

CHEMICAL COMPOSITION OF ESSENTIAL OILS FROM THE BUDS AND LEAVES OF CULTIVATED HAZELNUT

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Abstract. Essential oils are the volatile, aromatic oils obtained by steam or hydro distillation of botanical material. Different parts of the plants can be used to obtain essential oils, including the flowers, leaves, seeds, roots, stems, bark, wood, etc. There are many ways in which these aroma substances can be used in our life, but more importantly benefits are therapeutic properties of the oils. Distillation of buds and leaves of hazelnut (*Corylus L.*) grown in Poland revealed the presence of essential oils at efficiencies of 0.49% and 1.01%, respectively. The profile of physicochemical properties of achieved oils was determined, i.e.: density d_{20} (g · ml), refractive index, and optical rotation. The GC/MS analysis allowed for detecting 43 compounds in buds, among which 35 were identified, as well as 42 compounds in leaves, including 37 identified ones. Hydrocarbons and monoterpene alcohols were the main components of examined essential oils. Following compounds dominated in the oil made from hazel buds: nerol (20.6%), myrtenol (19.5%), α -campholenol (4.7%), and p-cymene (3.7%), while hazel leaves contained prevailing amounts of: nerol (13.0%), myrtenol (9.4%), α -campholenol (9.0%), menthol (6.7%), geraniol (4.8%), and limonene (3.9%). The qualitative differences between constituents of essential oils made of both studied materials were found.

Key words: *Corylus L.*, aroma substances, monoterpenes, GC/MS, anatomical parts

INTRODUCTION

Hazel (*Corylus L.*) is a plant species from Betulaceae family growing in moderate as well as moderate/subtropical climates. Only common hazel (*Corylus avellana L.*) can be found in Poland in natural habitats as an undergrowth of deciduous and mixed forests, whereas noble varieties, originating mainly from *Corylus avellana* var. *pontica* Winkl. and *Corylus maxima* Mill., or even their hybrids are grown on commercial plantations [Sękowski 1993; Zdyb 2003]. Only hazelnuts – that as similar as bark, buds, leaves, or

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male inflorescences show some medicinal properties – are used for consumption. In folk medicine, raw hazelnuts are recommended at mental exhausting and anemia, extracts made of bark and leaves are used in circulatory system disturbances, while balm prepared from male inflorescences shows some antibacterial and antiparasitic action [Wiszniewska 1991; Sarwa 1995; Yurttas et al. 2000; Sivakumar and Bacchetta 2005; Amaral et al. 2005; Kornsteiner et al. 2006; Oliveira et al. 2007].

There are no information on the content of biologically active substances in various anatomical parts of hazel plants in available domestic and foreign literature. Learning the chemical composition along with the action of active substances contained in hazel may contribute to the increase of the plant species significance as a medicinal specimen.

The essential oils have been known as the scent plant substances applied for cosmetics, disinfection, medicine, and spice purposes for a long time. In chemical respect, they are multi-component mixtures of terpene compounds and phenylpropane derivatives, as well as compounds containing nitrogen, sulfur, and acetylene-derivivative substances. Compounds present in essential oils are of hydrocarbon, alcohol, aldehyde, ketone, ester, and ether character. Over 2000 worldwide plant species belonging to 250 botanical families have the ability to synthesize the essential oils [Masango 2005; Sharififar et al. 2007]. Essential oils are a heterogenous group of plant-origin products with diverse chemical nature, and they show wide spectrum of pharmacological properties [Ludwiczuk et al. 2001; Wolski et al. 2002; Dyduch et al. 2003; Najda and Wolski 2003]. Contemporary medicine discovers that the scent is transported through nerve fibers straight to those brain parts that are responsible for intuition and emotions. The aroma substances affect the chemical balance of an organism, human's mood and disposition. Nowadays, essential oils and their components find more and more interests due to relatively safe status, wide consumer's acceptance, and wide applicability [Wolski and Najda 2004; Wolski et al. 2004; Dyduch and Najda 2005; Masango 2005; Smith et al. 2005; Sefidkon et al. 2007; Sharififar et al. 2007].

The present study aimed at evaluating the percentage, as well as qualitative and content composition of essential oils present in various anatomical parts of hazel plant, i.e. buds and leaves.

MATERIAL AND METHODS

Material for study was composed of buds and leaves of hazel plants originating from the experimental orchard in Końskowola near Lublin (Poland, 51°40' N, 22°06' E). The bud harvest was carried out in April, when plants had no leaves, while leaves were collected in June 2006. Collected material was dried at 35°C just after the harvest in a shadow and air till achieving the air-dry product.

Essential oils

The essential oils in studied samples were determined by means of indirect distillation with steam addition according to Polish Pharmacopoeia VI [2002]. 20 g of air-dry, buds and leaves were distilled during 4 hours in Deryng apparatus. The content of essential oils (X) was calculated from the equation:

$$X[\%] = \frac{a \cdot 100}{m}$$

where: m – mass of sample to be examined, g,
 a – value essential oils, cm^3 (1 line microscale = 0.01 cm^3).

The final content of essential oils was expressed in percents (% v/w) of dry matter.

Such achieved essential oil was dehydrated using anhydrous sodium sulfate, then analyzed by means of GC and GC/MS techniques and its qualitative and content composition was determined. Physicochemical parameters of a profile obtained is essential: the refractive index, density, optical rotation and solubility in ethanol.

Physicochemical parameters of essential oils

The profile of physicochemical properties of achieved oils was determined, i.e.: density d_{20} ($\text{g} \cdot \text{ml}$), refractive index, and optical rotation.

Density. The weight to volume ratio of examined oils was determined with a help of pycnometer of 5 ml capacity at 20°C [FP VI, 2002]. Measurements were performed using water as the immersion liquid. The density was calculated applying formula:

$$d_{20} = \frac{m}{w} \cdot 0.997 + 0.0012$$

where: d_{20} – density at 20°C , $\text{g} \cdot \text{ml}$,
 m – weight of tested substance determined in the air at 20°C , g,
 w – weight of the same water volume determined in the air at 20°C , g,
 0.997 – water density at 20°C ,
 0.0012 – correction for weighing in the air.

Refractive index. Ratio of light speed in the air to the same speed in studied oils was determined according to Polish Pharmacopoeia VI [2002]. Measurements were made with a help of refractometer in three replicates at 20°C in polychromatic light. The reading was made at 589.3 nm wavelength (line D for sodium). Refractive index was calculated applying formula:

$$n_D^{20} = \frac{v}{v_1} = \frac{\sin \alpha}{\sin \beta}$$

where: v – speed of light in the air,
 v_1 – speed of light in studied oil,
 α – angle of incidence,
 β – refraction in studied oil.

Optical rotation. Measurements of the rotation of polarization plane angle as well as rotation direction were carried out in visual polarimeter using line D for sodium at 20°C . The measurement precision was 0.05° [FP VI 2002]. The specific rotation of oils was calculated applying formula:

$$\alpha_D^{20} = \frac{10 \cdot \alpha}{l \cdot d}$$

where: α_D^{20} – specific rotation for line D for sodium (in degrees),

α – rotation angle of polarization plane (in degrees),

l – length of polarization tube (in cm),

d – density of studied oil (in g · ml).

GC and GC/MS determinations

The GC-MS instrument ITMS Varian 4000 GC-MS/MS (Varian, USA) equipped with a CP-8410 auto-injector and a 30 m × 0.25 mm; VF-5ms column (Varian, USA), film thickness 0.25 μm, carrier gas He 0.5 ml/min, injector and detector temperature were, at 220 and 200°C; split ratio 1:20; inject volume 1 μl, respectively. A temperature gradient was applied (60°C for 0.5 minute, then incremented by 3°C/min to 246°C, 246°C for 10 minutes); mass range: 40-1000 Da. GC: Varian 3800 Series (Varian, USA) with DB-5 column (J&W, USA) operated under the same conditions as GC-MS, FID – 256°C; split ratio 1:50. The qualitative analysis was carried out on the basis of MS spectra which were compared with the spectra of the NIST library [NIST/EPA/NIH 2002] and data available in literature [Joulain and König 1998; Adams 2001]. Identity of the compounds was confirmed by their retention indices taken from literature and own data [Joulain and König 1998; Adams 2001]. The quantity composition of oils was determined by GC (FID), by assuming total of all the particular oils to be 100%.

RESULTS AND DISCUSSION

Every oil-producing plant species with determined genotype synthesizes and accumulates essential oils with characteristic and unique chemical composition. However, great differences in oils contents and its composition can be recorded within a given species [Najda et al. 2005].

Analysis of achieved results revealed that hazel leaves contained over twice as much essential oils as compared to buds (tab. 1). Content of essential oils in hazel buds amounted from 0.48% to 0.50%, while in leaves from 0.98% to 1.01%.

Table 1. Yield of essential oils (%) made from buds and leaves of hazel
Tabela 1. Wydajność olejku eterycznego (%) otrzymanych z pąków i liści leszczyny

Sample Próba	Raw material – Badany surowiec	
	buds – pąki	leaves – liście
I	0.49	1.05
II	0.50	1.00
III	0.48	0.98
Mean – Średnio	0.49	1.01

Studies did not reveal remarkable differences between studied oils with regard to their physicochemical properties (tab. 2). The oils were characterized by density from 0.776 to 0.872, rotation from -2° to -27° , and refraction index from 1.4787 to 1.504.

Table 2. Physicochemical properties of essential oils isolated from hazel buds and leaves
Tabela 2. Właściwości fizykochemiczne olejków eterycznych wyizolowanych z pąków i liści leszczyny

Factor – Parametr		Raw material – Badany surowiec	
		buds – pąki	leaves – liście
Density Gęstość	d_{20} (g · ml)	0.776 – 0.798	0.851 – 0.872
Optical rotation Skręcalność	α_D^{20}	-2° do -15°	-16° do -27°
Refractive index Współczynnik załamania światła	n_D^{20}	1.4787 – 1.4794	1.4951 – 1.504

Oils extracted from hazel buds is a light yellow liquid with slightly lemon-menthol scent resulting from low concentration of citral (0.6%) and extremely high level of myrthenol 19.5% with characteristic green tune due to β -ionone (tab. 3).

Essential oil made of hazel leaves is a colorless converting into dark green liquid with a scent of a fresh plant with slight long-lasting flower aroma. That difference of the oil scent can be explained by relatively large amounts of geraniol and methyl salicylate.

The GC/MS analysis of essential oils revealed the presence of 43 compounds in buds, among which 35 were identified, including 13 ones that made up above 1% with the main components – nerol (20.6%) and myrthenol (19.5%), as well as 22 components at 0.01–1% concentrations. Leaves contained 42 compounds: 37 of them were identified, including 26 at above 1% of the content with main constituent – nerol (13.0%), as well as 10 components at 0.01–1% levels.

Following agents dominated in studied hazel buds: nerol, myrthenol, α -campholenol, and p-cymene, contents of which ranged to 20.6%, 19.5%, 4.7%, and 3.7%, respectively. Main constituents of the hazel leaves were: nerol (13.0%), myrthenol (9.4%), α -campholenol (9.0%), menthol (6.7%), geraniol (4.8%), and limonene (3.9%).

Based on literature data and own research, it can be concluded that specific and pleasant scent of raw buds and leaves of hazel plants is a mixture of over 10 fragrances compounds from mono and sesquiterpene groups.

In total, 45 compounds counted to various chemical groups were identified in examined materials, including the most important presented in Table 3. Among compounds identified in hazel buds essential oils, monoterpene hydrocarbons and monoterpene alcohols dominated (34.4% and 25.5%, respectively). Monoterpene hydrocarbons and alcohols were also the prevailing fraction in essential oils achieved from hazel leaves, and their percentage amounted to 42.8% and 21.3%, respectively (tab. 4).

Table 3. The share of identified components of essential oil distilled from hazel buds and leaves (%)
 Tabela 3. Udział zidentyfikowanych składników olejków eterycznych wydestylowanych z pąków i liści leszczyny (%)

No Lp.	Compound Składnik	RI	Buds Pąki	Leaves Liście
1.	2-pentyl-furan	992	0.5	0.3
2.	p-cymene	1025	3.7	2.4
3.	limonene	1028	0.1	3.9
4.	1.8-cineole	1031	0.1	1.0
5.	trans-linalool oxide	1073	0.4	1.6
6.	cis-linalool oxide	1089	0.3	1.0
7.	β -linalool	1100	0.9	1.5
8.	n-nonanal	1103	2.6	0.2
9.	trans-pinocarveol	1140	2.1	1.5
10.	L-menthone	1154	3.1	2.4
11.	isomenthone	1163	-	2.7
12.	n-nonanol	1171	0.5	2.0
13.	menthol	1173	2.9	6.7
14.	α -terpineol	1192	0.5	2.3
15.	methyl salicylate	1198	2.6	1.7
16.	myrtenol	1199	19.5	9.4
17.	α -Campholenol	1204	4.7	9.0
18.	nerol	1229	20.6	13.0
19.	neral	1237	0.3	1.6
20.	geraniol	1255	2.4	4.8
21.	myrtanol	1263	0.6	1.2
22.	geranial	1268	0.3	2.1
23.	menthyl acetate	1293	1.5	0.6
24.	thymol	1296	0.9	0.5
25.	perilla alcohol	1302	0.9	0.3
26.	carvacrol	1306	-	0.5
27.	eugenol	1361	0.2	0.7
28.	α -copaene	1376	0.2	1.2
29.	β -bourbonene	1386	0.2	0.4
30.	β -caryophyllene	1421	0.7	1.7
31.	germacrene D	1483	0.2	2.6
32.	β -ionone	1489	0.4	0.7
33.	δ -cadinene	1530	0.2	0.9
34.	spathulenol	1583	1.1	1.8
35.	caryophyllene oxide	1589	0.4	1.5
36.	hexahydrofarnesyl acetone	1842	0.4	2.3
37.	scareoloxide	2152	1.0	-

Table 4. The share of particular fractions of essential oils isolated from hazel buds and leaves (%)
 Tabela 4. Udział poszczególnych frakcji olejków eterycznych wyizolowanych z pąków i liści leszczyny (%)

Chemical group Grupa chemiczna	Raw material – Badany surowiec	
	buds – pąki	leaves – liście
Monoterpene hydrocarbons Węglowodory monoterpeneowe	25.5	21.3
Monoterpene aldehydes and ketones Aldehydy i ketony monoterpeneowe	19.8	11.5
Water-soluble Węglowodory rozpuszczalne w wodzie	7.9	16.9
Alcohol monoterpenes Alkohole monoterpeneowe	34.4	42.8
Sesquiterpene hydrocarbons Węglowodory seskwiterpenowe	1.5	6.8

The organic compounds constituting the essential oils are secondary metabolites of plants that show multi-directional pharmacological action towards human and animal's organisms. At present, it is recognized that monoterpenes play many roles in human organism. It was shown that they penetrate to the blood and act as medicinal agents and control the brain work. Components of the essential oils are also used in treatment of respiratory tract diseases – they show expectorate, antibacterial, and antiviral properties, while at gastric disturbances, they act as cholagogue and spasmolytic means [Setzer et al. 1999]. Many of them have disinfection and aseptic action to skin by stimulating its blood circulation, efficiently improve the mood, soothe the muscle pain, and show stimulating or soothing action [Stassi et al. 1996; El-Sawi et al. 2007]. Monoterpenes present in essential oils of some plant species are responsible for analgesic properties, which was confirmed by research performed in numerous worldwide scientific centers [Rajeswara et al. 2005; da Silva et al. 2005; Feng and Zheng 2007; Edris 2007].

Anticarcinogenic activity of limonene and perilla alcohol is at present the subject of the 1st phase of clinical studies [El-Sawi et al. 2007]. Moreover, a pilot clinical research within the 2nd phase aiming at evaluating the level of limonene and its metabolites accumulation in breast cancer, is carried out. It is indicated that orally administered limonene is completely absorbed and undergoes an intensive bio-transformation to active metabolites: perillic acid and 1,2-dihydroxy-limonene. Furthermore, limonene at clinical activity rates is well tolerated by patients with advanced tumour stages. The side effects such as: sickness, vomiting, or diarrhea depend on a monoterpene dose and quickly disappear not leading to any serious disfunctions of the organs [Vigushin et al. 1998]. Perilla alcohol is also studied as an inhibitor of UV-induced cancerogenesis. The anticarcinogenic activity is also shown by other monoterpenes, e.g.: carvone, carveol, germacren D, and geraniol. Their activity was confirmed during the studies upon breast, brain, uterine cervix, and lung cancers [Collins et al. 1985, Crowell et al. 1992, Buhagiar et al. 1999, El-Sawi et al. 2007].

CONCLUSIONS

1. Hazel leaves contained over twice as much essential oils as compared to buds.
2. Content of essential oils in hazel buds amounted from 0.48% to 0.50%, while in leaves from 0.98% to 1.01%.
3. Remarkable differences between studied oils with regard to their physicochemical properties did not proved.
4. The oils were characterized by density from 0.776 to 0.872, rotation from -2° to -27° , and refraction index from 1.4787 to 1.504.
5. The GC/MS analysis allowed for detecting 43 compounds in buds, among which 35 were identified, as well as 42 compounds in leaves, including 37 identified ones.
6. Following compounds dominated in the oil made from hazel buds: nerol, myrtenol, α -campholenol and p-cymene, while hazel leaves contained prevailing amounts of: nerol, myrtenol, α -campholenol, menthol, geraniol, and limonene.

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SKŁAD CHEMICZNY OLEJKU ETERYCZNEGO Z PĄKÓW I LIŚCI LESZCZYNY UPRAWNEJ (*Corylus L.*)

Streszczenie. Porównano skład jakościowy i ilościowy olejku eterycznego otrzymanego metodą bezpośredniej destylacji z parą wodną z pąków i liści roślin uprawnej leszczyny wielkoowocowej. Do badań użyto pąków, których zbiór przeprowadzono w okresie bezlistnym, tj. w kwietniu, natomiast liści w czerwcu 2006 r. Określono profil właściwości fizykochemicznych uzyskanych olejków, tj. gęstość d_{20} (g · ml), współczynnik załamania światła i skręcalność optyczną. Metodą chromatografii gazowej/spektrometrii masowej stwierdzono obecność 43 związków w pąkach, z których zidentyfikowano 35 oraz 42 związków w liściach, z których zidentyfikowano 37. Głównymi składnikami badanych olejków eterycznych były węglowodory i alkohole monoterpene. Dominującym związkami w kompozycji olejku z pąków były: nerol (20,6%), myrtenol (19,5%), α -kamfolenol (4,7%) i p-cymen (3,7%). Natomiast głównymi składnikami olejku z liści leszczyny były: nerol (13,0%), myrtenol (9,4%), α -kamfolenol (9,0%), mentol (6,7%), geraniol (4,8%) i limonen (3,9%). Stwierdzono różnice jakościowe pomiędzy składnikami olejków obu badanych surowców.

Słowa kluczowe: *Corylus L.*, substancje aromatyczne, monoterpene, analiza GC/MS, części anatomiczne

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