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PRELIMINARY STUDY ON THE COMPOSITION OF VOLATILE FRACTION OF FRESH FLOWERS AND LEAVES OF *ROBINIA PSEUDOACACIA* L. GROWING IN POLAND

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Abstract: The essential oils obtained by hydrodistillation from fresh flowers and leaves of *Robinia pseudoacacia* L. were analyzed by GC-FID-MS in respect to their chemical composition. Eighty volatile compounds (96.3% of the oil) contained in the flowers and sixty-three compounds (97.5%) derived from the leaves were identified. The major components in the flower oil were linalool (20.4%), 3-methyltetradecane (16.5%), hexahydrofarnesyl acetone (14.9%) and 1-octen-3-ol (13.4%). The leaf oil comprised an abundance of 1-octen-3-ol (57.9%), phytol (9.1%), (*Z*)-3-hexenyl acetate (6.0%) and (*Z*)-3-hexen-1-ol (5.2%).

Keywords: *Robinia pseudoacacia*, Fabaceae, essential oil composition, linalool, 1-octen-3-ol

The genus *Robinia* L. (Fabaceae) commonly known as locust comprises 10 species of trees and shrubs characterized by white or pink flowers with intensive, distinctive, sweet aroma (1, 2). *Robinia pseudoacacia* L. (black locust), originally native to the south-eastern USA, is widely distributed as wild and cultivated species growing in temperate regions throughout the world (1-3). As a species with little requirements, it has also adapted successfully to a diverse range of habitats in Poland (4). It is extensively used as a shade tree or for ornamental purposes. Black locust as large-sized tree, growing to around 20 meters tall can be distinguished by numerous white flowers, grouped in pendulous racemes, and pinnate leaves with nine to nineteen leaflets and a pair of short thorns at the base (1-3). Mainly the flowers of black locust are used in traditional medicine as diuretic, spasmolytic, sedative and cholagogic agents and relieve inflammation of the kidneys and biliary ducts (5). This biological activity can be connected with the presence of a non-volatile fraction which includes flavonoids such as robinin, biorobin, rhamnorobin, acaciin and other triglycosides of apigenin, luteolin and diosmetin with glucose and rhamnose (6-10). Moreover, the presence of coumarins (11), sterols (12) and fatty acids (13) has been reported.

The available literature indicates that black locust flowers, attracting a special interest due to its gentle fragrance, contain the essential oil whose composition depends on geographical region. The major volatile constituents of the flowers varied greatly among samples collected in China. According to Xie et al. linalool, *(Z)*-β-ocimene, *(E)*-β-bergamotene and formanilide are the main constituents isolated from the flowers by SPME method (14). In the essential oil obtained by Long et al. phenylethyl alcohol, linalool and geraniol were recognized as predominant compounds (15). Considering the results revealed by Wang et al. the main ones were limonene and γ-terpinene (16). The oil from the flowers grown in Iran was dominated by ethyl hexanoate, heptadecane and virdiflorol (17), whereas in the oil from the flowers collected in the USA, the main constituents were δ-3-carene, linalool and anthranilic aldehyde (18). This wide range of constituents found in the literature prompted the present study, whose aim is to determine the volatile constituents released from fresh flowers and leaves of *Robinia pseudoacacia* L. collected in Poland.

EXPERIMENTAL

Plant material

Fresh flowers and leaves of *Robinia pseudoacacia* L. were collected by hand in May 2012 from a ten-year-old black locust tree naturally growing in central Poland (Łódź). The voucher specimens

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(RPA-F12 and RPA-L12), identified and authenticated by Professor M. Wolbiś, were deposited in the Herbarium of the Department of Pharmacognosy, Medical University of Łódź.

Essential oil isolation

Fresh flowers (800 g) and leaves (700 g) were hydrodistilled separately for 3 h using a Clevenger type apparatus with *n*-pentane (1 mL) as collecting solvent. The essential oils were dried over anhydrous sodium sulfate and stored at 4°C until analyzed.

Analysis of essential oil

GC-FID-MS analyses were performed using a Trace GC Ultra apparatus (Thermo Electron Corporation) equipped with FID and MS DSQ II detectors and FID-MS splitter (SGE). Apolar capillary column Rtx-1MS (Restek), 60 m × 0.25 mm i.d., film thickness 0.25 μm; temperature program, 50-300°C at 4°C/min, SSL injection temperature 280°C, FID temperature 300°C, split ratio 1 : 20, carrier gas helium at a regular pressure 300 kPa. Polar capillary column TGWax-Gold (Thermo Scientific), 30 m × 0.25 mm i.d., film thickness 0.25 mm, temperature program 50-240°C (30 min) at 4°C/min; SSL injection temp. 250°C FID temperature 260°C; carrier gas, helium; 0.8 mL/min; split ratio 1 : 20. Mass spectra were acquired over the mass range 30-400 Da, ionization voltage 70 eV, ion source temperature 200°C.

Individual components were identified by the comparison of their MS spectra and GC retention indices (relative to *n*-alkane C_8-C_{26}) with those stored in NIST 98.1, Wiley Registry of Mass Spectral Data, 8th edn. and Mass Finder 3.1 libraries. The relative amount of the individual components was calculated from peak area without applying an FID response factor correction.

RESULTS AND DISCUSSION

The hydrodistillation of fresh flowers and leaves of *Robinia pseudoacacia* L. gave only trace yield of the oils, thus the volatiles were trapped in *n*-pentane. The previous report indicated that the flowers of *R. pseudoacacia* are poor in essential oil whose content is less than 0.001 mL/kg (19). In the present study, ninety-nine essential oil compounds were identified according to their retention indices (RI) and mass spectra. The list of identified volatile constituents, their retention indices (RI) and the percentages, in order of their elution from Rtx-1MS column are given in Table 1.

Chemical analysis of the flower essential oil revealed the presence of eighty components, representing 96.3% of the total oil. In the flowers, the monoterpene fraction amounted to 30.1% of the total composition and was particularly characterized by linalool (20.4%) with the following predominant compounds: geraniol (2.0%), terpinen-4-ol (1.7%) and α-terpineol (1.5%). The sesquiterpene fraction (13.1%) consisted of sesquiterpene alcohols and hydrocarbons with (*E,E*)-farnesol (4.6%), (*E*)-nerolidol (2.3%), (*E,E*)-α-farnesene (2.0%), β-bisabolol (1.5%) and (*E,Z*)-α-farnesene (1.1%) being the main compounds. Considerable was the content of hydrocarbons (20.2%) and carbonyl compounds (16.0%), particularly represented by 3-methyltetradecane (16.5%) and the C-18 ketone hexahydrofarnesyl acetone (14.9%). Other components of the essential oil were aliphatic alcohols (14.2%) including 1-octen-3-ol (13.4%) as the predominant compound.

The gentle fragrance characteristic of *R. pseudoacacia* flowers is mainly due to the presence of large amounts of linalool, 1-octen-3-ol and hexahydrofarnesyl acetone in the essential oil. Linalool depending on isomeric forms (3*R*-(-)- or 3*S*-(+)-linalool) has a lavender, lily-of-the-valley or more herbaceous and musty green odor, respectively. Furthermore, the enantiomeric distribution of linalool showed that 3*R*-(-) form is much more common in essential oils than its second isomer (20). Hexahydrofarnesyl acetone is associated with long lasting fresh jasmine fragrance (21, 22). In contrast, 1-octen-3-ol that has been cited as a marker for rotten grapes and wines, constitutes one of the main characteristic fungal odor (23, 24).

In the case of the leaf essential oil, sixty-three components were identified, which represented 97.5% of the total detected constituents. In the leaves, aliphatic alcohols $C_6 – C_{16}$ (65.1%) prevailed, particularly unsaturated chain compounds such as 1-octen-3-ol (57.9%) and (*Z*)-3-hexen-1-ol (5.2%). Besides, the leaves were characterized by the presence of (*E*)- and (*Z*)-3-hexen-1-ols and their ester derivatives (6.5%), which were not detected in the flowers. Within this group, (*Z*)-3-hexenyl acetate (6.0%), (*Z*)-3-hexenyl butyrate (0.1%), (*Z*)-3-hexenyl lactate (0.1%), (*Z*)- and (*E*)-3-hexenyl caproates (0.1%), (*Z*)-3-hexenyl benzoate (< 0.05%) and (*Z*)-3-hexenyl salicylate (< 0.05%) were identified. The other main components of leaf essential oil were terpenes (17.4%), among which monoterpene and sesquiterpene fractions amounted to 7.7% and 0.6%, respectively. The monoterpenes were characterized by a high content of linalool (3.5%) and geraniol (3.3%). Also considerable were phytol (9.1%) and hexahydrofarnesyl acetone contents (1.2%).

Table 1. Retention indices (RI) and the percentage composition of identified compounds in the essential oils from the flowers and leaves of *Robinia pseudoacacia* L.

Compound[a]	**RI_{Rtx}**[b]	**RI_{lit}**[c]	**RI_{Wax}**[b]	**Flowers**	**Leaves**
E)-3-hexen-1-ol	837	840	1363	–	0.1
(*Z*)-3-hexen-1-ol	840	846	1384	–	**5.2**
Hexanol	854	860	1351	0.1	0.6
(*E*)-3,5,5,-trimethyl-2-hexene	960	968	1484	–	0.9
1-Octen-3-ol	968	962	1452	**13.4**	**57.9**
2,3-Dehydro-1,8-cineole	979	985	1192	0.4	–
3-Octanol	984	981	1391	0.1	0.4
(*Z*)-3-hexenyl acetate	991	987	1320	–	**6.0**
Decane	1000	1000	1000	tr	0.1
α-Terpinene	1009	1013	1183	0.1	–
Limonene	1022	1025	1220	tr	tr
(*Z*)-β-ocimene	1029	1029	1237	tr	–
(*E*)-β-ocimene	1039	1041	1254	0.9	–
γ-Terpinene	1049	1051	1248	0.2	–
trans-Sabinene hydrate	1054	1053	1465	0.1	–
(*E*)-2-octen-1-ol	1054	1059	1614	0.2	0.5
Octanol	1058	1063	1555	–	0.6
trans-Linalool oxide (furanoid)	1058	1058		0.6	–
cis-Linalool oxide (furanoid)	1073	1072	1473	0.1	tr
Terpinolene	1079	1082	1287	0.1	–
Nonanal	1084	1084	1398	0.3	0.7
Linalool	1090	1086	1550	**20.4**	3.5
Undecane	1100	1100	1100	tr	tr
trans-p-Mentha-2,8-dien-1-ol	1105	1113	1628	0.2	–
cis-p-Mentha-2-en-1-ol	1107	1108	1565	0.2	–
cis-p-Mentha-2,8-dien-1-ol	1118	1125	1670	0.1	–
trans-p-Mentha-2-en-1-ol	1124	1123	1622	0.1	–
cis-Verbenol	1126	1132	1657	0.1	–
trans-Verbenol	1129	1136	1678	0.5	–
p-Mentha-1,5-dien-8-ol	1148	1148	1727	0.2	–
1,4-Dimethyl-δ-3-tetrahydroacetophenone	1155	1152	1565	0.1	0.1
Nonanol	1159	1156	1657	0.1	0.3
Terpinen-4-ol	1163	1164	1604	1.7	0.1
(*Z*)-3-hexenyl butyrate	1170	1170	1465	–	0.1
α-Terpineol	1174	1176	1698	1.5	0.4
Safranal	1176	1182	1652	–	0.1
Decanal	1186	1180	1503	0.5	0.5
(*E,E*)-2,6-dimethyl-3,5,7-octatriene-2-ol	1189	1187	1821	0.2	–
Nerol	1212	1210	1799	0.4	0.1
(*Z*)-3-hexenyl lactate	1217	1187	1722	–	0.1
Geraniol	1238	1235	1845	2.0	3.3
Decanol	1262	1259	1759	–	tr
Dihydroedulan II	1284	1290	1524	0.2	0.1

Table 1. Cont.

Compound[a]	**RI_{Rtx}**[b]	**RI_{lit}**[c]	**RI_{Wax}**[b]	**Flowers**	**Leaves**
Theaspirane (isomer 1)	1291	1299	1503	0.1	0.4
Tridecane	1299	1300	1299	tr	tr
Theaspirane (isomer 2)	1305	1313	1545	0.1	0.4
Methyl anthranilate	1320	1328	2248	0.3	–
(*E*)-β-damascenone	1363	1363		tr	–
(*Z*)-3-hexenyl caproate	1364	1362	1655	–	0.1
(*E*)-3-hexenyl caproate	1366	1368	1660	–	0.1
(*Z*)-jasmone	1371	1371	1950	0.1	0.3
Hexahydropseudoionone	1388	1389	1689	0.1	–
Tetradecane	1399	1400	1406	–	tr
Geranyl acetone	1432	1434	1856	0.1	–
Sesquisabinene A	1436	1435	1644	0.1	–
(*E*)-β-farnesene	1447	1446	1665	0.1	–
3-Methyltetradecane	1468	1462	1443	**16.5**	0.4
β-Ionone	1468	1468	1944	–	0.1
γ-Curcumene	1473	1475	1690	0.2	–
(*E,Z*)- α-farnesene	1479	1475	1685	1.1	tr
trans-β-Bergamotene	1483	1480	1580	0.1	–
(*E,E*)-α-farnesene	1497	1498	1749	2.0	0.1
Pentadecane	1499	1500	1500	–	tr
β-Curcumene	1502	1503	1741	0.4	–
(*Z*)-γ-bisabolene	1508	1505	1730	0.1	–
(*E*)-α-bisabolene	1533	1530	1773	0.1	–
(*E*)-nerolidol	1549	1553	2039	2.3	0.3
(*Z*)-3-hexenyl benzoate	1554	1554	2131	–	tr
cis-Sesquisabinene hydrate	1566	1565	2084	tr	–
Hexadecane	1598	1600	1600	–	tr
trans-Sesquisabinene hydrate	1600	1598	2112	0.1	–
α-Acorenol	1620	1623	2170	0.2	–
(*Z*)-3-hexenyl salicylate	1651	1648	2272	–	tr
β-Bisabolol	1659	1659	2155	1.5	–
α-Bisabolol	1668	1673		0.1	–
(*Z,E*)-farnesol	1682	1687	2311	tr	–
Heptadecane	1698	1700	1700	–	tr
(*E,E*)-farnesol	1704	1710	2354	4.6	0.3
(*E,Z*)-farnesol	1708	1718		0.1	–
Octadecane	1798	1800	1798	0.1	0.1
Hexahydrofarnesyl acetone	1835	1823	2135	**14.9**	1.2
6,10,14-Trimethylpentadecan-2-ol	1841	1843	2096	0.2	–
Hexadecanol	1867	1866	2374	0.2	–
Nonadecane	1898	1900	1900	tr	tr
Methyl palmitate	1909	1909	2216	0.4	0.1
Hexadecanoic acid	1946	1942	2618	0.2	0.4
Methyl linolate	2073	2087		0.3	tr

Table 1. Cont.

Compound[a]	**RI_{Rtx}**[b]	**RI_{lit}**[c]	**RI_{Wax}**[b]	**Flowers**	**Leaves**
Methyl linolenate	2077	2092	2566	0.3	tr
Heneicosane	2099	2100	2096	0.1	0.1
Phytol	2102	2114	2601	0.5	**9.1**
Methyl stearate	2110	2113	2422	0.1	0.1
Stearyl acetate	2193	2208	2506	0.2	0.1
Docosane	2199	2200	2200	–	tr
Tricosane	2298	2300	2293	0.3	0.7
Eicosanyl acetate	2393	2406	2585	0.1	tr
Tetracosane	2399	2400	2392	tr	tr
Docosanal	2412	2426	2618	0.1	0.1
Pentacosane	2501	2500	2499	1.1	0.6
Hexacosane	2610	2600	2606	2.1	0.6
Total				**96.3**	**97.5**
Terpenes:				**43.7**	17.4
Monoterpenes				30.1	7.7
Sesquiterpenes				13.1	0.6
Diterpenes				0.5	9.1
Aliphatic alcohols				14.2	**65.1**
(*E*), (*Z*)-3-hexen-1-ol derivatives				–	6.4
Aliphatic hydrocarbons				20.2	3.7
Carbonyl compound				16.0	2.9
Others				2.2	2.0

[a] Compounds are listed in order of their elution from Rtx-1MS column [b] RI_{Rtx}, RI_{Wax} – retention indices determined on Rtx-1MS and TGWax-Gold columns, respectively. [c] Literature values. – Not detected, tr – trace (percentage value less than 0.05%)

The literature review showed that linalool, commonly found in fragrances and essential oils, accounted for 20.4% (the flowers) and 3.5% (the leaves) of the present oils has been the subject of numerous studies investigating its anti-inflammatory, spasmolytic, sedative, anesthetic and antimicrobial activity. Moreover, linalool could enhance the permeability of a number of drugs through biological tissues like skin or mucus membranes (25, 26). Hexahydrofarnesyl acetone, present in flower oil as one of the major compound (14.9%), could be responsible for its antifungal, antibacterial and cytotoxic activity (27, 28). 1-Octen-3-ol prevailing in the present leaf and flower oils (57.9, 13.4%, respectively) was also considered as bioactive compound and indicated acaricidal and antifungal activity (29, 30). Moreover, green leaf volatiles including (*Z*)-3-hexen-1-ols and their ester derivatives characteristic for leaf oil may act as host-plant attractants, fruit ripeness indicators or as defensive secretions (31, 32).

Comparing previously available data, this report confirms that the composition of the essential oil of *R. pseudoacacia* flowers collected in Poland is not similar to that obtained from various regions of China, Iran and the USA (14-18). Although certain constituents of the oils such as (*Z*)-β-ocimene, limonene and γ-terpinene, detected by other researches as predominant, were also identified in the Polish essential oil, their concentrations were less than 0.2%. Exclusively, linalool and geraniol, at concentrations of 3.1–33.1% and 1.0– 2.1%, respectively, were among the major components also detected in flower oils from Poland, China and the USA. The chemical variations of the essential oils of *R. pseudoacacia* flowers collected from different locations might have been due to environmental factors (geographical, climatic and seasonal), storage duration of plant material and type of the methods used to produce the essential oils. Due to the fact, that in the current study the volatile constituents were investigated for plant samples collected in one year and from a single plant area, the present results could be considered as preliminary and will require further comparative analyses for the flowers and leaves derived at different stages of plant development and from various geographical regions of Poland. Therefore, the studying variability of natural population of *R. pseudoacacia* in various countries may have a great role in reliable evaluation of the essential oil composition of this species.

CONCLUSIONS

Summing up, the essential oil of *R. pseudoacacia* flowers collected in Poland was found to be rich in linalool (20.4%), 3-methyltetradecane (16.5%), hexahydrofarnesyl acetone (14.9%) and 1-octen-3-ol (13.4%). The leaf oil, which was analyzed in *R. pseudoacacia* for the first time, contained greater amounts of 1-octen-3-ol (57.9%), phytol (9.1%), (*Z*)-3-hexenyl acetate (6.0%) and (*Z*)-3-hexen-1-ol (5.2%). The qualitative profiles of the essential oils extracted from the flower and leaf are largely similar even though the leaves additionally include aliphatic alcohols C_6 – C_{16} and their ester derivatives. The further comprehensive studies of essential oil of *R. pseudoacacia* flowers and leaves of Polish origin would be desirable to clarify the relationship between variations of the essential oil composition and various geographical factors as well as morphological features in natural Polish populations.

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REFERENCES

1. Hegi G.: Illustrierte Flora von Mittel Europa. Bd. IV/3, Lehmanns, München 1924.
2. Tutin T.G., Heywood V.H., Burges N.A., Moore D.H., Valentine D.H., Walters S.M., Webb D.A.: Flora Europea, 2, Cambridge: University Press 1968.
3. Isely D., Peabody F.J.: Castanea 49, 187 (1984).
4. Rahmonov O.: Acta Ecol. Sin. 29, 237 (2009).
5. Strzelecka H., Kowalski J.: The Encyclopedia of Herbs and Herbalism [in Polish]. p. 477, PWN, Warszawa 2000.
6. Farkas L., Vermes B., Nógrádi M., Kálmán A.: Phytochemistry 15, 215 (1976).
7. Wagner H., Aurnhammer G., Hörhammer L., Farkas L.: Chem. Ber. 102, 1445 (1969).
8. Tian F., McLaughlin J.L.: Pharm. Biol. 38, 229 (2000).
8. Demeshko O.V., Kovalyov S., Komissarenko S.N.: Zhur. Organ. Farm. Khim. 3, 58 (2005).
10. Veitch N.C., Elliott P.C., Kite G.C., Lewis G.P.: Phytochemistry 71, 479 (2010).
11. Bratoeff E., Beauregard Cruz J.J., Alcala R., Sanchez R., Capella S., Gomez H.: Rev. Mex. Cienc. Farm. 14, 141 (1984).
12. Takatsuto S.: Yukagaku 46, 419 (1997).
13. Demeshko O.V., Zhuravel I.A., Komissarenko A.N.: Visn. Farm. 2, 23 (2004).
14. Xie J., Sun B., Yu M.: Flavour Fragr. J. 21, 797 (2006).
15. Long Ch., Xin K., Wang L., Long H., Ding J., Xuejian Y.: Nat. Prod. Res. Dev. 3, 84 (1991).
16. Wang M., Jing Z., Chen S., Zhang Z., Shen Z.: Sepu 9, 182 (1991).
17. Sefidkon F., Asli-Roodsari M.A., Aghavali J.A.: J. Essent. Oil-Bear. Pl. 5, 169 (2002).
18. Kamdem D.P., Gruber K., Barkman T., Gage D.A.: J. Essent. Oil Res. 6, 199 (1994).
19. Dutu L.-E., Dinu M.: Farmacia 54, 70 (2006).
20. Özek T., Tabanca N., Demirci F., Wedge D.E., Baser K.H.C.: Rec. Nat. Prod. 4, 180 (2010).
21. Eltz T., Hedenström E., Bång J., Wallin E.A., Andersson J.: J. Chem. Ecol. 36, 1322 (2010).
22. Kashima Y., Nakaya S., Miyazawa M.: J. Oleo Sci. 63, 149 (2014).
23. Nosaka S., Miyazawa M.: J. Oleo Sci. 63, 577 (2014).
24. La Guerche S., Dauphin B., Pons M., Blancard D., Darriet P.: J. Agric. Food Chem. 54, 9193 (2006).
25. Peana A.T., D'Aquila P.S., Panin F., Serra G., Pippia P., Moretti M.D.: Phytomedicine 9, 721 (2002).
26. Radulović N., Stojanović G., Palić R.: Phytother. Res. 20, 85 (2006).
27. Radulović N., Dekić M., Stojanović Radić Z., Palić R.: Turk. J. Chem. 35, 499 (2011).
28. Razavi S.M., Nejad-Ebrahimi S.: Nat. Prod. Res. 24, 1704 (2010).
29. Yang J.Y., Lee H.S.: Pest Manag. Sci. 68, 564 (2012).
30. Okull D.O., Beelman R.B., Gourama H.: J. Food Prot. 66, 1503 (2003).
31. Reddy G.V., Guerrero A.: J. Agric. Food Chem. 48, 6025 (2000).
32. Wei J., Kang L.: Plant Signal Behav. 6, 369 (2011).

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