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Storage-induced changes in essential oil composition of *Leonurus cardiaca* L. plants growing wild in Vilnius and of commercial herbs

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Wild plants of *Leonurus cardiaca* L. (motherwort) collected at full flowering in ten localities of Vilnius and two samples of commercial herbs were investigated. Essential oils produced by hydrodistillation were analysed using the GC and GC/MS methods. Sesquiterpene germacrene D was the major constituent in fresh and stored essential oils and in oils from plants stored for 12 months. Essential oils from commercial herbs and wild plants stored for more than 40 months contained compounds with higher boiling temperatures, phytol and caryophyllene oxide, as the main constituents, respectively. Oxidation of β-caryophyllene and α-humulene occurred during storage of essential oils. The amounts of compounds with caryophyllane (β-caryophyllene + caryophyllene oxide) and humulane (α-humulene + humulene oxide) carbon skeletons were nearly the same in fresh and stored essential oils. The main process in oil composition changes during storage of plants was evaporation of the compounds with lower boiling temperatures, mainly mono- and sesquiterpene hydrocarbons. The chemical composition of the oils obtained from commercial herbs was like that of long stored wild plants.

Key words: *Leonurus cardiaca* L., *Labiatae,* essential oil composition, germacrene D, α-humulene, β-caryophyllene, caryophyllene oxide, phytol

INTRODUCTION

Leonurus cardiaca L. (motherwort) grows wild in Lithuania [1]. This plant was first described in medicinal literature in the 10th century as a remedy for healing nervous and functional cardiac disorders [2, 3]. *L. cardiaca* is used for healing cardiac diseases till now in Germany, France, Russia, Hungary, Lithuania and some other countries [3–8]. The names of the plant in the above countries include the words that show connection with the heart. The English name 'motherwort' and the Latvian name 'matere' are indicative of the other uses of *L. cardiaca.* Motherwort is predominantly a womb remedy [9]. A combination of relaxant and uterotonic effects induced by alkaloids (stahydrine, etc.) gives motherwort a useful role in facilitating child-birth [9]. *L. cardiaca* might be prescribed for palpitation, to stimulate heart function, especially in conditions when the heart is weak [8–10]. It can be used for high blood pressure lowering [4, 8, 9]. Different meaning of *L. cardiaca* names and its different uses in healing in various countries may be caused by a quantitative variation of the constituents in the plant. *L. cardiaca* herbs biosynthesise flavonoids, alkaloids, iridoids, diterpenoids, cardenolids such as glycosides, tannins and other constituents in lower amounts [3, 5, 10–13]. Among other compounds, 0.01–0.05% belong to essential oils [5, 13, 14]. Some authors proposed that the main healing power of *L. cardiaca* depends on the content of flavonoids. The content of these compounds, expressed as hyperoxide, was determined in herbs according to European Pharmacopoeia [15]. Qualitative analysis of iridoids by thin layer chromatography is recommended beside the quantitative determination of flavonoids [15].

According to the data of Phytochemical and Ethnobotanical Databases, the main constituents of essential oils of *L. cardiaca* are sesquiterpene hydrocarbons β-caryophyllene and α-humulene and monoterpene hydrocarbon α-pinene [13]. The above major constituents were produced by *L. cardiaca* growing wild in Ontario (Canada) [14]. β-Pinene, benzaldehyde, limonene, 1-octen-β-ol, linalool, germacrene D, bicyclogermacrene and caryophyllene oxide were found in essential oils in low percentages [13, 14]. All above terpenoids are bioactive [16–23]. β-Caryophyllene exhibited antimicrobial and anti-inflammatory properties and cytotoxity against tumor cells [16–18]. The above compounds influenced the behaviour of insects [17, 19, 21–23]. β-Caryophyllene is approved by the U.S. Food and Drug Administration for food as an additive [18]. α-Pinene shows antimicrobial and mucolytic effects and relieves muscular pain [16, 17, 20]. β-Caryophyllene and α-humulene are easily oxidized during storage.

The aim of the present study was to investigate the influence of storage on the chemical composition of essential oil from *L. cardiaca* grown wild in Vilnius and from commercial herbs.

MATERIALS AND METHODS

Samples of motherwort (*Leonurus cardiaca* L.) were collected in ten localities of Vilnius district: A (Rokantiðkës 1999, 2000; Antakalnis), B (Virðupio, Regykla, Ðilo, Kraujo centras), C1 (Antakalnis, Sapiegynë), C2 (Nemenèinë), D – commercial herbs..Voucher specimens were deposited in the Herbarium of the Institute of Botany (BILAS), Vilnius, Lithuania (Numbers: A – 67292, 67287, B – 67288, 67291, 67289, 67290, C1 – 67287, 67290). *Leonurus cardiaca* L. plants were dried at room temperature (20–25 °C). Their essential oils were prepared by hydrodistillation for 2 h using a mixture of hexane and ethyl ether (1:1) as a collecting organic solvent. The yield of essential oils was ~0.02%

GC/MS analyses were performed using a chromatograph interfaced with an HP 5971 mass spectrometer (ionization voltage 70 eV) and equipped with a CP-Sil 8 CB capillary column (50 m × 0.32 mm i. d., film thickness 0.25 μm). The oven temperature was held at 60 °C for 2 min, then programmed from 60 to 160 °C at a rate of 5 °C min^{-1}, held for 1 min, and then programmed to 250 °C at the rate of 10 °C min^{-1}, held for 5 min using He as a carrier gas (1.0 ml min^{-1}). The injector and detector temperatures were 250 °C.

GC analysis was done on a HP 5890II chromatograph equipped with a FID, and a capillary column HP-FFAP (30 m × 0.25 mm i. d., film thicknes 0.3 μm) was used for quantitative analysis. The GC oven temperature was set at 70^0C for 10 min and then programmed from 70 to 210 °C at a rate of 3 °C min^{-1}, using He as a carrier gas (0.7 ml min^{-1}). The injector and detector temperatures were 200 and 250 °C, respectively.

The percentage composition of the essential oils was computed from GC peak areas without correction factors [24]. The qualitative analysis was based on a comparison of retention times and the mass spectra with the corresponding data in the literature [25] and the computer mass spectra libraries (Wiley and NBS 54K).

RESULTS AND DISCUSSION

Ten essential oils were obtained from tops (30–40 cm) with flowers of *Leonurus cardiaca* L. plants collected in ten localities of Vilnius (Table: A, B, C1, C2). Two oils were hydrodistilled from commercial herbs bought in chemist shops (D). Three essential oils (A) from fresh dried plants contained ~50% sesquiterpene hydrocarbons. The major constituents in the above oils were germacrene D (28.3–32.0%), β-caryophyllene (7.0–8.9%) and α-humulene (6.5–8.8%). The amounts of caryophyllene oxide (0.8–4.9%) and humulene epoxide II (tr – 1.7%) were markedly lower than those of corresponding hydrocarbons. The essential oils were stored in refrigerator, in stoppered glass vessels containing some air. After 7 months of storage of essential oils, oxidation of hydrocarbons was observed (Table 1, compare A and B). The quantities of caryophyllene oxide increased to 6.8–10.0% and humulene epoxide II to 3.6–6.4%. The content of hydrocarbons decreased about twofold. Part of them were oxidised and the other compounds were evaporated. The amounts of compounds with a caryophyllane carbon skeleton (β-caryophyllene + caryophyllene oxide) in fresh and stored essential oils were close to 8.8–13.9% and 8.8–14.1%, respectively. The same relates to compounds with a humulane carbon skeleton (α-humulene + humulene epoxide II): 6.5–10.5% (fresh oils) and 6.1–10.7% (stored samples). The percentage of constituents with a lower molecular weight decreased (evaporation, oxidation and other changes). The above decrease caused an increase of the conditional percentage of higher molecular weight compounds (Table: A and B).

Changes of essential oil composition during storage of plants in material or paper bags (Table, C1, C2) markedly differed from these of the essential oil samples (B). The conditions for evaporation of hydrocarbons in the above bags were better than in stoppered glass vessels (C1, C2). The essential oils from plants stored for 12 months (C1) contained notable amounts of monoterpene α-pinene (0.3–0.9%) and sesquiterpene β-caryophyllene (3.5–3.6%). The oil from plants stored for >40 months (C2) included only traces of α-pinene and 1.8% of β-caryophyllene. The part of β-caryophyllene that remained after 12 months was oxygenated during furt-

Table. **Changes of essential oil composition (main constituents, %) during storage of essential oils (A, B) and plants (C1, C2) of *Leonurus cardiaca* L. growing wild in Vilnius and in commercial herbs (D)**

Compound	RI	A	B	C1	C2	D
α-Pinene	939	0.5–1.0	0.3–0.9	0.3–0.9	tr.	tr.
β-Bourbonene	1384	0.8–1.8	tr.-1.0	0.4–0.7	0.1	tr.
β-Caryophyllene	1419	7.0–8.9	2.0–4.1	3.5–3.6	1.8	0.1–0.7
α-Humulene	1454	6.5–8.8	2.5–4.3	tr.-1.9	0.5	tr.-1.0
Germacrene D	**1485**	**28.3–32.0**	**10.4–18.9**	**9.2–12.0**	**3.9**	**tr.-1.3**
Caryophyllene oxide	1580	1.8–4.9	6.8–10.0	2.9–5.2	7.2	2.4–3.2
Humulene epoxideII	1608	tr.-1.7	3.6–6.4	1.4–2.4	4.3	0.8–1.4
n-Heptadecane	1700	0.7–1.7	tr.-1.0	1.0–1.8	1.1	tr.-2.0
Trimetilpentadecanone-2	1710	tr.-5.1	3.8–5.8	2.0–2.5	2.5	
Dibutyl phthalate	1790	7.1–11.2	10.1–14.9	10.9–15.4	5.1	1.6–2.2
n-Oktadecane	1800	tr.-0.2	tr.-3.2	1.4–2.7	1.4	0.2–2.1
n-Nonadecane	1900	tr.-0.8	tr.-1.0	2.5–2.6	2.9	0.5–1.0
Phytol	1943	2.5–4.6	tr.-0.8	2.9–3.1	3.1	8.5–15.6
n-Eicosane	2000	0.1–1.1	0.8–2.8	4.9–5.7	5.1	0.4–2.5
n-Heneicosane	2100	0.2–1.5	1.5–6.8	4.8–5.5	4.4	1.2–2.3
n-Docasane	2200	tr.-0.2	1.4–5.6	3.9–5.7	5.2	tr.-1.8
n-Tricosane	2300	0.1–0.3	2.1–3.8	5.1–6.4	7.1	tr.-4.3
n-Tetracosane	2400	tr.-0.4	0.7–2.0	1.9–6.1	1.5	tr.-0.7
n-Pentacosane	2500	tr.-1.9	tr.-2.5	4.5–5.8	4.7	tr.

Notes. RI – retention index on unpolar column CP-Sil 8CB, tr. – traces (<0.05%); n – number of samples, m – number of storage months; A – n = 3, m = 1; B – n = 4, m = 7; C1 – n = 2, m = 12; C2 – n = 1, m > 40; D – n = 2, m = 25–30.

her storage. The amounts of compounds with a caryophyllane carbon skeleton after 12 and >40 months of storage were close to 8.8 and 9.0%, respectively.

The commercial herbs of *L. cardiaca* may be used for healing up to 36 months [2]. The essential oil composition of the above herbs was like that of long stored wild plants (Table, C2, D). Oils of commercial herbs contained low amounts of mono- and sesquiterpene hydrocarbons and included higher quantities of diterpenoid phytol (8.5–15.6%) than did samples produced from wild plants. The essential oils of the study plants and of commercial herbs included notable amounts of butyl phthalate. The origin of the above compound is artifact [26], it could be accumulated from polluted soils and / or enviroment.

CONCLUSIONS

Contact with air induced oxidation of β-caryophyllene and α-humulene during storage of *Leonurus cardiaca* L. essential oils in stoppered glass vessels in refrigerator. The amounts of compound with caryophyllane (β-caryophyllene + caryophyllene oxide) and humulane (α-humulene + humulene epoxide II) carbon skeletons were similar in fresh and stored essential oils. The main process during storage of plants was evaporation of compounds with a lower boiling temperature, mainly of mono- and sesquiterpene hydrocarbons.

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VILNIUJE AUGANÈIOS IR VAISTINËSE PARDUODAMOS SUKATÞOLËS (*LEONURUS CARDIACA* L.) ETERINIØ ALIEJØ CHEMINËS SUDËTIES KITIMØ PRIKLAUSOMYBË NUO LAIKO

S a n t r a u k a

Tirta sukatþolës þydinti antþeminë dalis, surinkta deðimtyje augavieèiø Vilniuje (1999–2003), ir du pavyzdþiai, pirkti vaistinëse. Eteriniai aliejai, iðgauti hidrodistiliacijos bûdu, buvo analizuojami dujø chromatografijos ir dujø chromatografijos–masiø spektroskopijos metodais. Ðvieþiuose ir laikytuose eteriniuose aliejuose, taip pat 12 mënesiø saugotø augalø eteriniuose aliejuose pagrindinis komponentas yra seskviterpenas germakrenas D. Vaistinëse pirktø ir laukiniø augalø, saugotø >40 mënesiø, eteriniuose aliejuose vyrauja junginiai, kuriø aukðtesnës virimo temperatûros, – fitolis ir kariofileno oksidas kaip pagrindiniai komponentai. Laikui bëgant eteriniuose aliejuose oksiduojasi β–kariofilenas ir α humulenas. Junginiø, kuriø sudëtyje yra kariofilano (β–kariofilenas + kariofileno oksidas) ir humulano (α–humuleno + humuleno oksidas) anglies skeletø, kiekiai ðvieþiuose bei iðlaikytuose eteriniuose aliejuose yra panaðûs. Ilgai saugotø augalø eteriniø aliejø sudëties pasikeitimus daugiausia sàlygoja junginiai, kuriø þemesnë virimo temperatûra, mono- ir seskviterpeniniø angliavandeniliø garavimas.