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Composition and Antimicrobial Activity of *Myrica gale* L. Leaf and Flower Essential Oils and Hydrolates

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Abstract: *Myrica gale* L. (sweet gale) leaves and flowers were subjected to industrial steam distillation in order to obtain essential oil and hydrolate. Obtained products were investigated to determine their chemical composition and antimicrobial activity. The main components found in both leaf and flower essential oils were monoterpene hydrocarbons: α-pinene (12.3, 23.5.%), *p*-cymene (12.8, 4.9%), and limonene (11.0, 5.6%), respectively. While oxygenated monoterpenes: 1,8-cineole (28.6, 44.2%), terpinen-4-ol (14.3, 13.4%), and α-terpineol (15.6, 11.3%) were dominant compounds in leaf and flower hydrolates. Essential oil and hydrolate from *M. gale* leaves exhibited antimicrobial activity against obligatory and opportunistic bacterial pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and *Enterococcus faecalis*. Interestingly, the leaf essential oil, but not the hydrolate, was also active against *Candida albicans* and *Candida glabrata* – yeast included in human skin and mucous membrane microbiota and simultaneously important fungal pathogens.

Keywords: Sweet gale; monoterpenes; antimicrobial activity.

1. Introduction

Myrica gale L. known as myrique baumier or sweet gale is a species of aromatic shrub from the family of *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 [1]. In Poland sweet gale is the only representative of the *Myriaceae* family and occurs naturally only in the narrow coastal strip of Baltic Sea [2]. Myrique baumier is a protected plant, however, it can be successfully cultivated.

Research concerning essential oil composition of *M. gale* from different origins revealed considerable differences as it was presented previously by Svoboda et al. [3] and recently by Wawrzyńczak *et al*. [4]. Essential oils of the plants collected from most of the locations were rich in α-pinene (12.2-38.9%), apart from those originating from Canada and Japan (α-pinene content 2.2 and 1.9%, respectively) [3-7]. Leaf essential oil from Canadian myrique baumier was the only one in which considerable amount of myrcene (11.3%) was identified [5]. This compound was also present in essential oils from the plants of Polish origin, up to 2.2% in the case of senescent leaves [4]. Germacrone, the compound that possesses some valuable properties, including antitumor effect

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against human hepatoma and antimicrobial activity against early stage of influenza [8-9], was present at high quantities in leaf and fruit essential oils of the plants from France (25.1, 14.2%, respectively) [7] and in flower essential oil of the plants from Poland – up to 8.9% [4]. Essential oils obtained during different development stages of the plants were also assessed. It was proven that *M. gale* senescent leaves were more valuable source of essential oils than matured leaves [3-4].

In present study a composition of *M. gale* leaf and flower essential oils and hydrolates produced in an industrial scale and their antimicrobial activity were assessed. The changes in hydrolate volatiles' content and composition during distillation time were also indicated.

2. Materials and Methods

2.1. Collection of Plant Material

Myrica gale male flowers (4.5 kg) and leaves (11 kg) were collected from organic farm in Połczyno, Poland (54°42'N 18°20'E) in June and September 2015, respectively. The plant material identification was done by Dr. Jeremi Kołodziejek from Department of Geobotany and Plant Ecology, University of Lodz and a voucher specimen No 157386 is deposited at the Herbarium Universitatis Lodziensis LOD– Poloniae.

2.2. Steam Distillation of Essential Oils and Hydrolates

Essential oils and hydrolates were obtained by industrial steam distillation using Innotec-Tetekov TWE 250-2000 VA apparatus (distillation cylinder 2 m x 0.25 m, volume 98 l; working pressure 0.3 bar; steam quantity 12 kg/h, distillate quantity 200 mL/min.). Fresh flowers (4 kg) and leaves (10.5 kg) were steam distilled for 90 min., which is a routine time indicated by the manufacturer in respect to the process profitability. The hydrolates were collected in 4 L containers. Four hydrolate fractions were obtained from the flowers and five from the leaves of *M. gale*. Representative total hydrolates were prepared by mixing 500 mL of all respective fractions, separately for the flower and leaf hydrolates. After the distillation neat essential oils were separated in the Florentine-type flask receiver. Essential oils were dried over magnesium sulfate.

2.3. Isolation of Volatile Compounds from Hydrolate

Volatile constituents of hydrolates fractions and total hydrolates were extracted with diethyl ether pure p.a. (CHEMPUR, Piekary Slaskie, Poland), previously rectified. Samples, 500 mL each, were salted-out with 180 g of sodium chloride prior to extraction, in order to reduce solubility of volatile compounds in water. Four portions 100 mL each of diethyl ether were used as an extractant. Combined extracts were dried over anhydrous magnesium sulfate and filtered. The solvent was removed using rotary evaporator at 40°C. The remaining mixture of volatile constituents of hydrolates was weighed and the content of volatiles was reported as mg/L. Each extraction was repeated twice.

2.4. GC-FID-MS Analysis and Identification of the Components

Volatile compounds isolated from hydrolate as well as essential oil were analyzed by gas chromatography coupled with mass spectrometry (GC–FID–MS) [10]. Apparatus: Trace GC Ultra gas chromatograph coupled with DSQ II mass spectrometer (Thermo Electron Corporation). A simultaneous GC-FID and MS analysis was performed using MS-FID splitter (SGE, Analytical Science, Austin, TX, USA). Operating conditions: nonpolar capillary column Rtx-1ms (60 m x 0.25 mm, 0.25 μm film thickness), programmed temperature: 50 (3 minutes)–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. Identification of the components was based on a comparison of their mass spectra and relative retention indices with data stored in computer libraries NIST 98.1, Wiley 8th Ed. and MassFinder 4.1

as well as literature [11]. Retention indices (RI, apolar column) were determined with relation to a homologous series of alkanes (C8–C26) under the same conditions with linear interpolation. The percentages of constituents were computed from the GC peak area without using a correction factor.

2.5. Antimicrobial Activity Assessment

Staphylococcus aureus ATCC 29213 (reference strain, MSSA – methicillin susceptible *S. aureus*), *S. aureus* H9 (clinical strain, MRSA – methicillin resistant *S. aureus*), *Enterococcus faecalis* ATCC 29212, *Escherichia coli* NCTC 8196, *Pseudomonas aeruginosa* ATCC 15442, *Candida albicans* ATCC 10231, and *Candida glabarata* ATCC 90030 were used in the study. Antimicrobial activity of *M. gale* leaf essential oil (EO_{leaf}) and hydrolate (H_{leaf}) were tested using microdilution method in broth to determine the minimal inhibitory concentration (MIC), followed by the culture on solid media to determine the minimal bactericidal concentration (MBC) or minimal fungicidal concentration (MFC). Microbial suspensions at the density of 5×10^5 CFU/mL in Mueller-Hinton Broth (for bacteria; Graso Biotech, Poland) or RPMI-1640 with 1% L-glutamine (for yeast; Sigma, Germany) were exposed on *M. gale* leaf essential oil at the final concentration range of 0.097-1.56% (v/v) or the hydrolate at the final concentration range of 6.5-75% (v/v) for 24 h at 37°C (bacteria) or 48 h at 35°C (yeast). Microbial suspensions at the same density (5×10^5 CFU/mL) in appropriate media alone (Mueller-Hinton Broth for bacteria or RPMI-1640 with 1% L-glutamine for yeast) culture at the same conditions served as positive control. Visual and densitometric method (assessment of microbial growth based on the changes in absorbance of samples at λ = 600 nm) was used to determine MIC values. MIC was defined as the lowest concentration of tested preparation inhibiting bacterial/fungal growth compared to the appropriate positive control. MBC/MFC of the preparations means the lowest concentration killing 99.9% of bacteria or yeast after subculturing 10 µL of samples on Tryptic-soy agar (TSA; BTL, Poland; for bacteria) or Sabouraud's dextrose agar (SDA; BTL, Poland; for fungi) and further incubation for 24 h at 35-37°C. Two experiments for each sample and each microbial strain were performed.

3. Results and Discussion

3.1. Essential Oils and Hydrolates Composition

Fresh leaves of *M. gale* (10.5 kg) yielded 4.2 mL (0.04%) of essential oil and five fractions 4 L each of hydrolate (20 L). From 4 kg of fresh flowers 25 mL of essential oil (0.62%) and four fractions (4 L each) of hydrolate were obtained (16 L). *M. gale* leaves and flowers both yielded pale green essential oil with fresh, green, herbal smell. Hydrolates were transparent liquids with characteristic smell resembling the essential oil smell. Yield of laboratory hydrodistilled essential oil of *M. gale* leaves varies between collection locations and development stage of the plant material in the range from 0.13 % [3] to 0.55 % [6]. The highest measured flower essential oil yield was 1.46% from the plant cultivated in Finland [3]. Laboratory distillation of dried leaves and flowers from the same origin as in the research presented here yielded 0.16% and 1.23% essential oils, respectively [4]. Usually the yield of essential oil in industrial steam distillation is lower than that observed in laboratory hydrodistillation.

The content of volatile compounds in hydrolates was assessed by extraction of salted hydrolate samples with diethyl ether. Different solvents were previously used for volatiles extraction from hydrolates, dichloromethane [12,13], hexane [14], pentane [15], and diethyl ether [10, 16,17] being the most common. Diethyl ether was chosen according to literature data as well as our previous experiences.

Table 1. Composition of *Myrica gale* essential oils and hydrolate volatiles obtained from leaves and flowers in industrial steam distillation

No	Compound	RI_{exp}	RI_{lit}	EO_{leaf} [%]	EO_{flower} [%]	H_{leaf} [%]	H_{flower} [%]
1	Hex-3-en-1-ol	838	838	-	-	1.1	-
2	Octane	800	800	-	0.1	-	-
3	Tricyclene	921	927	-	0.2	-	-
4	α-Thujene	927	932	0.1	0.1	-	0.3
5	Benzaldehyde	929	935	-	-	0.6	-
6	**α-Pinene**	933	936	**12.3**	**23.5**	-	-
7	Camphene	945	950	0.5	0.9	-	-
8	β-Pinene	971	978	2.1	2.3	-	-
9	Myrcene	984	987	0.5	1.2	-	-
10	α-Phellandrene	996	1002	-	3.2	-	-
11	3-Carene	1005	1010	0.1	-	-	-
12	α-Terpinene	1008	1013	-	0.4	-	-
13	Benzyl alcohol	1010	1006	-	-	0.8	-
14	***p*-Cymene**	1013	1015	**12.8**	**4.9**	-	0.2
15	**1,8-Cineole**	1021	1024	2.2	**8.0**	**28.6**	**44.2**
16	**Limonene**	1023	1025	**11.0**	**5.6**	-	-
17	(*Z*)-β-Ocimene	1028	1029	-	0.9	-	-
18	(*E*)-β-Ocimene	1040	1041	-	0.2	-	-
19	γ-Terpinene	1050	1051	-	1.1	-	-
20	*trans*-Linalool oxide (f)	1058	1058	-	-	0.1	-
21	*cis*-Linalool oxide (f)	1072	1072	-	-	t	-
22	Nonanal	1076	1076	-	0.4	-	-
23	Terpinolene	1081	1082	-	0.4	-	-
24	Linalool	1086	1086	-	-	1.6	1.1
25	α-Fenchol	1102	1099	-	0.1	-	0.9
26	α-Campholenal	1104	1105	-	-	t	-
27	*cis*-*p*-Menth-2-en-1-ol	1111	1108	-	-	0.9	1.1
28	*trans*-*p*-Menth-2-en-1-ol	1123	1116	-	t	1.0	1.1
29	*cis*-Verbenol	1133	1132	-	-	0.1	0.1
31	Camphene hydrate	1135	1143	-	-	0.4	0.7
32	δ-Terpineol*	1150	1155	-	-	1.6	-
32	Borneol*	1151	1150	-	0.2	1.3	3.3
33	**Terpinen-4-ol**	1166	1164	-	0.4	**14.3**	**13.4**
34	**α-Terpineol**	1172	1176	-	0.2	**15.6**	**11.3**
35	*cis*-Piperitol	1183	1181	-	-	0.3	0.1
36	*trans*-Piperitol	1192	1193	-	t	0.3	-
37	β-Cyclocitral	1197	1195	-	-	0.1	-
38	2α-Hydroxy-1,8-cineole	1120	1196	-	-	0.7	0.4
39	Benzylacetone	1210	1207	-	-	1.6	-
40	Citronellol	1212	1213	-	-	1.7	-
41	*cis*-Carveol	1216	1210	-	-	0.4	-
42	Carvotanacetone	1222	1220	-	-	0.1	-

Table 1 continued..

43	Piperitone	1228	1226	-	-	0.2	-
44	Nonanoic acid	1260	1263	-	-	0.1	-
45	Bornyl acetate	1273	1270	1.0	0.8	0.2	0.3
46	Carvacrol	1281	1278	-	-	0.4	-
47	Terpinen-4-yl acetate	1285	1289	-	0.2	-	-
48	α-Terpinyl acetate	1336	1335	2.9	4.0	0.3	1.0
49	(*E*)-Methyl cinnamate	1354	1354	-	-	0.2	-
50	Geranyl acetate	1363	1362	0.1	0.4	-	0.1
51	Benzyl isovalerate	1370	1370	-	-	0.1	-
52	α-Ylangene	1377	1376	-	0.1	-	-
53	α-Copaene	1380	1379	1.2	0.4	-	-
54	β-Elemene	1391	1389	0.1	-	-	-
55	α-Gurjunene	1410	1413	-	0.1	-	-
56	(*E*)-β-Caryophyllene	1422	1421	0.5	0.9	-	-
57	γ-Elemene	1431	1429	2.0	0.5	-	-
58	α-Humulene	1454	1455	0.2	t	-	-
59	7aH,10bH-Cadina-1(6),4-diene	1469	1472	-	0.2	-	-
60	γ-Muurolene	1474	1474	0.5	-	-	-
61	*cis*-Eudesma-6,11-diene	1480	1484	0.2	0.3	-	-
62	β-Selinene	1486	1486	1.3	0.4	-	-
63	α-Selinene	1499	1494	0.3	0.6	-	-
64	*trans*-Calamenene	1513	1517	1.6	0.4	-	0.2
65	δ-Cadinene	1515	1520	-	3.4	-	0.3
66	β-Cadinene	1517	1526	1.7	0.1	-	0.2
67	**Selina-4(15),7(11)-diene**	1534	1534	**9.8**	**8.1**	-	1.3
68	**Selina-3,7(11)-diene**	1543	1542	4.4	**7.9**	-	1.2
69	**(*E*)-Nerolidol**	1551	1553	**6.3**	2.1	1.0	1.1
70	Spathulenol	1575	1572	1.6	-	0.1	-
71	Caryophyllene oxide	1578	1578	1.2	-	-	t
72	β-Elemenone	1586	1589	1.7	4.5	1.4	3.7
73	Isospathulenol	1614	1625	-	-	2.5	1.5
74	1-*epi*-Cubenol	1619	1623	0.7	0.3	0.6	0.4
75	Germacrone*	1678	1684	0.8	3.6	1.7	3.0
76	Eudesm-7(11)-en-4α-ol*	1681	1676	0.7	0.7	0.7	0.9
77	Heptadecane	1700	1700	-	-	-	0.2
78	Benzyl benzoate	1728	1730	0.1	-	0.3	-
	Total identified			**82.5**	**94.3**	**83.0**	**93.6**
	Monoterpene hydrocarbons			**39.4**	**44.5**	-	0.5
	Oxygenated monoterpenes			6.2	14.7	**71.9**	**79.1**
	Sesquiterpene hydrocarbons			**23.8**	**23.4**	-	3.2
	Oxygenated sesquiterpenes			13.0	11.2	8.0	10.6
	Others			0.1	0.5	3.1	0.2

t – traces, <0.5%, - – not identified, * – the order of compounds was reversed in comparison to MassFinder data

Composition of *M. gale* essential oils and volatiles extracted from representative total leaf and flower hydrolates' is presented in the Table 1. The content of volatile compounds and percentage composition of the main constituents of hydrolate fractions and total hydrolate is presented in Table 2 and Table 3 for leaf and flower hydrolate, respectively. Summarizing, 33 compounds were identified in leaf essential oil and 47 in flower essential oil. While 38 and 30 different compounds were found in leaf and flower hydrolate, respectively.

The essential oils composition was to broad extent consistent with a composition of previously investigated essential oils from the same origin. Some differences of quantitative nature occurred [4]. The main constituents of the industrial *M. gale* leaf and flower essential oil were monoterpene and sesquiterpene hydrocarbons that constituted 63.2% and 67.9%, respectively. The major compound were α-pinene (12.3, 23.5%, respectively), *p*-cymene (12.8, 4.9%), limonene (11.0, 5.6%), selina-4(15),7(11)-diene (9.8, 8.1%), and selina-3,7(11)-diene (4.4, 7.9%). Most of the major compounds have been previously identified [3,4,6-8] with some differences between the essential oils isolated from the plants originating from different locations. 1,8-Cineole that was present in large quantities in the leaf and flower essential oils obtained in laboratory hydrodistillation of the same materials (18.1, 20.9%) [4] was found in smaller amounts in both industrial essential oils and amounted to 2.2% and 8.0%, respectively. According to previous reports the contents of sesquiterpene ketones β-elemenone and germacrone were in the broad range of 0-25.1%. In this research β-elemenone and germacrone percentages were higher in flower essential oil (4.5 and 3.6%) than in leaf essential oil (1.7 and 0.8%).

Volatile organic compounds of *M. gale* leaves and flowers hydrolates were rich with oxygenated monoterpenes (71.9 and 79.1% of all compounds, respectively). 1,8-Cineole (28.6, 44.2%), terpinen-4-ol (14.3, 13.4%), and α-terpineol (15.6, 11.3%) were the main constituents of hydrolates. β-Elemenone and germacrone were present in the amounts similar to that in respective essential oils. Only trace amounts of monoterpene hydrocarbons were present in the hydrolates. Surprisingly nonpolar selina-4(15),7(11)-diene and selina-3,7(11)-diene and other sesquiterpene hydrocarbons (which were present in both essential oils) were found in the hydrolate obtained from flowers. In this hydrolate hex-3-en-1-ol, benzaldehyde and benzyl alcohol were identified as minor components. These oxygenated compounds were not observed in essential oil due to high solubility in water. In previous research *cis*-hex-3-en-1-ol (9.0%) and germacrone (13.5%) were identified as the main constituents of *M. gale* hydrolate and small content of mono- and sesquiterpene alcohols [18]. It is obvious that pronounced differences in the composition of volatiles in essential oil and hydrolate occur, especially when essential oils consisted mainly of hydrocarbons. This is the case of the essential oils and hydrolates of two spruce and fir [19], *Laurus nobilis* [15] and many others. The amount of organic compounds in hydrolates depends on their content in the plant material however, it is limited by the solubility of individual compounds in water. The relative concentration of volatiles in both hydrolates was with good agreement with their solubility, measured at 25°C is the following: for 1,8-cineole 3500 mg/L, for α-terpineol 710 mg/mL, and for α-pinene 2.49 mg/mL [19].

3.2. The Influence of Distillation Time on the Quality of Hydrolates

In this research 1.9 L of total leaf hydrolate and 4 L of total flower hydrolate were produced from 1 kg of fresh plant material. These proportions were in agreement with indication that 1 to 5 L of hydrolate can be obtained from 1 kg of plant material, the amount varies depending on the particular plant [20]. It was proven that distillation time influenced both the content of volatile organic compounds in hydrolates and their percentage composition. As it is revealed in Table 2 and Table 3, showing the compounds present in amount of 1% or higher, longer time of distillation caused a reduction of volatile content in the hydrolate fraction. The fractions (438 to 92 mg/L) and total hydrolate (186 mg/L) from the flowers were more abounded with the volatiles than the fractions from the leaves (142 to 44 mg/L and 66 mg/L). This corresponded to the essential oil content in both plant parts. Previously Collin and Gagnon [18] produced 50 kg of hydrolate from 300 kg of *M. gale* aerial parts. The hydrolate contained 110 mg/L of volatile compounds. Taking into account the low volatile content in total leaf hydrolate it would be advised to mix only three first fractions. Such hydrolate would contain ca. 100 mg/L of volatiles and 1L would be obtained from ca. 1 kg of plant material.

A prolongation of distillation time caused an increase of the content of the compounds with higher mass (as sesquiterpenes) and opposite effect on low mass compounds. Good example of this phenomenon is the main component – 1,8-cineole which represents oxygenated monoterpenes. In the first fraction of flower hydrolate it reached of 58.0%, while in the fourth fraction only 22.0% of the total volatiles content. Similar effect was observed for leaf hydrolate – in the first fraction as much as 41.1% of all compound was 1,8-cineole, while in fifth fraction only 21.5% of this compound was present. The changes in the presence of higher mass compounds can be illustrated by germacrone, being one of the oxygenated sesquiterpenes. Germacrone content varied from 0.8% to 9.3% between the first and the last fraction of the flower hydrolate, and from 0.6% to 3.4% between those fractions of the leaf hydrolate.

Table 2. Composition of main volatile compounds of hydrolate fractions and total hydrolate of *Myrica gale* leaf

No	Compound	Fraction No					Total hydrolate H_{leaf}
		1	2	3	4	5	
		[%]	[%]	[%]	[%]	[%]	[%]
1	Hex-3-en-1-ol	1.4	0.3	0.1	0.5	1.2	1.1
2	Benzaldehyde	0.6	1.2	0.5	0.8	1.5	0.5
3	**1,8-Cineole**	**41.1**	**27.7**	**20.0**	**23.0**	**21.5**	**28.6**
4	Linalool	2.0	1.1	1.0	0.9	1.0	1.6
5	trans-*p*-Menth-2-en-1-ol	1.0	0.6	t	0.3	0.4	1.0
6	δ-Terpineol	1.7	0.6	1.0	0.4	0.3	1.6
7	Borneol	1.8	1.7	1.0	1.2	1.0	1.3
8	**Terpinen-4-ol**	**14.6**	**13.7**	**10.5**	**7.70**	**6.4**	**14.3**
9	**α-Terpineol**	**17.6**	**14.8**	**13.1**	**9.7**	**8.0**	**15.6**
10	Benzyl acetate	0.3	2.5	2.3	1.9	1.5	1.5
11	Citronellol	1.9	1.2	1.5	1.1	0.9	1.7
13	(*E*)-Nerolidol	0.5	1.2	1.8	1.5	1.8	1.0
14	Spathulenol	t	0.7	1.3	1.0	0.1	0.1
15	**β-Elemenone**	**0.6**	**1.5**	**3.0**	**3.0**	**3.2**	**1.4**
16	**Isospathulenol**	**0.7**	**1.8**	**3.0**	**3.5**	**5.0**	**2.5**
17	1-*epi*-Cubenol	0.2	0.6	1.0	1.2	1.2	0.6
18	**Germacrone**	**0.6**	**1.5**	**2.8**	**3.2**	**3.4**	**1.7**
19	Eudesm-7(11)-en-4α-ol	0.2	0.6	1.3	1.4	1.6	0.7
20	Benzyl benzoate	-	0.4	0.7	0.9	1.1	0.3
	Volatiles content (mg/L)	**142**	**81**	**73**	**53**	**44**	**66**

3.3. Antimicrobial activity

Antimicrobial activity of *M. gale* leaf essential oil and hydrolate was tested against both Gram-positive (*S. aureus*, *E. faecalis*) and Gram-negative bacteria (*E. coli*, *P. aeruginosa*), as well as fungi (*C. albicans*, *C. glabarata*). MIC and MBC values of essential oil are presented in Table 4. It was shown, that essential oil expressed the strongest activity against yeast achieving MIC at as low as 0.39%. Leaf essential oil also inhibited the growth and viability of Gram-positive bacteria and *E. coli* showing the same activity against all of these microorganisms.

Table 3. Composition of main volatile constituents of hydrolate fractions and total hydrolate of *Myrica gale* flower

No	Compound	Fraction No				Total hydrolate H_{flower}
		1	2	3	4	
		[%]	[%]	[%]	[%]	[%]
1	**1,8-Cineole**	**58.0**	**31.7**	**32.4**	**22.0**	**44.2**
2	*trans*-*p*-Menth-2-en-1-ol	1.0	1.0	0.6	1.1	1.1
3	Camphene hydrate	1.0	0.4	0.3	-	0.7
4	**Borneol**	**2.5**	**1.8**	**2.0**	**1.8**	**3.3**
5	**Terpinen-4-ol**	**12.9**	**6.8**	**6.8**	**5.8**	**13.4**
6	**α-Terpineol**	**11.4**	**6.5**	**6.0**	**5.2**	**11.3**
7	α-Terpinyl acetate	0.8	1.4	0.9	1.2	1.0
8	Selina-4(15),7(11)-diene	1.0	2.7	1.5	1.1	1.3
9	Selina-3,7(11)-diene	0.9	2.6	2.0	1.1	1.1
10	(*E*)-Nerolidol	0.4	2.6	2.1	2.9	1.1
11	β-Elemenone	0.7	7.8	10.7	12.3	3.7
12	Isospathulenol	0.4	3.1	3.3	4.8	1.5
13	1-*epi*-Cubenol	-	0.9	1.0	1.3	0.4
14	**Germacrone**	**0.8**	**6.3**	**9.7**	**9.3**	**3.0**
	Volatiles content (mg/L)	**438**	**176**	**98**	**92**	**186**

However, there was no effect of *M. gale* leaf essential oil on *P. aeruginosa* in a whole range of tested concentrations. Similar results were obtained by Nakata *et al.* [21], who demonstrated antimicrobial activity of essential oil from cultivated *M. gale* var. *tomentosa* against Gram-positive bacteria including *S. aureus* and *B. subtilis*, as well as fungi such as *Saccharomyces cerevisiae* and *C. albicans* using disc diffusion method in agar. The strongest activity was observed against *B. subtilis* with the only one given MIC value at 61 ppm (0,0061%), since the MIC for other microorganisms was above range of concentrations tested (> 1500 ppm) [21].

Table 4. Minimal inhibitory concentration (MIC) and minimal bactericidal/fungicidal concentration (MBC/MFC) of *M. gale* leaf essential oil

Strain	**MIC** [%]	**MBC/MFC** [%]
S. aureus ATCC 29213	0.78	1.56
S. aureus H9 (clinical MRSA)	0.78	1.56
E. faecalis ATCC 29212	0.78	>1.56
E. coli NCTC 8196	0.78	1.56
P. aeruginosa ATCC 15442	>1.56	>1.56
C. albicans ATCC 10231	**0.39**	**0.78**
C. glabrata ATCC 90030	**0.39**	**0.78**

Popovici *et al.* [22] showed inhibitory activity of the essential oils obtained from fruits of *M. gale* L. against a panel of foodborne fungi: *Aspergillus flavus*, *Cladosporium cladosporioides* and *Penicillium expansum*. A complete fungicidal effect was observed against *C. cladosporioides* at a concentration of 1000 ppm (0.1% *versus* 0.78% for *Candida* sp. in our study). The authors suggest possible use of *M. gale* essential oils as an additive in food or cosmetics for their flavour, odour and conservative properties. Because of our results indicating on leaf essential oil activity against

pathogenic bacteria and fungi, also disinfectants for usable industrial surfaces can be included to this list.

M. gale leaf hydrolate was less active in this respect, nevertheless, 75% solution of hydrolate resulted in a significant reduction in the viability of Gram-positive bacteria – in the range of 31-66% (Table 5). Interestingly, tested hydrolate in contrast to essential oil was also active against *P. aeruginosa* reducing the viability of these bacteria by 30% and 63% when used at the concentration of 50% and 75%, respectively. Moreover, there was no effect of leaf hydrolate against fungi in a whole range of concentrations tested. Taking into account the fact that the hydrolates are used in undiluted form it should be stated that the antibacterial potential of *M. gale* hydrolate is good despite the small amounts of organic volatile compounds (66 mg/L). It is known that the alcohols are amongst the most active antimicrobial essential oils' constituents beside phenols while the hydrocarbons reveal a poor activity [23]. Terpinen-4-ol and α-terpineol, which were identified here in significant amounts in hydrolates' volatiles were among five out of twenty-one oxygenated monoterpenes showing the broadest spectrum of antibacterial efficacy measured by disc diffusion method, while 1,8-cineole appeared not active [24]. Hence, it is clear that these two alcohols were the key antimicrobial constituents of the hydrolates.

Table 5. Antimicrobial activity of *M. gale* leaf hydrolate measured as microbial viability in the presence of the preparation at different concentrations

Strain	Hydrolate concentration [%]	Viability [%]
S. aureus ATCC 29213	**75**	**69**
	50	100
	25	100
S. aureus H9 (clinical MRSA)	**75**	**68**
	50	100
	25	100
E. faecalis ATCC 29212	**75**	**34**
	50	**61**
	25	100
E. coli NCTC 8196	**75**	**92**
	50	100
	25	100
P. aeruginosa ATCC 15442	**75**	**37**
	50	**70**
	25	**94**
C. albicans ATCC 10231	75	100
	50	100
	25	100
C. glabrata ATCC 90030	75	100
	50	100
	25	100

Essential oils are natural products largely employed due to their beneficial properties [25]. MIC values of different essential oils lie in a broad range from 0.008% for tea tree oil to 2% for juniper oil [25]. In this respect both *M. gale* essential oils should be consider as middle active. Recently there is a growing interest in hydrolates that antimicrobial activity and potential were reviewed by D'Amato et al. [26]. Hydrolates can be used as face and air refreshers, cosmetic natural ingredients [16], fruit and vegetable sanitizers [27,28], and insecticides [29]. It can be concluded that *M. gale* leaves and flowers are valuable source of essential oils and hydrolates with fresh herbal smell and antimicrobial activity. These products can be used in wide variety of products.

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