

# Chemical composition of apple-tree (*Malus domestica* Borkh.) leaf essential oils

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Despite that essential oils play very important and multifunctional roles (mainly protection function against pests) for apple-tree, the number of studies of the fruiter leaf oils is still very limited.

This research represents the chemical composition of apple-tree (*Malus domestica* Borkh.) leaf essential oils. Plant material was collected from fruit trees growing in different parts of Lithuania. The oils, prepared by simultaneous hydro-distillation and extraction with organic solvents in a Clevenger-type apparatus, were analysed using GC(FID) and GC/MS methods. The compositions varied both by individual principal constituents and by an amount of fractions. The sesquiterpenoid fraction (26.9–45.7%) was the biggest one in all investigated oils, which contained sesquiterpenes from 13.7 to 36.0%, and oxygenated sesquiterpenes from 9.7 until 25.0%. The percentage of monoterpenoids varied from 5.3 to 10.1, the amount of diterpenes differed from 3.6 to 27.3%, and the quantity of aliphatic hydrocarbons was 5.2–24.4%. The first three major compounds were found to be (iso)phytol ( $\leq 27.3\%$ ), (E, E)- $\alpha$ -farnesene ( $\leq 22.0\%$ ), n-hexyl benzoate ( $\leq 13.5\%$ ), tridecane (11.0%), and  $\alpha$ -cadinol (10.5%) in all the investigated apple-tree leaf oils.

One oil under study could be attributed to the (iso)phytol and another one to the (E, E)- $\alpha$ -farnesene chemotype, while other oils were of mixed types.

**Keywords:** *Malus domestica* Borkh., leaf essential oils, (E, E)- $\alpha$ -farnesene, (Z)-3-hexenyl benzoate, (iso)phytol

## INTRODUCTION

Apple-tree (*Malus domestica* [L.] Borkh., family Rosaceae Juss.) is a worldwide cultivated fruit tree. The original homeland of this leafy tree is Central Asia, where its predecessor *Malus sieversii* is still growing wild. The apple-tree could be 3–12 m in height, the blooming season is April–May; and the fruit ripening period is from July to October [1]. Fruits are of different colours, shapes, tastes and odour. Their resistance to pests, diseases and rotting varies depending on the cultivar. The worldwide production of apples in 2014 was 84.6 million tonnes, with China accounting for 48% of the total [2]. Apple fruits have several brown seeds (containing small amounts of poisonous cyanogenic glycoside, amygdalin), and the trees are larger if grown from seeds. Usually, *M. domestica* cultivars are propagated by grafting on rootstocks. Apple-tree fruit flavour is an important quality parameter; and often it

is a goal during plant breeding. A huge number (about 7.500) of cultivars with different desired characteristics is known; hundreds of them are cultivated in Lithuania.

One species *M. sylvestris* Mill. (also called as the European crab apple or the Forest apple) of the genus *Malus* grows spontaneously over all the territory in Lithuania [1]. The tree has an expanded crown and looks like more as a bush than a tree, and, as a truly wild tree, it has thorns. The plant could grow as a single individual or in small groups in mixed or leafy forests, at the edge of forests, in farmlands or abandoned places, and requires a lot of light. A wild apple-tree is quite rare in most European countries.

Apple-tree (especially its fruits) has been an object of many phytochemical studies. Apples are among vegetal foods rich in flavonoids and other phenolic compounds (e.g. procyanidins, polyphenolic acids), responsible for its nutrition value and medical properties.

Volatile organic compounds (VOC) contain a large group of different terpenoids, fatty acid derivatives, benzenoids,

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phenylpropanoids, and amino acid derivatives, as well as many other constituents like alkanes, alkenes, alcohols, esters, aldehydes, and ketones [3]. The main function of volatile organic compounds synthesized in the plants is to protect plants against herbivores and pathogens, to attract pollinators, and to have a communication role among plants and other organisms. Some monoterpenoids, sesquiterpenes, esters and aliphatic hydrocarbons composed aroma of apple-tree flowers [4–8]. Major compounds detected in apple fruits are ethyl esters; propyl and butyl esters; propanoate and 2-methylbutanoate, and acetate esters; terpenoids; aliphatic compounds, and alcohols [5, 6, 9–12]. Foliage emission or constituents in leaves were characterized by terpenoids, esters, alkanes, and diterpenes [5, 6, 13]. A summary of the main volatile organic compounds emitted by the plant or determined in different apple-tree organs (flowers, leaves and fruits) is presented in Table 1. Recently, for the first time, the chemical composition and the *in vitro* cytotoxic activity of the leaf essential oil of *M. domestica* were presented [13]. Besides, the analysis of apple peel oil (VOC obtained by hydro-distillation from apple peels and identified by GC and GC-MS) showed the following major constituents:  $\alpha$ -phellandrene (20.6%),  $\alpha$ -pinene (17.4%),  $\beta$ -pinene (17.1%), mentha-1(7), 8-diene (16.1%) and sabinene (9.5%); and some appreciable amounts of o-cymene (4.3%),  $\beta$ -cubebene (3.1%), and  $\beta$ -ocimene (2.5%) [14].

Many research studies have been focused how various factors (both of biotic and abiotic nature) influence the emission of VOC from apple-trees. Headspace analyses (thermal desorption and GC-MS technique) allowed one to identify 62 volatile compounds in changing quantities from

apple-bearing twigs with leaves, and it was evaluated how rainfall, relative humidity and temperature influence their amounts [6]. Besides, the changes in volatiles emitted from apple-trees in response to larvae and adult female codling moths (*Cydia pomonella*), one of the principal pest insects of apple fruits worldwide, were investigated [9, 15–17]. Additionally, an analysis of the volatiles emitted by apple fruit-lets infested by larvae of the European apple sawfly was performed and the changes of (E, E)- $\alpha$ -farnesene, a common compound of herbivore-induced plant volatiles, were investigated [18]. Actively growing potted apple trees were subjected to drought stress, which was imposed over a 2-week period, in order to determine changes in the emissions of leaf volatile compounds [19]. The emission rates of hexanal, (E)-2-hexenal, (E)-2-hexen-1-ol, 1-hexenol, hexyl acetate, and (E)-2-hexenyl acetate were 5 to 310 times higher for the stressed trees compared to those of the controls [19].

The research of essential oils obtained from leaves of apple-trees is scarce, despite that the oils play very important and multifunctional roles for the fruiter. The aim of the present research is to determine the chemical composition of leaf essential oils of *Malus domestica*, which is a popular fruit tree not only in Lithuania, but in the middle climatic zones as well.

## EXPERIMENTAL

### Plant material

Leaves were collected from the apple-trees (*Malus domestica* Borkh., name of cultivars not specified) growing in the Kaunas District (Babtai), and Jonava, Mažeikiai and N. Akmenė

Table 1. Main volatile organic compounds (VOC) emitted by or determined in different apple-tree (*M. domestica*) organs (flowers, fruits and leaves)

During blooming or in apple flowers	Apple fruits VOC	In apple-tree leaves or during foliage emission
$\alpha$ , $\beta$ -pinene, camphene, $\delta$ -3-carene, $\alpha$ -terpinene, limonene, p-cymene, perillene, $\alpha$ -terpinolene, $\alpha$ -humulene, linalool, menthol, $\beta$ -caryophyllene, $\beta$ -ionone, (E, E)- $\alpha$ -farnesene, eugenol, geraniol, thymol, (E)-, (Z)-citral, bornyl acetate, geranyl acetate, benzaldehyde, cinnamaldehyde, nonanal, decanal, (Z)-3-hexen-1-ol, benzyl alcohol, 2-phenylethyl alcohol, benzophenone, hexanol, octanoic acid, palmitic acid, butyl acetate, isopropyl myristate, butyl myristate, isopropyl palmitate, n-decane, n-eicosane, 1-dodecene, dodecane, hexadecane [4–8, and therein cited]	$\alpha$ , $\beta$ -pinene, camphene, $\beta$ -myrcene, $\delta$ -3-carene, $\alpha$ -terpinene, limonene, $\gamma$ -terpinene, $\alpha$ -terpinolene, linalool, camphor, p-cymene, $\beta$ -caryophyllene, (E, E)- $\alpha$ -farnesene, butyl acetate, 2-methylbutyl acetate, hexyl acetate, butyl butanoate, butyl 2-methyl butanoate, hexyl butyrate, propyl hexanoate, butyl hexanoate, hexyl hexanoate, butyl propanoate, butyl laurate, butyl myristate, hexyl propanoate, 1-methylethyl butanoate, ethyl octanoate, ethyl hexanoate, ethyl 2-methyl butanoate, ethyl butanoate, ethyl pentanoate, 2-methyl butanol, methyl 2-methyl butanoate, propyl 2-methyl butanoate, propyl butanoate, pentyl acetate, hexyl 2-methyl butanoate, geranyl acetate, estragole, hexanol, hexanal, (E)-2-hexenal, heptanol, butanol, octanol, nonanal, decanal, dodecanal, acetophenone, benzophenone, nonanoic acid, n-tridecane, n-tetradecane [5, 6, 9–12; and therein cited]	$\alpha$ , $\beta$ -pinene, camphene, limonene, 1,8-cineole, $\delta$ -3-carene, $\alpha$ -phellandrene, linalool, 4-terpineol, $\beta$ -caryophyllene, spathulenol, hexanal, (E)-2-hexenal, heptanal, (Z)-3-hexen-1-ol, 2- $\beta$ -damascenone, 2-dodecanone, (E, E)- $\alpha$ -farnesene, 1,6,10-dodecatrien-3-ol-3,7,11-trimethyl, cis-3-hexenyl benzoate, n-hexyl benzoate, benzyl benzoate, 6,10, 4-trimethyl-2-pentadecanone, podocarpene A, phytol, n-eicosane, undecanone, tricosane, pentacosane [5, 6, 13; and therein cited]

city surroundings. Raw material was taken immediately to the laboratory to be dried at room temperature (20–25 °C) and shade conditions.

### Essential oil isolation

The essential oils were prepared by a simultaneous hydro-distillation and extraction with organic solvents. A hydro-distillation procedure (for 2 h) was applied for crushed air-dried apple-tree leaves in a Clevenger-type apparatus according to the European *Pharmacopoeia* and a mixture of pentane and diethyl ether (1:1) was used as a collecting solvent. The obtained solutions had a strong pleasant apple-like odour; they were dried over anhydrous sodium sulphate, kept in closed dark vials and stored in a refrigerator until their analysis.

### GC (FID) analysis

Quantitative analyses of the essential oils were carried out by GC on a DB-Wax capillary column (polyethylene glycol 30 m × 0.25 mm i. d., film thickness 0.25 µm) using a Perkin-Elmer Clarus 500 chromatograph equipped with a FID. The oven temperature was programmed from 60 to 280 °C at a rate of 3 °C/min. The injector and detector temperatures were 250 °C; the carrier gas helium 1 mL/min; the ratio split 1:60. At least 2 repetitions ( $n \geq 2$ ) per analysis were performed.

### GC-MS analysis

Qualitative analyses were performed by GC-MS using a chromatograph HP 5890 interfaced to an HP 5971 mass spectrometer (ionization voltage 70 eV, scan time 0.6 s, scan range 35–400 Da) and equipped with a capillary column DB-5 (50 m × 0.32 mm i. d., film thickness 0.25 µm).

The GC oven temperature was programmed from 60 °C (isothermal for 2 min) to 160 °C (isothermal for 1 min) at a rate of 5 °C/min, then increased to 250 °C at a rate of 10 °C/min, and the final temperature was kept for 3 min. The temperature of the injector and detector was 250 °C. The flow rate of carrier gas (helium) was 1 mL/min, the ratio split 1:40. The mass spectra in the electron mode were generated at 70 eV, 0.97 scans/s, the mass range 35–650 m/z.

### Identification of compounds

The percentage composition of the essential oils was computed from the GC peak areas without correction factors. The qualitative analysis was based on the comparison of retention indexes on both columns, the co-injection of some terpenes references and  $C_8$ – $C_{28}$  n-alkane series; and the mass spectra with corresponding data in the literature [20] and computer mass spectra libraries (Wiley and NBS 54K). The identification has been approved when computer matching with the mass spectral libraries was with a probability above 90%. The relative proportions of the oil constituents were expressed as percentages obtained by peak

area normalization, all relative-response factors being taken as one.

## RESULTS AND DISCUSSION

The composition of essential oils from apple-trees can be related to the genetic, phenological characteristics and environmental factors, the interspecies interactions in ecosystems, the impact of damaging factors, etc. The compositions of four leaf apple-tree oils are presented in Table 2. The yield of the oils was very low, so for this reason a simultaneous hydro-distillation and extraction with organic solvents was performed. In total, up to 28 components were identified by GC (FID) and GC-MS techniques; and they comprised 83.9–95.8% of the oil content. The essential oils differed not only by individual principal components, but also by an amount of fractions. The sesquiterpenoid fraction (26.9–45.7%) was the biggest one in all the oils, where quantities of sesquiterpenes differed from 13.7 to 36.0%, and of oxygenated sesquiterpenes from 9.7 until 25.0%. The percentage of monoterpenoids varied from 5.3 to 10.1, the amount of diterpenes varied from 3.6 to 27.3%, and the quantity of aliphatic hydrocarbons was 5.2–24.4%.

Table 2. Leaf essential oil chemical composition (%) of *Malus domestica* cultivated in Lithuania

Compound	RI DB-5	A	B	C	D
Pinane*	980	0.5	0.3	1.2	0.6
n-Undecane	1100	2.6			0.1
trans-Pinene hydrate	1123	0.4	1.2	0.5	1.0
cis-Pinene hydrate	1144		4.0	0.2	2.5
(E)-2-Hexenyl butyrate	1193	5.3	0.3	tr.	4.8
Tridecane	1300	0.3	11.0	0.2	4.2
β-Caryophyllene	1419	2.9		0.9	7.8
Neryl propionate	1460	2.2			1.0
Germacrene D	1480	1.0	0.3	1.5	5.0
(E)-β-Ionone	1485	0.9	2.4	4.3	4.4
Neryl isobutyrate	1491	1.3		tr.	0.6
α-Muurolene	1500	0.5	0.3	3.9	0.9
n-Pentadecane	1500	0.3	2.1	1.4	0.3
(E, E)-α-Farnesene	1506	11.2	12.6	22.0	14.0
γ-Cadinene	1514	1.0	0.2	0.5	0.9
δ-Cadinene	1523	1.3	tr.	6.5	0.5
α-Cadinene	1539	0.4	0.3	0.7	1.5
(E)-Nerolidol	1563	2.1	0.3	1.5	0.9
(Z)-3-Hexenyl benzoate	1567	8.4	7.5	2.7	1.5
n-Hexyl benzoate	1580	2.1	13.5	8.1	5.5
(E)-2-Hexenyl benzoate	1585	1.0	2.5	2.1	3.0
epi-α-Muurolol	1641	9.0	0.9	1.3	3.5

$\alpha$ -Muurolol	1654	1.8	0.6	0.9	3.8
$\alpha$ -Cadinol	1655	10.5	7.4	3.3	3.0
Farnesol*	1697	1.6	4.0	2.7	4.2
(iso)Phytol*	1943	27.3	3.6	7.5	7.9
n-Eicosane	2000	0.9	4.5	3.0	4.5
n-Tricosane	2300	1.1	6.8	7.0	6.0
Total		95.8	86.6	83.9	93.9
Monoterpenoids		5.3	7.9	6.2	10.1
Sesquiterpenes		18.3	13.7	36.0	30.6
Oxygenated sesquiterpenes		25.0	13.2	9.7	15.4
Diterpenes		27.3	3.6	7.5	7.9
Aliphatic hydrocarbons		5.2	24.4	11.6	15.1

Collecting sites of *M. domestica* leaves: A is Babtai, Kaunas District; B is Jonava; C is Mažeikiai; D is N. Akmenė.

\* Specific isomers were not identified, RI is the retention index on apolar column DB-5. tr. is traces ( $\leq 0.05\%$ ).

The oil indicated by A was characterized by a big amount (27.3%) of (iso)phytol, while the second and the third main compounds were (E, E)- $\alpha$ -farnesene and  $\alpha$ -cadinol, with the quantities 11.2 and 10.5%, respectively. Phytol, an acyclic diterpene alcohol, is a decomposition product of chlorophylls. Amounts of this compound varied from 3.6 to 7.9% in the rest leaf oils under study. Additionally, appreciable amounts (over 5.0%) of epi- $\alpha$ -muurolol (9.0%), (Z)-3-hexenyl benzoate (8.4%) and E-2-hexenyl butyrate (5.3%) were determined in the oil A. The leaf essential oil A could be attributed to the (iso)phytol chemotype.

The leaf oil B contained almost equal quantities of the compounds such as: n-hexyl benzoate (13.5%), (E, E)- $\alpha$ -farnesene (12.6%), tridecane (11.0%), (Z)-3-hexenyl benzoate (7.5%),  $\alpha$ -cadinol (7.4%), and n-tricosane (6.8%). The origin of aliphatic hydrocarbons in leaf oils could be from apple leaf epicuticular waxes.

Apple tree oils indicated by letters C and D were characterised by (E, E)- $\alpha$ -farnesene as the first principal constituent with quantities 22.0 and 14.0%, respectively. n-Hexyl benzoate (8.1%), (iso)phytol (7.5%), and n-tricosane (7.0%) were identified in appreciable amounts in the sample C, while (iso)phytol (7.9%) and  $\beta$ -caryophyllene (7.8%) were found to be the second and the third major compound in the oil D. The oil C containing the predominant quantity of (E, E)- $\alpha$ -farnesene over other constituents could be described as the oil of the (E, E)- $\alpha$ -farnesene chemotype. Sesquiterpenene (E, E)- $\alpha$ -farnesene is a characteristic compound (with green apple odour) not only for apple-tree leaves, but for flowers and fruits as well.

To the best of our knowledge, the number of published available data on apple-tree leaf essential oils is very limited. A research of fresh leaves of the *M. domestica* tree,

growing in Dhauladhar (Himachal Pradesh, India; 3300 m above the sea level located in the Himalayas) was conducted [13]. The light pale-coloured essential oil, obtained in 0.004% yield, had a characteristic eucalyptol dominant fragrance. Totally, 17 compounds were identified there and major compounds of the oil were 1,8-cineole (43.7%), phytol (11.5%),  $\alpha$ -farnesene (9.6%), and pentacosane (7.6%), while the following ones were tricosane (4.2%), podocarpene A (3.8%), and *cis*-3-hexenyl benzoate (3.1%). Even more, the oil was tested against three cancer cell lines, namely, C-6 (glioma cells), A549 (human lung carcinoma), CHOK1 (Chinese hamster ovary cells), and THP-1 (human acute monocytic leukemia cell). The tests evaluated by sulforhodamine B (SRB) assays showed the highest activity by the oil on C-6 cell lines. According to the authors [13], it was the first paper in literature not only on the chemical composition but also the cytotoxic activity of the essential oil of *M. domestica* leaves.

It is very interesting that leaf essential oils from *M. domestica* growing in Lithuania and India contained among the main constituents the same compounds such as phytol, (E, E)- $\alpha$ -farnesene, and 3-hexenyl benzoate. Even more, some other compounds identified were common for the oils obtained from apple-tree (cultivar not specified) leaves of totally different plant origins (North Europe and mid-hills of Western Himalayas).

It should be mentioned that many constituents (mainly esters, sesquiterpenes and aliphatic hydrocarbons), identified in the leaf essential oils under this study, have been determined in the VOC emission from apple-trees cultivated in other countries [5, 6 and therein cited], but differed by their amounts.

## CONCLUSIONS

The chemical composition of the leaf essential oils of *Malus domestica* Borkh. growing in Lithuania varied not only by individual principal components, but also by a quantity of different compound classes. Among four investigated oils, one could be attributed to the (iso)phytol and another one to the (E, E)- $\alpha$ -farnesene chemotype, while other oils were of mixed types.

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## OBELŲ (*MALUS DOMESTICA* BORKH.) LAPŲ ETERINIŲ ALIEJŲ CHEMINĖ SUDĖTIS

### Santrauka

Naminių obelų (*Malus domestica* [L.] Borkh.) lapų eteriniai aliejai nėra iki šiol plačiai tirti, nors augalas daug kur pasulyje auginamas dėl vaisių, o lakūs lapų organiniai junginiai atlieka daugybę labai svarbių funkcijų, bene pagrindinė – apsauga nuo kenkėjų.

Pateikta obelų, augančių skirtingose Lietuvos vietose, lapų eterinių aliejų sudėtis. Aliejai išgauti iš džiovintos susmulkintos žaliavos hidrodistiliacijos būdu Klevendžerio aparatu, lakiuosius junginius surenkant į organinių tirpiklių mišinį. Cheminė analizė atlikta dujų chromatografijos metodu, panaudojant liepsnos jonizacinį ir masių spektrometrijos detektorius. Šių aliejų sudėtis tarpusavyje skyrėsi ne tik pagal atskirus junginius, bet ir pagal junginių klases. Nustatyti vyraujantys junginiai yra šie: (izo)fitolis ( $\leq 27,3$  %), (E, E)- $\alpha$ -farnezenas ( $\leq 22,0$  %), n-heksyl benzoatas ( $\leq 13,5$  %), tridekanas (11,0 %) ir  $\alpha$ -kadinolis (10,5 %). Lietuvoje auginamų obelų lapų eterinis aliejus pagal dominuojančius junginius yra panašus į eterinį aliejų, kuris paruoštas iš šviežių lapų, nuskintų nuo obelų, augančių vakarinėje Himalajų dalyje (3 300 m. virš jūros lygio).

Iš keturių tirtų mėginių vienas aliejus galėtų būti priskirtas (izo)fitolio, vienas (E, E)- $\alpha$ -farnezeno chemotipui, o likusieji – mišriam tipui.