DOI 10.34658/bfs.2019.83.1.87-96

Biotechnology and Food Science

Research article

Composition of leaf and flower essential oil of *Myrica gale* L.

Karolina Wawrzyńczak*, Alicja Jakiel, Danuta Kalemba

Institute of General Food Chemistry, Lodz University of Technology
Stefanowskiego 4/10, 90-924 Lodz

*karolina.wawrzynczak@edu.p.lodz.pl

Received: 11 March 2019 / Available on-line: 8 April 2019

Abstract: *Myrica gale* L. *leaves were collected from two plantations in Poland and flowers from one plantation. Essential oil yield and composition were assessed according to plantation site and leaf development stage. Main components of essential oils were: 1,8-cineole, α-pinene, limonene, selina-3(7)-diene, and (E)-nerolidol. It is proven that senescent leaves are valuable source of essential oil.*

Keywords: *sweet gale, plant part, vegetation stage, GC-MS, NMR*

Introduction

Myrica gale L., called myrique baumier or sweet gale, is a species of aromatic shrub in the family Myriaceae. *M. gale* is found world-wide in the northern hemisphere. It is widely spread at higher latitudes. In Europe it is distributed in the east-central Highlands and is often associated with oceanic climates [1]. In Poland it is the only representative of the Myriaceae family. It occurs naturally only in the narrow coastal strip of Baltic sea [2]. It is protected by law; however, it can be successfully cultivated.

M. gale reaches up to 2 m high [3]. It is, for the most part, dioecious, but within a single population monoecious, and hermaphrodite flowers may also occur [4]. Plants may change sex from year to year. Its main stems are brown. Twigs are reddish-brown with scattered, shiny, yellowish glands, becoming dark. Leaves are oblanceolate or oblong-obovate. Flowers are borne on the bare wood of the previous year's growth and appear before the leaves, usually in May-June. Male catkins are unbranched,10 mm long, with red-brown bracts. Female catkins are slightly smaller (6-7 mm long), but thicker and closely set with green bracts. Fruit is a dry resinous two-winged drupe [3].

Although *M. gale* has very limited value in terms of wood production it has found many applications, for example being used in programs of reclamation and stabilization of peatlands or possibly to increase forest productivity in wet soils, because of its potential as a dinitrogen-fixing plant [4]. Moreover, dried leaves and fruits have been used as a spice in soups and stews and as a flavouring for beer; roots and bark are used as a source of yellow dye for calfskin and wool;

catkins and fruits as a source of wax for candles; and leaf and fruit infusions as an insecticide. It has been also used in traditional medicine as a remedy for stomach and cardiac disorders. Furthermore, use as an abortifacient has been reported [3].

Research concerning essential oil composition of *M. gale* from different origins revealed considerable differences as was shown previously by Svoboda *et al.* in 1998 [3] and is presented according to the more recent research in Table 1.

Table 1. Composition of essential oil of *Myrica gale* depending on different part plants and different places (%)

Compound	Canada	Japan	Finland		Scotland		France	
	leaf [5]	leaf [6]	leaf[a] [3]	flower [3]	leaf[a] [3]	flower [3]	leaf [7]	fruit [7]
α-Pinene	2.2	1.9	17.4	28.1	32.2	38.9	12.2	22.6
β-Pinene	0.2	5.0	1.7	1.8	3.4	3.9	-	-
Myrcene	11.3	-	-	-	-	-	-	-
α-Phelandrene	7.1	1.3	-	-	-	-	8.0	1.7
α-Terpinene	0.3	0.6	5.5	6.2	0.4	3.8	-	-
p-Cymene	3.3	-	-	-	-	-	2.0	2.4
Limonene	5.3	9.8	11.4	5.3	6.5	3.4	8.1	-
1,8-Cineole	0.3	7.3	18.7	14.7	3.7	6.0	5.3	18.9
(Z)-β-Ocimene	2.1	-	1.9[b]	2.1[b]	2.6[b]	2.6[b]	-	-
(E)-β-Ocimene	2.2	-	-	-	-	-	-	-
α-Terpineol	-	-	4.7	0.2	1.3	1.7	-	-
(*E*)-β-Caryophyllene	8.4	0.4	-	-	-	-	-	-
α-Humulene	2.9	-	-	-	-	-	-	-
β-Selinene	2.9	-	-	-	-	-	-	-
γ-Cadinene	-	-	8.4	12.8	-	-	-	-
Selina-3,7(11)-diene	-	-	-	-	-	-	0.2	2.7
(*E*)-Nerolidol	1.1	1.7	1.4	1.1	4.9	0.8	-	-
Eudesm-11-en-1-ol	11.5	11.0	-	-	-	-	-	-
β-Elemenone	-	13.3	2.2	3.0	1.7	4.3	-	-
Germacrene					6.2[b]	0.4[b]		
Germacrone	4.5	-	-	-	-	-	25.1	14.2
EO yield	-	-	0.13	1.46	0.12	0.97	0.38	1.44

[a] leaves collected in September
[b] isomer not specified

Irregular variation in leaf volatiles composition within a population [8] as well as during vegetation period were reported [3]. Independently of the plant part (leaf, flower, fruit) monoterpene hydrocarbons (α-pinene, limonene) and

1,8-cineole are found in all samples and belong to the main constituents. Leaf essential oil from wild population of *M. gale* in Scotland and Finland collected throughout five month period in eight development stages had the same monoterpene hydrocarbons (up to 28.1% and 17.4% in Scotland and Finland, respectively) and 1,8-cineole (up to 23.9% and 18.7%). The main difference concerned the content of sesquiterpenes; germacrene predominated in Scottish sample (up to 13.2%) and γ-cadinene in Finnish sample (up to 21.0%) [3]. Other compounds that were identified in pronounced amounts in essential oils from other sites were β-caryophyllene, 8.4% [9], eudesm-11-en-1-ol, 11.5%, 11.0% [5, 6], β-elemenone 2.2-13.3%, and germacrone 4.5-25.1%) [7,9,10].

Essential oil of *M. gale* leaves has presented anticancer activity against human lung carcinoma A-549 and colonadenocarcinoma DLD-1 cell lines [5], antifungal activity [11], and repellent effects on midges [3]. Fruit essential oil with composition similar to leaf oil was proven to have antifungal activity against food spoilage agents such as *Penicillium expansum, Cladosporium cladosporioides, Aspergillus flavus* [7].

In this study composition of leaf and flower essential oil of *M. gale* cultivated in two places in Poland was investigated in order to determine influence of time and place of cultivation on the essential oil quality.

Experimental

Materials

M. gale leaves and male flowers were collected from ecological plantations Biofarm in Northern Poland (Pomerania) and commercial plantation Future Gardens Burdziałowska in Silesia, Poland. Leaves were collected in three development stages: young leaves (July 2014), matured leaves (September 2014), falling partly yellowish leaves (October 2014). Flowers were collected in June 2014. Materials were dried at room temperature.

Methods

Hydrodistillation of essential oils

Essential oils from dried leaves (100 g) and flowers (50 g) were isolated by hydrodistillation in Clevenger-type apparatus for 4 hours.

Isolation of essential oil components

To isolate the compounds of interest, the leaf oil (1.1 g) was subjected to flash-chromatography (FC) on silica gel 60 (0.040-0.063 mm, Merck) with pentane and increasing amounts of diethyl ether. The separation was monitored by TLC and GC-MS. The following fractions were obtained: 1 (283 mg), selina-3,7(11)-diene (25%), α-pinene-(8%), limonene (8%), *p*-cymene (5%); 2 (320 mg), 1,8-cineole (17%), germacrone (20%), β-elemenone (15%), α-terpinyl acetate (7%), benzyl benzoate (5%); 3 (247 mg), (*E*)-nerolidol (19%), terpinen-4-ol (8%), selin-7(11)-en-4-ol (7%); 4 (97 mg), α-terpineol (10%), citronellol (8%). Fractions were subjected to NMR analysis.

GC-FID-MS analysis

Constituents of essential oils were analyzed by gas chromatography coupled with mass spectrometry (GC-FID-MS) using Trace GC Ultra gas chromatograph with FID and DSQ II mass spectrometer (Thermo Electron Corporation, Italy). Operating condition: apolar capillary column Rtx-1ms (60 m × 0.25 mm, 0.25 m film thickness), programmed temperature: 50 (3 min)–300°C, 4°C/min, injector (SSL) temperature 280°C, detector (FID) temperature 300°C, transfer line temperature 250°C, carrier gas – helium, flow with constant pressure 200 kPa, split ratio 1:20. The mass spectrometer parameters: ion source temperature 200°C, ionization energy 70 eV (EI), scan mode: full scan, mass range 33–420. The percentages of constituents were computed from the GC peak area without using a correction factor. Identification of components was based on comparison of their linear retention indices (RIs) and MS spectra stored in computer libraries NIST 98.1, Wiley 275.1 and MassFinder 4.1. Retention indices were determined with reference to a series of n-alkanes C8-C26.

NMR analysis

The ^{1}H-NMR spectra were recorded on a Bruker Avance II 400 spectrophotometer at 400 MHz, using $CDCl_3$ as solvent and TMS as internal standard. Identification was based on comparison of spectra with literature data or a homemade NMR data base.

Results and Discussion

Both leaves and flowers of *M. gale* yield pale green essential oil with fresh, green, herbal smell. The oil yield and composition were presented in Table 2. Flower oil yield (1.23%) was significantly higher than leaf oil (0.13-0.33%). The results were similar to previously reported (Table 1). Both yield and composition of leaf oil were more dependent on the cultivation place than on the stage of development. Main constituents of all investigated leaf and flower essential oils were monoterpenes: 1,8-cineole, α-pinene and limonene as well as sesquiterpenes: (*E*)-nerolidol, selina-3,7(11)-diene, and germacrone. GC-MS identification of these compounds and other main constituents was confirmed by ^{1}H-NMR analysis of fractions obtained by flash chromatography of the leaf oil. All main compounds were previously identified in sweet gale essential oil of different origin. In presented research more detailed analysis enabled identification of 60-80 constituents that amounted to more than 90% in individual oils.

Slight increase of monoterpene hydrocarbons, and quite stable content of other groups of constituents (oxygenated monoterpenes and sesquiterpenes) were observed during leaf development. The only exception was (*E*)-nerolidol. Content of this sesquiterpene alcohol in leaf oil from Pomerania in two stages (3.0%) was similar as in other research (Table 1).

Table 2. Composition of *Myrica gale* leaf and flower essential oils according to place of cultivation and time of collection (%)

No.	Compound	$RI_{exp.}$	$RI_{lit.}$	Pomerania		Silesia			
				Leaves					Flowers
				Mature	Senescent	Young	Mature	Senescent	
1	α-Thujene	930	932	0.1	0.2	tr	0.2	0.1	0.1
2	**α-Pinene [a]**	**933**	**936**	**15.9**	**17.9**	**9.6**	**10.1**	**11.2**	**6.5**
3	Camphene	948	950	0.7	0.7	0.3	0.3	0.4	0.3
4	Sabinene	969	973	tr	tr	tr	0.1	0.1	tr
5	β-Pinene	975	978	1.4	1.8	0.8	1.2	1.1	1.2
6	Myrcene	983	987	1.2	2.2	1.4	1.7	1.4	1.3
7	α-Phellandrene	999	1002	3.1	4.5	2.2	3.5	2.6	4.4
8	3-Carene	1006	1010	0.8	0.8	-	-	-	-
9	α-Terpinene	1009	1013	0.4	0.6	0.4	0.4	0.4	0.9
10	***p*-Cymene[a]**	**1013**	**1015**	**4.8**	**3.8**	**3.8**	**3.3**	**3.6**	**2.1**
11	**1,8-Cineole[a]**	**1022**	**1024**	**18.1**	**18.6**	**27.1**	**16.2**	**19.4**	**20.9**
12	**Limonene [a]**	**1024**	**1025**	**6.5**	**6.0**	**3.6**	**6.0**	**5.0**	**-**
13	(*Z*)-β-Ocimene	1029	1029	1.5	2.6	5.0	3.9	3.5	5.2
14	(*E*)-β-Ocimene	1039	1041	0.5	0.9	1.5	1.1	1.2	1.2
15	γ-Terpinene	1050	1051	1.5	2.3	2.8	2.2	2.3	2.0
16	α-Terpinolene	1079	1082	0.3	0.5	0.4	0.4	0.3	0.7
17	Nonanal	1082	1076	-	0.2	0.1	0.1	0.1	0.1
18	Linalol	1084	1086	0.2	0.3	0.2	0.5	0.4	0.2
20	Fenchol	1098	1099	0.1	0.1	0.1	0.1	0.1	-

21	Campholene aldehyde	1104	1105	-	tr	-	tr	-	-
22	*cis-p*-Menth-2-en-1-ol	1106	1108	0.1	0.1	0.1	0.3	0.2	0.3
23	*allo*-Ocimene	1116	1113	0.1	0.1	0.1	0.1	0.2	0.1
24	*trans-p*-Menth-2-en-1-ol	1123	1116	0.1	0.1	0.1	0.2	0.2	0.2
25	Isopulegol	1129	1132	-	tr	-	0.1	tr	-
26	*cis-p*-Mentha-2,8-dien-1-ol	1128	1125	-	-	-	-	-	0.1
27	Camphene hydrate	1139	1143	0.1	tr	0.1	0.1	0.1	0.1
29	Isoborneol	1142	1142	-	0.1	-	tr	-	-
30	Borneol	1149	1150	0.4	0.2	0.3	0.2	0.4	0.1
31	δ-Terpineol	1159	1155	-	tr	-	0.1	0.1	-
32	Terpinen-4-ol[a]	1163	1164	1.8	1.5	2.8	1.8	2.0	2.7
33	Methyl salicylate	1169	1172	-	0.1	-	-	-	-
34	α-Terpineol[a]	1174	1176	1.9	1.4	3.2	1.9	2.1	1.4
35	*cis*-Piperitol	1180	1181	tr	tr	tr	0.1	tr	-
36	*cis*-Sabinol	1182	1179	0.2	-	0.1	0.1	0.1	-
37	*trans*-Piperitol	1190	1193	0.1	tr	0.1	0.1	0.1	-
38	β-Cyclocitral	1196	1195	tr	tr	-	tr	-	-
39	Fenchyl acetate	1208	1205	tr	-	tr	0.1	-	0.1
40	Citronellol[a]	1210	1213	0.1	0.3	0.1	0.8	0.5	tr
41	Piperitone	1226	1226	-	tr	tr	tr	-	tr
42	Bornyl acetate	1269	1270	0.5	0.4	0.2	0.2	0.2	0.5
44	Terpinen-4-yl acetate	1283	1286	0.1	tr	tr	tr	tr	0.2
45	δ-Terpinyl acetate	1298	-	0.1	-	tr	tr	0.1	0.2

46	α-Terpinyl acetate [a]	1332	1335	1.4	1.2	1.0	1.4	1.4	4.0
47	Neryl acetate	1343	1342	-	-	-	-	-	0.1
48	(*E*)-Methyl cinnamate	1351	1354	-	-	0.1	0.1	0.1	0.2
49	Geranyl acetate	1359	1362	0.1	0.1	-	0.1	0.1	tr
51	α-Ylangene	1372	1376	0.1	tr	tr	tr	tr	0.1
52	α-Copaene	1376	1379	0.2	0.3	0.1	0.2	0.1	0.1
54	β-Elemene	1388	1389	0.1	0.1	0.1	0.1	0.1	tr
55	α-Gurjunene	1411	1413	0.1	-	-	0.1	0.1	tr
56	β-Caryophyllene	1418	1421	0.6	0.6	0.3	0.5	0.3	1.2
57	γ-Elemene	1429	1429	0.8	0.7	0.7	0.9	1.2	1.6
58	Aromadendrene	1447	1443	-	-	tr	-	-	0.1
59	β-Jonone	1465	1468	0.1	tr	-	-	-	tr
60	Cadina-1(6),4-diene	1471	1472	-	-	-	-	-	0.2
61	*cis*-β-Guaiene	1478	1488	-	0.2	0.2	0.1	0.2	0.2
62	γ-Muurolene	1474	1474	0.3	0.1	0.1	0.1	0.1	0.2
63	*cis*-Eudesma-6,11-diene	1480	1484	0.2	0.1	-	0.1	0.2	0.2
64	β-Selinene	1483	1486	0.5	0.5	0.9	0.3	0.5	0.4
66	δ-Selinene	1486	1490	-	tr	0.1	tr	0.1	0.1
67	α-Selinene	1493	1494	0.9	0.9	0.7	0.7	0.9	0.6
68	γ-Cadinene	1508	1507	0.2	0.1	-	tr	0.1	-
69	*cis*-Calamenene	1514	1517	0.3	0.3	0.1	0.3	0.1	0.1
70	δ-Cadinene	1517	1520	1.2	1.5	0.6	1.5	0.8	1.0
71	Guaia-3,9-diene	1519	1522	-	0.1	0.1	0.7	-	-

72	β-Cadinene	1522	1526	-	0.7	0.6	0.1	0.7	0.6
73	**Selina-3,7(11)-diene** [a]	**1542**	**1542**	**6.9**	**6.3**	**6.6**	**5.9**	**6.8**	**11.9**
74	**(*E*)-Nerolidol**[a]	**1554**	**1553**	**3.0**	**3.0**	**tr**	**16.5**	**12.4**	**1.7**
75	Spathulenol	1571	1572	0.4	0.1	0.7	-	tr	-
76	Caryophyllene epoxide	1573	1578	0.3	0.2	-	0.2	0.3	0.1
77	Gleenol	1575	1574	-	-	-	0.2	-	-
78	β-Elemenone [a]	1586	1589	3.0	1.3	1.9	0.5	1.5	3.6
79	1-*epi*-Cubenol	1620	1623	0.6	0.4	0.2	0.6	0.2	-
83	α-Eudesmol	1655	1653	0.6	0.2	0.2	0.3	0.2	0.7
84	**Germacrone**[a]	**1679**	**1684**	**4.9**	**4.9**	**6.9**	**2.8**	**2.6**	**8.9**
85	Selin-7(11)-en-4-ol[a]	1680	1676	0.9	0.5	0.7	0.8	1.5	1.0
86	Benzyl benzoate[a]	1727	1730	-	0.4	1.4	0.9	1.0	0.3
87	Phytol	2110	2100	0.1	0.3	0.1	0.7	0.1	-
88	Alkanes C22-C30			-	0.7	-	0.4	0.3	0.4
	Total identified			90.5	94.1	90.9	94.6	93.5	92.7
	Monoterpene hydrocarbons			38.8	44.9	31.9	34.5	33.4	26.0
	Oxygenated monoterpenes			7.3	5.9	8.5	8.3	8.2	10.4
	Sesquiterpene hydrocarbons			12.4	12.5	11.2	11.6	12.3	18.6
	Oxygenated sesquiterpenes			13.8	11.0	12.0	22.8	19.7	16.3
	EO yield [%]			*0.16*	*0.14*	*0.33*	*0.13*	*0.29*	*1.23*

tr < 0.05%; [a]- identified additionally by ^{1}H-NMR

On the contrary, in Silesia, (*E*)-nerolidol was present in trace amount in young leaf oil and in small amount in flower oil (1.7%), while abundant in mature and senescent leaf oil (up to 16.5%). It is worth mentioning that nerolidol has floral, fruity, woody flavour with green notes and is used in perfumery. Recently, various pharmacological and biological activities of nerolidol were reviewed [12]. Svoboda *et al.* [3] did not observe any regularity in constituent percentages during vegetation period of sweet gale in Scotland and Finland. This may be explained by natural origin of plant material, what is supported by finding of Carlton *et al.* [8] that shown variation of leaf volatiles within population. It is important to mention, that leaves and flowers for our research were collected from 5-8 randomly selected plants from two plantations. Hence, it can be concluded that cultivated *M. gale* plants did not differ significantly within plantations. However, some differences between plantations were observed.

Leaf essential oil was compared in three stages of development. The collection of young and mature leaves for essential oil production can adversely influence plant development. Hence, it was interesting to learn that senescent, yellowish, partly fallen leaves are as good source of essential oil as mature leaves. The oil yield was a little lower and only slight differences were observed in essential oil composition between mature and senescent leaves.

Flower essential oil differed from leaf oil in terms of both yield and composition. It could be stated that, although the oil yield is higher, using sweet gale flowers as a source of essential oil is not economically justified because of relatively small amount of flowers.

Conclusion

It was proven that place of plant cultivation as well as time of leaf collection influenced both the yield and composition of *M. gale* essential oil only to small extent. It is important that senescent leaf can be a good source of this essential oil. Literature up to this point can be a good basis for further research in this topic.

References

[1] Skene K, Sprent J, Raven J, Herdman L. *Myrica gale* L. J Ecol **2000**, 88:1079-1094.
[2] Ciaciura M, Stępień E. Distribution and actual state of *Myrica gale* L. population on the Szczecin Coastland area. Acta Biol **2007**, 14: 54-61.
[3] Svoboda K, Inglis A, Hampson J, Galambosi B, Asakawa Y. Biomass production, essential oil yield and composition of *Myrica gale* L. harvested from wild populations in Scotland and Finland. Flavour Fragr J **1998**, 13:367-372.
[4] Davey A, Gibson C. Note on the distribution of sexes in *Myrica gale*. New Phytologist **1917**, 74:147-151.
[5] Sylvestre M, Legault J, Dufour D, Pichette A. Chemical composition and anticancer activity of leaf essential oil of *Myrica gale* L. Phytomedicine **2005**, 12:299-304.

[6] Nakata M, Myoda T, Wakita Y, Sato T, Tanahashi I, Toeda K, Fujimori T, Nishizawa M. Volatile components of essential oil from cultivated *Myrica gale* var. *tomentosa* and its antioxidant and antimicrobial activities. J Oleo Sci **2013**, 62:755-762.
[7] Popovici J, Bertrand C, Bagnarol E, Fernandez MP, Comte G. Chemical composition of essential oil and headspace-solid microextracts from fruits of *Myrica gale* L. and antifungal activity. Nat Prod Res **2008**, 22:1024-1032.
[8] Carlton R, Waterman P, Gray A. Variation of leaf gland volatile oil within a population of sweet gale (*Myrica gale*) (*Myricaceae*). Chemoecology **1992**, 3:45-54.
[9] Sylvestre M, Legault J, Lavoie S, Pichette A. Investigation of leaf essential oil of *myrica gale* L. from Quebec: purification and analysis of oxygenated fractions. J Essent Oil Res **2006**, 18:38-41.
[10] Tavares F, Abreu I, Salema R. Regeneration of the actinorhizal plant *Myrica gale* L. from epicotyl explants. Plant Sci **1998**, 135:203-210.
[11] Carlton R, Waterman P, Gray A, Deans S. The antifungal activity of the leaf gland volatile oil of sweet gale (*Myrica gale*) (*Myricaceae*). Chemoecology **1992**, 3: 55-59.
[12] Weng-Keong Chan, Loh Teng-Hern Tan, Kok-Gan Chan, Learn-Han Lee, Bey-Hing Goh. Nerolidol: a sesquiterpene alcohol with multi-faceted pharmacological and biological activities. Molecules **2016**, 21: 529-569