetoides* (Boiss.) Fiori. In their literature survey on costmary Bestmann et al. (1984) suggest a third chemotype, with camphor and thujone as prevailing components in the essential oil. Göckeritz (1968) and

Zielinska-Sowicka and Wolbis (1970) have studied the essential oils of camphor chemotype *B. major*. They found that the content of oil varied from 0.31 to 1.25% in leaves and that camphor acts as a major component constituting 72–91% of the total oil content.

Several authors studied the essential oil composition of carvone chemotype *B. major*. Voigt et al. (1938) determined the essential oil content in this costmary chemotype, which varied from 0.38 to 0.64% in dry matter. Juknevičienė et al. (1973) investigated separate anatomical parts of *B. major* at different plant growth phases and found that essential oil content varied from 0.06 to 2.20 mL 100 g⁻¹ of dry matter. Three years later Paris et al. (1976) determined 1.3% of essential oil in the flowers of *B. major* and 0.7% in its aerial parts. In both studies carvone was shown to be a dominating compound because it constituted 65–80% of the total oil depending on the growth stage of the plant.

Bestmann et al. (1984) and Lukic et al. (1965) studied the insecticidal activity of costmary essential oil. They identified and quantified >20 constituents in the essential oil of *Chrysanthemum balsamita* and found each of them possessing insecticidal properties against aphids.

Other published studies were focused on particular components of *B. major*; for example, five compounds were identified in the *o*-dihydroxyphenolic fraction of *Chrysanthemum balsamita* L. var. *tanacetoides*, which included caffeic, chlorogenic, and ferulic acids (Tāmaš et al., 1989). Todorova and Ognyanov (1989) isolated seven C-9 β -hydroxylated or esterified germacranolides from the flowers of a *B. major* population cultivated in Bulgaria and found these lactones differ from the presently known eudesmanolides in a *B. major* population cultivated in Poland.

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A study on the seasonal dependence of oil content and composition of main constituents of *C. balsamita* L. was carried out by Strobel et al. (1987), who identified 29 compounds with carvone (55–60%) and thujone (10%) as the major ones. This study showed that the composition of the oil from *C. balsamita* L. leaves changes more distinctly during the season than does that of the oil from flower head.

Agrotechnological studies of *B. major* at the Lithuanian Institute of Horticulture (Baranauskienė, 1995) showed that the crop of fresh costmary leaves varies from 30 to 45 t ha⁻¹, of which the yield of dry herb was 16.8%. Therefore, the plant seems to be of interest as a source of natural flavorings. On the other hand, published data on *B. major* essential oil composition and its seasonal changes are rather scarce. Considering these points, the present work was undertaken to study seasonal changes of the essential oil composition of *B. major* grown in Lithuania.

MATERIALS AND METHODS

Plant Material and Isolation of Volatile Compounds.

Plant material was grown in the experimental garden of the Lithuanian Institute of Horticulture, Babtai, in 1995. The whole herb was harvested every second week, a total of six times from the end of May until the middle of August as follows: blossoms at the beginning of and during full flowering, and leaves at different times of growing phase of the plant. The plants were sorted by botanical part (leaves, stems, flowers), depending on the growth period, and naturally dried at room temperature. The oils were obtained by hydrodistillation of the different raw materials in a Clevenger type European Pharmacopoeia apparatus during 2 h (Richard et al., 1992). Three replicate analyses were carried out for every oil sample. Simultaneously, the content of moisture in air-dried herb was determined by distilling 5 g of the sample with toluene for 1.5 h (AOAC, 1990). The yields of oil were expressed in percent (w/w) of dry weight.

Identification and Quantification of Volatile Compounds. The essential oils were diluted with Et₂O (1% v/v) and analyzed on a Fisons 8160 series gas chromatograph with the FID heated at 250 °C using a DB-5 (J&W, Folsom, CA) (5% phenyldimethyl polysiloxane) fused silica capillary column (30 m length, 0.32 mm i. d., 0.25 μm film thickness). The oven temperature was programmed from 50 °C (5 min hold) to 250 °C at a rate of 3 °C/min with a final hold of 10 min. Helium was used as carrier gas with a linear velocity 35 cm/s. Injector temperature was set up at 260 °C, split injection mode with the split ratio of 1:25. Four replicate GC analyses were carried out for each essential oil.

Gas chromatography–mass spectrometry (GC-MS) analyses were performed on a combined GC-MS instrument (Varian 3400, Finnigan MAT 95) using a BP-5 (Ringwood, Australia) (5% phenylmethyl polysiloxane) capillary column (25 m length, 0.25 mm i. d., 0.25 μm film thickness). The GC oven temperature was programmed from 60 to 260 °C at a rate of 3 °C/min, the injection port temperature was 200 °C, and split injection mode with the split ratio of 1:25 was used. The mass spectrometer was operated in the 70 eV EI mode with scanning from 24 to 300 amu at 0.5 s decade⁻¹.

Identification was based mainly on the comparison of retention indices (RI) (Adams, 1995; Davies, 1990) and mass spectra (NIST/EPA/NIH Mass Spectral Database NBS75K and the Wageningen Collection of Mass Spectra of Natural Products). When available, pure chemicals were used as a reference compounds. Costic acid methyl esters were prepared from α- and γ-costic acids with diazomethane/ether. The content of the individual constituents was expressed as a peak area percent computed by the GC system integrator and also in arbitrary units (au), which were calculated by multiplying the percentage content of essential oil by 10⁴ (to convert it into mg kg⁻¹

Table 1. Yields (Percent, w/w of Dry Weight) of Essential Oils from Costmary at Different Harvest Periods

	growth phase and date of harvest	leaves	blossoms
1	growing, May 25	0.18 ± 0.03	
2	growing, June 10	0.71 ± 0.04	
3	growing, June 25	0.81 ± 0.03	
4	growing, July 10	0.65 ± 0.03	
5	bud formation, July 25	1.15 ± 0.04	1.34 ± 0.04
6	blooming, August 8	0.86 ± 0.03	1.15 ± 0.03

of dried herb). The obtained figure is multiplied by the percentage content of a particular constituent and divided by 100 (Bylaitė et al., 1998).

RESULTS AND DISCUSSION

The yields of essential oil at different harvesting periods in costmary leaves and blossoms are given in Table 1. The dried leaves and flowers of *B. major* yielded yellowish oil, the content of which varied from 0.18 to 1.34% in the period of monitoring. A very low content of the oil (0.18 ± 0.03%) was found in the leaves at the starting phase of growth (May 25). Later, essential oil was intensively biosynthesized, and the highest amount of it was accumulated during bud formation (July 25) both in leaves (1.15 ± 0.04%) and in blossoms (1.34 ± 0.04%). The results obtained are in agreement with those reported by Strobel et al. (1987), who determined in the fresh leaves of costmary 0.02 and 0.2% in May and the second half of July, respectively. The content of the oil in the flowers was slightly higher than in the leaves. For comparison it should be said that the content of the oil in blossoms determined earlier in *B. major* from Lithuania by Juknevičienė et al. (1973) was ~2 times higher than in leaves. Very low yields of volatiles were recovered from the stems of the plant (~0.005%), and therefore their composition was not analyzed by GC.

All 78 identified oil compounds in *B. major* are listed in Table 2 in order of their elution time from the capillary BP-5 column. Among them, 58 constituents, 39 identified positively (mass spectrum plus retention index) and 19 identified tentatively, were not reported in *B. major* in previously published papers. A typical gas chromatogram of the essential oil from costmary leaves is presented in Figure 1. Preliminary information on the identification of costic acid derivatives (compounds at peak numbers 77, 79, and 80, Table 2) was obtained by comparison and interpretation of mass spectra of unknown relevant costmary peaks with the reduced spectrum of ilicic acid methyl ester (Sanz et al., 1990). So far as such comparison was not sufficient for reliable identification and mass spectra of costic acid derivatives were not available in the literature sources, isocostic acid methyl ester (or γ-costic acid methyl ester) and α-costic acid methyl ester were prepared from costic acids with diazomethane/ether. Costic acids were prepared from α- and β-costal. The compounds obtained were analyzed by GC-MS under the same conditions, and spectra recorded were exactly equal to the spectra of the costmary essential oil constituents 77 and 79.

The percentage composition of essential oil at different growing phases is presented in Table 3; the changes of the amount of the main constituents in arbitrary units are shown in Figure 2. Carvone was the dominating constituent at all vegetation periods both in leaves (51.83–68.01%) and in flowers (59.5 and 61.67%). The differences in the percentage content of carvone at different harvesting times were not considerable except

Table 2. Identification Data of Volatile Compounds in Costmary Leaves and Flowers

no. ^a	constituent ^b	identification ^c	amount ^d (min–max), au	
			leaves	flowers
1	α -thujene*	a, b	tr	tr
2	α -pinene	a, b	tr–1.7	17.3–17.4
3	S-methyl pentanethioate*	a	tr	tr
4	camphene	a, b	tr–13.8	tr–11.5
5	benzaldehyde*	a, b	tr	tr
6	sabinene	a, b	15.7–47.2	64.3–75.9
7	β -pinene	a, b	tr–16.1	16.1
8/9	myrcene* + 2,3-dehydro-1,8-cineole*	a, b	tr–16.3	tr–13.8
10	isobutyl-2-methylbutanoate*	a	tr	tr
11	p-mentha-1,3,8-triene*	a	tr	tr
12	3-methylbutyl butanoate*	a		tr
13	2-methylbutyl 2-methylpropanoate*	a	tr	tr
14	α -terpinene*	a, b	tr	tr
15	p-cymene*	a, b	3.6–28.8	21.4–24.2
16/17	limonene+ 1,8-cineole	a, b	96.7–415.2	28.2–54.2
18	butyl 2-methylbutanoate*	a	tr	tr
19	γ -terpinene	a, b	tr	tr
20	phenyl acetaldehyde*	a, b	tr–12.7	15.0–16.1
21	3-methylbutyl 2-methylbutanoate*	a	tr	tr
22	2-methylbutyl 2-methylbutanoate*	a	tr–13.8	60.3–42.6
23	α -thujone	a, b	289.8–1261.6	1211.4–1239.7
24	3-methyl-3-butenyl 2-methylbutanoate*	a		tr
25	β -thujone	a, b	15.7–225.4	143.8–187.6
26	trans-p-mentha-2,8-dien-1-ol*	a	39.8–125.4	121.9–138.0
27	cis-p-mentha-2,8-dien-1-ol*	a	8.1–80.5	55.2–67.0
28	trans-pinocarveol	a, b	tr	tr
29	cis-verbenol*	a, b	tr	tr
30	trans-verbenol*	a, b	tr–19.6	tr–18.4
31	pinocarvone*	a, b	tr–16.1	29.5–32.2
32	borneol*	a, b	tr	tr
33	p-mentha-1,5-dien-8-ol*	a	tr	tr
34	terpinen-4-ol	a, b	tr–20.7	10.7–29.9
35	trans-p-mentha-1(7),8-dien-2-ol*	a	tr	tr
36	α -terpineol*	a, b	tr–17.2	29.5–31.1
37	p-cymen-8-ol*	a, b	tr	tr
38	cis-dihydrocarvone*	a, b	5.4–65.6	59.8–72.4
39	cis-piperitol*	a, b	tr	tr
40	methyl chrysanthemate*	a		tr
41/42	trans-piperitol* + verbenone*	a, b	8.6–89.7	17.4–21.9
43	trans-carveol*	a, b	tr–26.7	15.0–17.4
44	cis-p-mentha-1(7),8-dien-2-ol*	a	tr–16.3	tr–10.4
45	cis-carveol	a, b	6.3–39.1	26.8–38.0
46	carvone	a, b	932.9–7637.2	7092.1–7973.0
47	2-phenylethyl acetate	a, b	tr	tr
48	cis-chrysanthenyl acetate*	a, b	14.–69.0	57.5–113.9
49	trans-carvone oxide*	a, b	9.7–27.6	23.0–24.1
50	bornyl acetate	a	tr	tr
51	unidentified	c	tr	tr–8.0
52	trans-carvyl acetate	a, b	8.1–25.3	21.9–40.2
53	α -terpinyl acetate	a, b		tr
54	δ -elemene*	a, b	tr	tr
55	cis-carvyl acetate	a, b	10.7–23.0	44.9–57.6
56	α -copaene	a, b	tr–16.1	24.1–24.2
57	cis-jasmone*	a, b	tr	tr
58	β -caryophyllene	a, b	tr	tr
59	4-epi-cubebol*	a, b	16.3–34.5	58.7–120.6
60	α -muurolene*	a, b		tr
61	β -bisabolene*	a, b	80.1–185.8	116.2–143.4
62	cubebol*	a, b	tr	tr
63	cis-calamenene*	a, b	27.0–115.0	84.0–113.9
64	δ -cadinene*	a, b	27.5–51.1	113.9–115.0
65	trans-calamenene*	a, b	tr	tr
66	cadina-1,4-diene*	a, b	tr	tr
67	trans-nerolidol*	a, b	tr	tr
68	spathulenol*	a, b	tr	tr
69	caryophyllene oxide*	a, b	tr	tr
70	1-epi-cubebol*	a, b	6.5–35.7	36.8–38.9
71	sesquilandulol (E)*	a, b		tr
72	T-muurolool	a, b	24.1–77.1	248.4–505.2
73	β -eudesmol*	a, b		tr
74	selin-11-en-4- α -ol*	a, b	9.1–69.0	138.0–142.6
75	unidentified	c	tr–9.7	21.9–33.7
76	sesquilandulyl acetate (E)*	a, b		tr
77	methyl isocostate*	c	tr–19.6	71.3–144.7
78	6,10,14-trimethyl 2-pentadecanone*	a		tr
79	methyl costate*	c	tr	tr
80	4-hydroxy 4,5-dihydroisocostate*	d	7.7–28.8	92.0–241.2

^a Constituents are listed in order of elution from BP5 column. ^b Asterisks indicate that the compound was not previously reported in costmary. ^c Identification: (a) mass spectrum; (b) retention index on DB-5 column; (c) not in Adams, not in NIST 98, not in WAU collection (identification is based on the comparison with mass spectra of reference compounds prepared in the laboratory); (d) not in Adams, not in NIST 98, not in WAU collection (tentative identification is based on the comparison with mass spectra of costic acid methyl esters). ^d Amounts are calculated from GC-FID peak area percent. tr (trace) < 5.0 au.

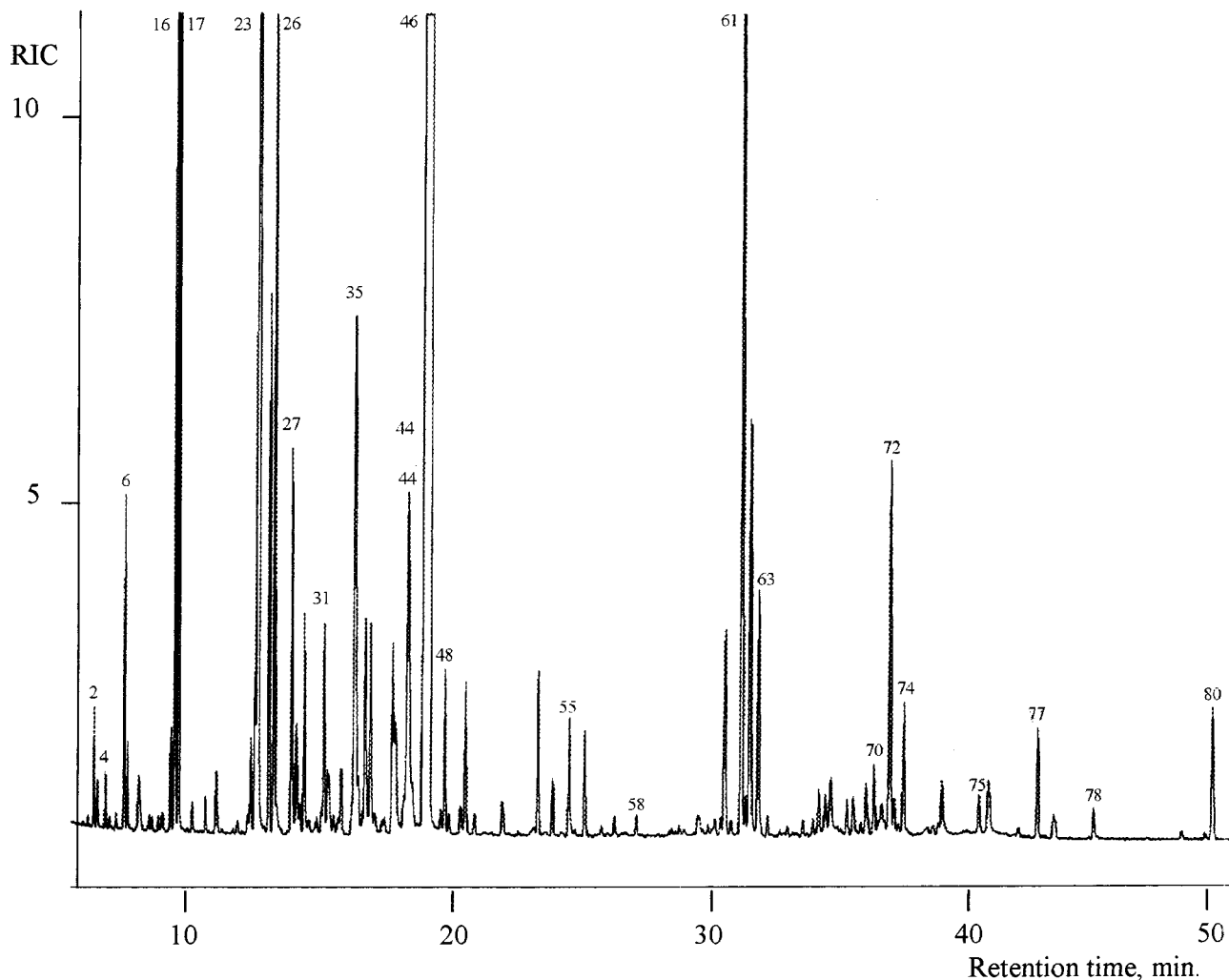


Figure 1. Gas chromatogram of the essential oil of costmary leaves. See Materials and Methods for GC conditions and Table 2 for peak identification.

for May 25, when it was lowest. The absolute amount (Figure 2) of this constituent increased with the increase of the total oil content until bud formation with some exception on July 10, when it was slightly reduced as compared with previous harvesting on June 25. The results presented in Table 2 show that it is possible to obtain up to 8 g of natural carvone from 1 kg of dried costmary herb.

The second major compound in the analyzed *B. major* was α -thujone, the content of which exceeded 10% in most cases. It is interesting to note that the percentage content of α -thujone, in contrast to that of carvone, was highest on May 25 (16.1%). However, its absolute amount also was highest during bud formation, that is, 1262 au in leaves. According to the two major constituents the chemotype of costmary analyzed in our study seems to be similar to the chemotype of *C. balsamita* reported in the work of Strobel et al. (1987).

The content of some other constituents in costmary essential oil was higher than 1%: limonene, 1,8-cineole, β -thujone, *trans-p*-mentha-2,8-dien-1-ol, β -bisabolene, T-muurolol, and selin-11-en-4-ol (flowers only). Limonene and 1,8-cineole were not separated on the DB-5 column, which was used for the analysis of all samples on GC-FID; however, their peaks were baseline separated on the BP-5 column by GC-MS analysis. The ratio of limonene and 1,8-cineole calculated from MS data was 1.8 to 1 in the leaves and 1 to 1.4 in the flowers.

The differences in percentage composition between leaves and blossoms were not considerable, except for sesquiterpenes, especially the sesquiterpene alcohol T-muurolol, the content of which was considerably higher in blossoms than in leaves at the flowering period (July 25, August 8). The absolute amount of sesquiterpenes in costmary flowers was ~ 2 times higher than in the leaves at the same growing phase. The content of other identified constituents was $<1\%$ in most cases both in leaves and in flowers.

It is interesting to note that the highest percentage content of all identified sesquiterpenes in leaves was much higher at early growth phase (May 25) as compared with later harvesting periods. For instance, the major sesquiterpene β -bisabolene on May 25 constituted 4.45%, whereas on June 10 it constituted 2.33% and on June 25, 1.26%. The absolute amount of sesquiterpenes increased with accumulation of essential oil, however, less considerably as compared with the major compound carvone (Figure 2). Strobel et al. (1987) identified β -cubebene as one of the major sesquiterpenes in costmary, the percentage content of which also decreased in the period of May–June. We have not identified β -cubebene in our work, and its presence in *B. major* seems to be rather doubtful; in the chromatogram provided in the paper above-mentioned, the peak of β -cubebene is shown eluting after β -caryophyllene and β -farnesene peaks. However, retention data obtained

Table 3. Percentage Composition of Essential Oils from Costmary Leaves and Flowers at Different Growth Phases (1–6 as in Table 1)

component	leaves						flowers	
	1	2	3	4	5	6	5	6
α -pinene	0.25	0.24	0.15	0.22	0.15	0.12	0.13	0.15
camphene	tr ^a	0.14	0.10	0.12	0.12	0.14	tr	0.10
sabinene	0.87	0.65	0.45	0.67	0.41	0.45	0.48	0.66
β -pinene	0.19	0.18	0.13	0.18	0.14	0.13	0.12	0.14
myrcene + 2,3-dehydro-1,8-cineole	0.15	0.23	0.10	0.10	tr	tr	tr	0.12
<i>p</i> -cymene	0.20	0.26	0.18	0.25	0.25	0.29	0.16	0.21
limonene + 1,8-cineole	5.37	5.42	4.30	4.95	3.61	2.88	2.82	3.75
phenylacetaldehyde	tr	0.13	tr	tr	0.11	tr	0.12	0.13
2-methylbutyl 2-methylbutanoate	tr	0.17	0.12	0.18	0.12	0.13	0.45	0.37
α -thujone	16.10	12.37	10.79	11.83	10.97	13.75	9.04	10.78
β -thujone	2.21	1.28	1.16	1.20	1.09	1.35	1.03	1.06
<i>trans-p</i> -mentha-2,8-dien-1-ol	0.87	2.03	1.82	1.90	1.96	2.05	1.40	1.25
<i>cis-p</i> -mentha-2,8-dien-1-ol	0.45	0.92	0.75	0.80	0.70	0.64	0.50	0.48
<i>trans</i> -verbenol	tr	0.15	tr	0.14	0.17	0.15	tr	0.16
pinocarvone	0.17	0.13	tr	0.18	0.14	0.15	0.22	0.28
<i>cis</i> -dihydrocarvone	0.3	0.69	0.75	0.78	0.57	0.45	0.54	0.52
verbenone + <i>trans</i> -piperitol	0.48	0.49	0.59	0.61	0.78	0.69	0.13	0.19
<i>trans</i> -carveol	tr	0.21	0.33	0.15	0.14	0.18	0.13	0.13
<i>cis</i> -carveol	0.35	0.19	0.36	0.25	0.34	0.38	0.20	0.33
carvone	51.83	63.32	68.01	65.78	66.41	65.36	59.5	61.67
<i>cis</i> -chrysanthenyl acetate	0.79	0.56	0.70	0.59	0.60	0.40	0.85	0.50
carvone oxide	0.54	0.24	0.32	0.20	0.24	0.32	0.18	0.20
<i>trans</i> -carvyl acetate	0.45	0.23	0.24	0.31	0.22	0.11	0.30	0.19
<i>cis</i> -carvyl acetate	0.91	0.15	0.18	0.33	0.20	0.15	0.43	0.39
α -copaene	0.39	0.21	0.12	0.16	0.14	tr	0.18	0.21
4- <i>epi</i> -cubebol	1.10	0.23	0.25	0.28	0.30	0.26	0.90	0.51
β -bisabolene	4.45	2.33	1.26	1.60	1.59	2.16	1.07	1.01
<i>cis</i> -calamenene	1.50	0.82	0.36	0.41	1.00	0.96	0.85	0.73
δ -cadinene	1.53	0.72	0.49	0.55	0.44	0.42	0.85	1.00
1- <i>epi</i> -cubanol	0.36	0.28	0.25	0.11	0.31	0.24	0.29	0.32
T-murolol	2.19	0.34	0.73	0.47	0.67	0.44	3.77	2.16
selin-11-en-4-ol	0.69	0.35	0.49	0.14	0.60	0.50	1.03	1.24
methyl isocostate	0.43	0.12	0.22	0.19	0.17	tr	1.08	0.62
methyl 4-hydroxy-4,5-dihydroisocostate	0.43	0.15	0.26	0.13	0.25	0.27	1.80	0.80
total	95.55	95.93	95.96	95.76	94.91	95.52	90.55	92.36

^a tr (trace) < 0.1%.

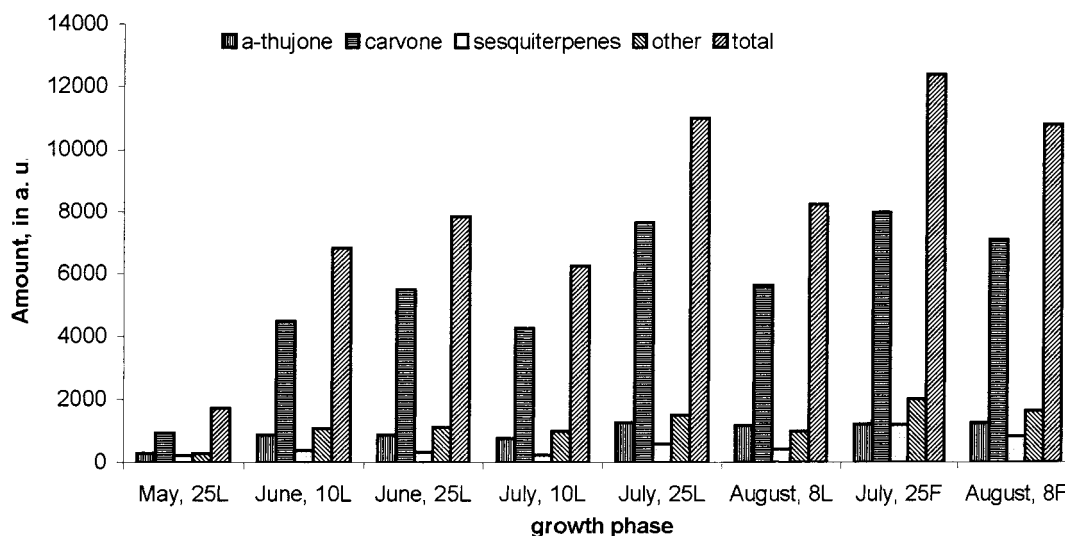


Figure 2. Amounts of major compounds in leaves (L) and flowers (F) of costmary at different growth phases. au = [essential oil yield (%) \times percentage content of individual component \times 100].

from different sources using different polarity capillary columns demonstrate that β -cubebene always elutes earlier than β -caryophyllene and β -farnesene (Adams, 1995; Boelens, 1995; Davies, 1990).

CONCLUSIONS

The highest amount of essential oil in *B. major* leaves and blossoms was accumulated during bud formation.

Knowledge of costmary essential oil composition was significantly extended by identifying 58 compounds not previously reported in this plant.

The plants of *B. major* analyzed in this study can be defined as belonging to the carvone chemotype and producing considerable amounts of α -thujone as well.

In general, the compositions of essential oils were quite similar for all harvest periods except for the early

growing phase in May when carvone content was lower, whereas the contents of α -thujone and sesquiterpene were higher.

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