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Significant Chemical Compounds, Antimicrobial activity of the Essential oils from *I. verbascifolia* (Wild). Hausskn

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ABSTRACT

Plant oils have attracted interest for centuries as natural remedies in treatment of various diseases. The *Inula verbascifolia* (Willd.) Hausskn isgrowing wild plant in Bosnia and Herzegovina. Aromatic natural oils are one of the most significant sources of natural organic components. The natural vegetable oil of the selected plant (Inula verb.) was obtained by the hydrodistillation method. A comparison was made between the fragrant vegetable oil from the aerial parts of the plant in the flowering period (summer) and after the flowering period (autumn). In this study, chemical compounds were tested, comparing the content and composition of natural oils from the plant *Inula verbascifolia*. The aerial parts of the plant contained a fragrant and yellow essential oil. The identified 125 constituents accounted for 86.87% and 88.38% of the oil. The dominant compounds of both EOs were tridecanal, (3Z)-hexenyl benzoate, α-murolol, hexadecanoic acid, linalool and undecanal. Since essential aromatic oils possess a number of antimicrobial properties, an analysis of antimicrobial activity was also performed in this work. The antimicrobial activity of a mixture of EOs was determined on selected ATCC strains of microorganisms. Results of antimicrobial activity indicated that all used the microorganisms were sensitive to the EO. No data about antimicrobial activity of *Inula verbascifolia* has been published yet.

Keywords: *Inula verbascifolia*, Essential oils, Isolation, Antimicrobial activity.

INTRODUCTION

Inula verbascifolia is a plant that is found in parts of Italy, Dalmatia, Balkans and parts Crete, Asia Minor, Pontus region and Syria. It characterized by greyish leaves with yellow flowers. It is up to 50 cm tall (Fig. 1). It grows in coastal cliffs in the coastal area. It is also found on dry stony pastures and rocks. The plant species Inula verbascifolia contains secondary metabolites such as essential oils, terpenes[1,2]. It also contains polyphenolic compounds and exhibit antioxidant properies[3]. The variability of the composition and content of EOs in aromatic plants depends on the individual genetic variations, developmental stages and environmental factors. Environmental factors include soil composition, heat, sunlight, humidity[4,5]. It is well known that the genus Inula has different biological activities. Gokbulut *et al.,* 2013 investigated antioxidant properties, Bae *et al.,* 2019 beside antioxidant investigated hepatoprotective properties while Bar-Shalom *et al.,* 2019 reported anticancer properties of the genus Inula[6,7,8]. Seka *et al.,* 2014 published etnopharmacological and medicinal use of genus Inula such as antimicrobial, anti-inflammatory, cytotoxic and insecticidal activities[9]. As far as we know, no results have been reported on the antimicrobial activity of essential oils from parts of the *Inula verbascifolia*. In this study, the aspect is based on the compounds present in the essential oil from parts of Bosnia and Herzegovina during the flowering period and after the flowering period. The study is also based on the antimicrobial activity of the corresponding ATCC strains.

Although it is known that there are research results on the action of natural oils, isolation of compounds and antimicrobial activity, the mechanism of action itself has not been fully explained. Essential oils due to their lipophilic nature, pass through the cell membrane and affect changes in ion concentration, pH gradient. They also damage lipids and proteins in the bacterial cell. It all leads to cell damage and cell lysis[10].

Fig. 1. *Inula verbscifolia* (orginal photo original picture from the picking site)

MATERIALS AND METHODS

The floral parts of the plant *I. verbascifolia* were taken in the area Bosnia and Hercegovina (Podveležje, Mostar City) (N43°18'36.0" E17° 54'40.6") during and after flowering period in 2020 and were air-dried. Typical specimens, identified by Prof. Cilovic Kozarevic, at the University of Tuzla, Department of Pharmaceutical Botany, Bosnia and Herzegovina.

Reagents and chemicals

The chemicals used in this research are Streptomycin and Iodonitrotetrasolium chloride (Sigma-Aldrich), Ampicillin and Ketoconazole, purchased with the support of Serbia.

The process of distillation of essential oil

The aerial parts of *I. verbascifolia* were crushed. According to Ph. Eur. certain amount of the plant was subjected to distillation on an apparatus for determining essential oils (Clevenger Apparatus). Natural essential oils were obtained by distillation, dried with anhydrous Na_2SO_4 and left in the refrigerator (-20°C) until analysis.

Analysis of chemical compounds

The analysis of essential oils was done on a GC-MS/FID. Peaks of the individual chemical compounds within the obtained chromatogram were identified. After identification, their retention indices (RI) were compared with the baseline data. Analyses were performed at Agilent GC(6890N). Capillary column HP-5MS was used (length 30m, 250µm, film thickness 0.25µm).

GC was equipped with an Agilent MSD-5975 and FID. The temperature of FID was 300°C. Helium gas was used as a carrier in a constant flow regime of 1.0 mL min^{-1}. The injection volume of EOs dissolved in ethanol was 1 µL at temperature 200°C. The oven was programmed at a temperature from 60°C to 280°C from 3°C/min^{-1} and then at 280°C for 5 minute. The MSD was operated from 35 to 55 m/z with transfer line at 250°C. NIST, literature and other spectral libraries were used for data analysis. The results are expressed as a percentage (%) of each component in the EO.

Antimicrobial activity assessment

Three reference bacterial strains

Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and fungus *Candida albicans* were used to determine antimicrobial activity assessment of essential oils made of *I. verbascifolia* aerial parts mixture.

The MO reference strains were made in the laboratory Mycology of the Institute for Biological Research "Siniša Stankovi", at the University of Belgrade in Serbia.

Minimum inhibitory concentration and minimum bactericidal/fungicidal concentration was determined utilizing microdilution method, using 96-well microtiter plates[11]. Namely, microorganisms were cultivated overnight on selective media, and then inoculated into bacteria and fungus on microtiter plates. Serial dilutions of the EO were made and then added to the nutrient medium.

As a negative control medium without EO was used, and a positive controls we used the commercial antimicrobial agents streptomycin, ampicillin, and ketoconazole. Microplates were incubated with a sterile film cover for bacteria on 24 h at 37°C and fungus on 72 h at 28°C.

The lowest concentration after incubation that showed no growth of microorganisms was determined as MIC. The minimum bactericidal/ fungicidal concentration was determined by serial reinoculation of 10µL of the wells without growth into 100µL of sterile nutrient medium.

After reincubation (24 h/37°C for bacteria, 72 h/28°C for fungus), 40 µL of microbial growth indicator, purple p-iodonitrotetrasolium chloride (0.2 mg/mL), was added to the wells, which were then incubated for 30 minute. The results were compared with the control wells.

RESULTS AND DISCUSSION

Results of isolation and identification of chemical compounds from natural essential oils

After the analysis, the results showed that the aerial parts of the *I. verbascifolia* plant contain 0.01% essential oil during the flowering period (summer) where 62 components were identified, which accounts for 86.87% of the oil presence. Also, the results were analysed after flowering period (in autumn). At that time, 60 components were identified, which makes up 88.38% of the oil, and the aboveground parts of the plant contained 0.005% EO, which are shown in Table 1. Essential oils isolated in summer at room temperature were solid, and at body temperature in a liquid aggregate state, while the EO isolated in autumn was in a solid aggregate state. Both EOs were yellow and fragrant.

The EOs of aerial parts of *I. verbascifolia* were abundant in the presence of high concentration of non-terpene (other) compounds and constituted 58.62–62.30% of the oils, followed by oxygenated sesquiterpnes (16.07-16.95%), oxygenated monoterpenes (6.59-12.71%) and finally sesquiterpene hydrocarbons (0.98-1.03%) in Table 1.

Table 1: Present compounds in essential oil *I. verbascifolia* collected in summer and autumn

No	Retention period	Constituent	RIL	RIE	EO IVA_S (%)	EO IVA_A (%)
2	12,857	Linalool	1095	1101.4	3.06	5.43
3	12,983	*n*-nonanal	1100	1104.3	0.61	0.78
4	15,878	Menthol	1167	1173.9	-	0.23
5	16,013	Octanoicacid	1176	1176.9	0.29	0.26
6	16,637	α-terpineol	1186	1191.7	0.49	0.87
7	16,925	Dodecane	1200	1198.5	0.46	-
8	17,219	*n*-decanal	1201	1205.4	0.76	1.44
9	17,713	methyl ether	1219	1216.9	-	0.43
10	18,202	Nerol	1227	1228.4	-	0.59
11	19,331	Geraniol	1249	1254.7	0.39	0.99
12	20,138	*n*-decanol	1266	1272.4	0.28	0.64
13	20,261	nonanoic acid	1275	1276.6	0.69	1.01
15	21,197	Tridecane	1300	1298.3	0.24	-
16	21,603	*n*-undecanal	1305	1308.0	3.38	6.17
17	21,963	(*2E, 4E*)-decadienal	1315	1316.4	0.50	0.39
18	22,454	isobutyl benzoate	1327	1328.1	0.29	-
19	23,707	Eugenol	1356	1357.6	-	0.35

20	24,372	decanoic acid	1370	1373.7	1.70	1.48
22	24,839	(E)-β-damascenone	1383	1384.6	-	0.29
24	25,178	(*Z*)-jasmone	1392	1392.3	0.41	0.34
25	25,656	methyl eugenol	1403	1403.9	0.89	0.32
28	25,811	*n*-dodecanal	1408	1408.1	1.46	2.56
29	26,292	(*E*)-caryophyllene	1417	1420.2	0.36	-
31	26,776	Dictamnol	1428	1432.6	-	0.27
33	27,581	geranyl acetone	1453	1452.5	0.62	2.14
34	27,914	Dehydroaromadendrane	1460	1461.1	-	0.20
35	28,252	undecanoic acid	1467	1469.4	1.54	-
37	28,504	*n*-dodecanol	1469	1475.5	-	3.46
39	28,838	*ar*-curcumen	1479	1484.1	-	0.35
41	28,972	(*E*)-β-ionone	1487	1487.4	0.62	1.53
44	29.466	benzyl tiglate	1497	1499.9	0.58	-
46	29,858	*n*-tridecanal	1509	1509.9	6.53	10.06
47	30,08	γ-cadinene	1513	1515.8	0.45	0.23
49	30,885	italicene ether	1536	1536.5	-	0.23
50	31,958	(*E*)-nerolidol	1561	1564.0	0.55	0.38
52	32,25	(*3Z*)-hexenyl benzoate	1565	1571.4	7.97	2.39
53	32,501	*n*-hexyl benzoate	1579	1578,3	1.02	-
54	32,569	*ar*-turmerol	1582	1579.9	-	0.67
55	32,781	caryophyllene oxide	1582	1585.3	2.67	1.72
57	33.248	*n*-hexadecane	1600	1597,3	0.36	-
58	33,747	humulene epoxide II	1608	1611.0	0.54	-
59	33,755	Tetradecanal	1611	1611.1	-	0.56
60	34,369	Benzophenone	1626	1627.8	-	0.41
61	34.429	1-epi-Cubenol	1627	1629,6	0.27	-
62	34,441	Muurola-4,10(14)-dien-1-β-ol	1630	1630.1	0.27	0.38
64	34,784	Caryophyll -4(12),8(13)-dien-5-α-ol	1639	1639.0	0.30	-
65	34.759	Caryophyll -4(12),8(13)-dien-5-β-ol	1639	1638.7	0.86	-
67	34,993	α-murolol	1644	1645.0	7.89	8.41
69	35.174	(Z)-methyl jasmonate	1648	1650.1	0.31	-
72	35,466	α-cadinol	1652	1657.9	2.98	3.21
73	35,586	14-hydroxy-(Z)-caryophyllene	1666	1661.5	0.20	-
75	36,03	tridecanoic acid	1678	1673.5	1.47	4.53
76	36,012	14-hydroxy-9-epi-(E)-caryophyllene	1668	1674.0	0.42	-
77	36,138	hexyl salicylate	1674	1676.7	-	0.21
78	36,138	Cadalene	1675	1676.7	0.22	0.20
79	36,261	*n*-tetradecanol	1676	1679.6	-	0.42
81	36,925	*n*-heptadecane	1700	1697.5	-	0.29
84	37,458	*n*-pentadecanal	1711	1713.1	0.67	0.34
85	37,741	3-methoxy-cuminyl isobutyrate	n/a	1721.1	0.46	-
87	38,154	Isobicyclogermacrenal	1733	1733.0	-	0.31
88	38,679	Fukinone	1756	1748.0	-	0.48
90	39.281	benzyl benzoate	1759	1765,4	3.95	-
92	39,444	tetradecanoic acid	1769	1770.1	-	2.94
94	40,414	Octadecane	1800	1798.3	-	0.20
95	40,742	dehydrofukinone	1813	1808.1	-	0.28
96	41,972	hexahydrofarnesyl acetone	1845	1844.6	1.52	0.56
97	42.300	phenyl ethyl octanoate	1854	1854,8	0.60	-
98	42,567	pentadecanoic acid	1869	1862.7	0.51	0.58
99	42.766	benzyl salicylate	1864	1868,9	1.47	-
103	44,389	(*5E,9E*)-farnesyl acetone	1913	1918.3	0.45	0.45
106	46,072	hexadecanoic acid	1975	1971.7	6.74	6.95
107	46.662	1-eicosene	1987	1991.0	0.36	-
111	49,451	*n*-octadecanol	2077	2081.7	0.22	0,25
112	49,977	*n*-heneicosane	2100	2099.0	0.27	-
113	50,479	*(E)*-phytol	2111	2116.2	-	0.33
115	51,425	9*(Z)*,12*(Z)*-Octadecadienoic acid	2140	2148.6	-	1.56
116	53.528	*(E)*-phytol acetate	2218	2222.1	0.63	-

117	55.602	*n*-tricosane	2300	2297.3	0.87	-
119	58.268	*n*-tetracosane	2400	2395.8	0.36	-
120	60,943	*n*-pentacosane	2500	2498.7	2.48	0.31
121	63.327	*n*-hexacosane	2600	2595.1	0.24	-
122	65,84	*n*-heptacosane	2700	2698.9	3.26	1.31
123	68,159	*n*-octacosane	2800	2799.1	0.42	0.26
124	70,425	*n*-nonacosane	2900	2902.3	5.85	3.97
125	74,759	Untriacontane	3100	3100.8	0.64	0.48
Total identified					86.87	88.38
Oxygenated monoterpenes					6.59	12.71
Sesquiterpene hydrocarbons					1.03	0.98
Oxygenated sesquiterpenes					16.95	16.07
Other					62.30	58.62

EO IVA_S-Essential oil of *Inula verbascifolia* aerial parts in summer
EO IVA_A-Essential oil of *Inula verbascifolia* aerial parts in autumn
RIE–retention index experimental
n/a–data is not available
„-„–not detected
Bold components are presented in amounts over 1%

Dominant components in both EOs were linalool (3.06-5.43%), undecanal (3.38-6.17%), dodecanal (1.46-2.56%), tridecanal (6.53-10.06%), (3Z)-hexenyl benzoate (2.39-7.97%),-murolol (7.89-8.41%), α-cadinol (2.98-3.21%), tridecanoic acid (1.47-4.53%), hexadecanoic acid (6.74-6.95%), *n*-nonacosane (3.97-5.85%). Benzyl benzoate was presented only in EO of *I. verbascifolia* parts in summer in amount 3.95%, while *n*-dodecanol and tetradecanoic acid were presented only in EO of *I. verbascifoliaaerial* parts in autumn in amounts 3.46% and 2.94%, respectively. As far as we know, studies about essential oils for wild growing plant *I. verbascifolia* were done only in Croatia and two locations in Greece. Dominant components in *I. verbascifolia* aerial parts essential oil from Croatia (Badija island) are hexadecanoic acid with 10.4%, followed by 9(Z),12(Z)-Octadecadienoic acid (6.5%), tetradecanal (4.5%), germacrene D (4.4%) and δ-cadinene (3.3%)[12]. Dominant components in *I. verbascifolia* aerial parts essential oil from Greece location Viotia were: methyl salicylate (23.4%), cis-chrysanthenol (17.3%), β-caryophyllene (13.2%), linalool (7.1%), tridecanal (5.3%) while dominant components in *I. verbascifolia* aerial parts essential oil from Greece location Attiki were: linalool (21.2%), epi-α-cadinol (19.5%), (Z)-Nuciferol (16.6%), α-murolol (9.4%), (3Z)-hexenyl benzoate (6.4%)[1]. In comparison with EOs from Croatia and from two locations in Greece, the composition of *I. verbascifolia* aerial parts EO from Bosnia and Herzegovina presents a combination of the above mentioned Eos constituents. Based on the obtained results, after comparing the flowering period of the plant (summer) and after flowering period of the plant (autumn), the presence of the compound (3Z)-hexenyl benzoate is reduced in autumn by 5.58%, while the presence of the other listed dominant compounds: linalool, *n*-undecanal, *n*-tridecanal, tridecanoic acid is increased in autumn by the maximum 3.53%.

Table 2: The values of essential oil antimicrobial activity (*I. verbascifolia*)

Strain	ATCC	MIC (mg/mL)	MBC/MFC (mg/mL)
Staphylococcus aureus	6538	4	7
Pseudomonas aeruginosa	27853	3	4
Escherichia coli	35210	7	15
Candida albicans	10231	11	15

Minimum inhibitory concentration (MIC)
Minimum bactericidal concentration (MBC); Minimum fungicidal concentration (MFC)

Results of antimicrobial activity assessment

EO made from *I. verbascifolia* parts mixture showed the strongest antimicrobial effect on *Pseudomonas aeruginosa* and *Staphylococcus aureus* with MIC values 3 $mgmL^{-1}$ and 4 $mgmL^{-1}$, respectively. Inhibition of growth has also been reported for *E. coli* and *Candida albicans* with slightly higher MIC and MBC/MBC values. After the results obtained, it can be observed that *I. verbascifolia* essential oil possesses antimicrobial activity against the microorganisms used, only in different concentrations. Based on research, we know that data on the antimicrobial activity of *Inula verbscifolia* essential oil are not available.

CONCLUSION

Aromatic essential oils are known to be used in medicine, the pharmaceutical industry and the food industry. It is precisely because of their aromatic properties that they were chosen for research. The EOs from *I.verbascifolia* aerial parts during and after flowering period was isolated. The chemical composition of EOs were determined. They were abundant in the presence of high concentration of non-terpene (other) compounds. After the research studies, it is noticed that the results obtained with the essential oil of the plant *Inula verbascifolia* are new and therefore special. Antimicrobial activity assessment of *I. verbascifolia* aerial parts EO have been done for the first time, as far as we know. The obtained antimicrobial tests showed all positive results and that the essential oil inhibits the growth of microorganisms.

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Conflicts of interest

The mentioned authors have no conflicts of interest regarding the publication of this paper.

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