GC/MS analysis and antimicrobial activity of essential oils of *Telekia speciosa* (Schreb.) Baumg.

ERMINA CILOVIĆ KOZAREVIĆ[1,*], BROZA ŠARIĆ-KUNDALIĆ[1], MERIMA IBIŠEVIĆ[1], EMIR HOROZIĆ[2], JASMINA GLAMOČLIJA[3], MARINA SOKOVIĆ[3], JELENA ARSENIJEVIĆ[4], AND ZORAN MAKSIMOVIĆ[4]

[1] *University of Tuzla - Faculty of Pharmacy, Urfeta Vejzagića 8, 75000 Tuzla, Bosnia and Hercegovina*
[2] *University of Tuzla - Faculty of Technology, Urfeta Vejzagića 8, 75000 Tuzla, Bosnia and Hercegovina*
[3] *Institute for Biological Research "Siniša Stanković"-National Institute of Republic of Serbia, University of Belgrade, Department of Plant Physiology, Bulevar Despota Stefana 142, 11000 Belgrade, Serbia*
[4] *University of Belgrade - Faculty of Pharmacy, Department of Pharmacognosy, Vojvode Stepe 450, 11221 Belgrade, Serbia*
[*] *Corresponding author: ermina.cilovic@untz.ba*

Published: December 25, 2021
Received: April 19, 2021
Accepted: July 8, 2021
Published on-line: November 30, 2021

Telekia speciosa (Schreb.) Baumg., Asteraceae, is widespread in Eastern and Central Europe and the Balkan Peninsula. Previous phytochemical investigations have revealed _T. speciosa_ as a rich source of sesquiterpene lactone – isoalantolactone, especially in its underground parts. The aim of the present study was to analyze the essential oils from aerial and underground parts of _T. speciosa_ and investigate their antimicrobial activity. Chemical composition of essential oils was determined by GC-FID/MS method leading to the identification of 67 compounds in total, with 15.77 % oxygenated monoterpenes, 7.77 % sesquiterpene hydrocarbons, 49.14 % oxygenated sesquiterpenes, and 12.37 % other compounds from aerial parts, and 3.80 % oxygenated monoterpenes, 3.13 % sesquiterpene hydrocarbons, 90.33 % oxygenated sesquiterpenes from underground parts essential oil. The main components from aerial parts were (_E_)-nerolidol (11.54 %) and caryophyllene oxide (10.54 %), while isoalantolactone was the predominant component from essential oil underground parts (83.41 %). The minimum inhibitory concentration (MIC), minimum bactericidal/fungicidal concentration of the essential oils were evaluated against six strains of bacteria and two strains of fungus using _in vitro_ microdilution method. Both oils presented antimicrobial properties against pathogens _Staphylococcus aureus_, _Bacilus cereus_, _Pseudomonas aeruginosa_, _Escherichia coli_, and _Candida albicans_. Inhibition of growth of tested microorganisms by _T. speciosa_ underground parts essential oil was achieved with MICs ranging from 1.0 to 11.0 mg mL^{-1}, while MICs of aerial parts essential oil varied from 4.0 to 30.0 mg mL^{-1}. The obtained results contribute to the knowledge of antimicrobial properties of _T. speciosa_, which support traditional uses underground parts of the plant.

Key words: *Telekia speciosa*; Asteraceae; essential oil composition; antimicrobial activity; isoalantolactone

http://dx.doi.org/10.5937/leksir2141035C

1. INTRODUCTION

Telekia speciosa (Schreb.) Baumg. (Asteraceae) is a perennial herbaceous plant widespread in Eastern and Central Europe and the Balkan Peninsula. It is up to 200 cm high and it has alternating, wide, whole leaves and large heterogeneous yellow flowers with a diameter of 5-8 cm which can be individual or in cluster inflorescence. It inhabits wet and shady positions in mountain woodlands (Chalchat et al., 2004; Domac, 1994), and somewhere it is planted as a decorative plant. It is closely related to genus Inula, including *Inula helenium* L., a well-known medicinal plant (Stojakowska et al., 2011). The underground parts of *T. speciosa* are traditionally used as a remedy for bronchial asthma in Balkan countries (Serbia, Bosnia and Herzegovina). For example, smoke after burning underground parts is inhaled for the cure of asthma (Redzić, 2007).

The underground parts of *T. speciosa* contain essential oil, bitter compounds and inulin (Marković et al., 2010). Phytochemical investigations have revealed *T. speciosa* as a rich source of sesquiterpene lactone – isoalantolactone, especially in its underground parts which amount is equivalent to *I. helenium* underground parts (Radulović et al., 2010; Stojakowska et al., 2011; 2015a). The aerial parts extracts have been found to contain fatty acids, namely palmitic, linoleic, oleic, and caproic

acids, and sterols (Deliorman et al., 2002; Orhan and Sener, 2003). Aerial parts of *T. speciosa* accumulated miscellaneous sesquiterpene lactones, mainly of guaiane, pseudoguaiane, xanthane, and eudesmane type (Stojakowska et al., 2015b).

Isoalantolactone, a bioactive compound present in *T. speciosa* underground parts and aerial parts essential oils, has been found to have various pharmacological activities including anti-inflammatory, antimicrobial, and anticancer properties, with no significant toxicity. Alantolactone and isoalantolactone have been extensively investigated on several cancer cell lines, such as colon, melanoma, ovary, prostate, lung, and leukemia (Rasul et al., 2013). A previous study also revealed that extracts from both leaves and flowers of *T. speciosa* showed high antiproliferative activity against the cancer cell lines tested (Yuan et al., 2018). In addition, several *in vitro* and *in vivo* studies evaluated antimicrobial properties of isoalantolactone against methycillin-resistant *Staphylococcus aureus* (MRSA) and α-toxin, a product of most *S. aureus* microorganisms and essential for the pathogenesis of pneumonia (Qiu et al., 2011; Zhou et al., 2020). Unlike *T. speciosa*, for which, as far as we know, there are no available data on antimicrobial activity, essential oil of *I. helenium* underground parts was active against several Gram-positive and Gram-negative bacteria and *Candida* strains (Deriu et al., 2008).

Increasing bacterial resistance to antibiotics, antimicrobials, and antifungal agents is a growing concern facing the medical, pharmaceutical, sanitation, and food industries (Krist et al., 2015). An alternative to reduce the use of synthetic chemicals is the search for antimicrobials from medicinal plants. In this context, plants constituents, such as essential oils and their main components, terpenoids, have attracted considerable interest (Swamy et al., 2016).

The aim of the present study was to analyze the essential oils of both, aerial and underground parts of *T. speciosa* and to evaluate antimicrobial activity of essential oils.

2. MATERIALS AND METHODS

2.1. Plant material

Aerial and underground parts of *T. speciosa* were collected at the location of Karaula, Olovo municipality (Bosnia and Herzegovina) (N44°10′22.3", E18°38′58.6") during the flowering period in July 2018. The plant material was identified according to Flora Croatica (Domac, 1994) by authors and the voucher specimen was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, the University of Tuzla. The plant material was cleaned, cut, and air-dried.

2.2. Isolation and GC-FID/MS analyses of volatiles

The dried aerial and underground parts of *T. speciosa* were chopped and subjected to hydrodistillation for 3 h using a Clevenger-type apparatus. The obtained essential oils were separated, dried over anhydrous sodium sulfate, and stored at -20 °C until the analysis. The constituents of essential oils were determined by the GC-FID/MS method, as described previously (Cilović et al., 2019).

2.3. Antimicrobial activity of essential oils

The antimicrobial activity (AMA) of *T. speciosa* essential oils were tested against six strains of bacteria, i.e. Gram-positive *Staphylococcus aureus* (ATCC 6538 and clinical isolate) and *Bacillus cereus* (clinical isolate), and Gram-negative *Pseudomonas aeruginosa* (ATCC 27853 and clinical isolate) and *Escherichia coli* (ATCC 35210), as well as against two strains of fungus *Candida albicans* (ATCC 10231 and clinical isolate). The microorganisms were obtained from the Mycology Laboratory of the Institute for Biological Research "Siniša Stanković", University of Belgrade, Serbia.

The antibacterial assay was done by microdilution method (Cazella et al., 2019) utilizing 96-well microtiter plates to determine the minimal inhibitory concentration (MIC) and minimal bactericidal /fungicidal concentration (MBC/MFC). The inoculum was cultivated in a solid medium to verify the absence of contaminations, and for validation. Nutrient media were Tryptic Soy Broth (TSB) for bacteria and Sabouraud Dextrose Broth (SDB) for fungi. The microorganism suspensions (inocula) were adjusted with sterile saline until the concentration of 1.0×10^5 CFU mL^{-1}. The inoculum was prepared daily and stored at 4 °C until its utilization. The essential oil was added to the nutrient medium for the growth of microorganisms. Then the microorganism inocula were added and the plates were incubated for 24 h at 37 °C for bacteria and 72 h at 28 °C for fungi. The lowest concentration without visible microbial biomass growth under a binocular magnifying glass was defined as MIC.

Determining the absence of growth of microorganisms, i.e. determination MBC/MFC, was performed by serial reinoculation of 10 μL of inoculated medium from wells where no growth of the microorganism was recorded in 100 μL of sterile nutrient medium and reincubation for 24 h at 37 °C for bacteria and for 72 h at 28 °C for fungi. The results were confirmed after adding 40 μL of purple *p*-iodonitrotetrasolium chloride (Sigma), microbial growth indicator solution (0.2 mg mL^{-1} distilled water) to each well and incubation for 30 minutes at 37 °C (Tsukatani et al., 2012). Comparison of color intensity was performed with control wells in which unhindered growth of microorganisms was enabled, and commercial antimicrobial agents streptomycin (Sigma), ampicillin (Panfarma, Belgrade, Serbia), and ketoconazole (Zorka farma, Šabac, Serbia) were used as positive controls. Inoculated medium without added essential oil was used as the negative control. The antimicrobial tests were carried out in triplicate. The results were expressed in values of arithmetical average ± standard deviation.

3. RESULTS AND DISCUSSION

3.1. Content and composition of essential oils

The aerial parts of *T. speciosa* contained 0.02 % (v/w) of yellow, liquid fragrant essential oil. The identified 49 constituents accounting for 85.05 % of the oil are presented in Table 1. The essential oil of aerial parts (AEO) of *T. speciosa* was characterized by the presence of a high concentration of oxygenated sesquiterpenes (49.14 %). The major components were (*E*)-nerolidol (11.54 %), caryophyllene oxide (10.54 %), and (2*Z*,6*E*)-farnesol (4.52 %). Sesquiterpene hydrocarbons constituted 7.77 % of the oil with the dominant compound β-caryophyllene (4.90 %). Nerol (4.77 %) was the major representative of oxygenated monoterpenes, which constituted 15.77 % of the oil. Non-terpene (other) compounds presented an appreciable amount of essential oil (12.37 %) with dominant (*E*)-phytol (4.14 %).

The amount of essential oil found in the underground parts (UEO) of *T. speciosa* (0.31 %, v/w) was higher than in the aerial parts. The oil was yellowish semi-solid mass, with aromatic odor. By cooling and standing it crystallizes in the form of opaque needle-like crystals. The identified 35 constituents accounting for 97.26 % of the oil are presented in Table 1. This essential oil was characterized by the presence of a high concentration of oxygenated sesquiterpenes (90.33 %) with isoalantolactone (IAL) being the major component (83.41 %). Alantolactone, an oxygenated sesquiterpene, was also presented, but in a substantially lower amount (2.56 %).

Table 1. Chemical composition of essential oils from *T. speciosa* aerial and underground parts

No.	Ret. time	Compound	RIE[a]	AEO[b] [%m/m]	UEO[c] [%m/m]
1	12.844	linalool	1101.1	3.15	0.15
2	15.109	nerol oxide	1155.2	0.52	-
3	16.674	*α*-terpineol	1192.5	0.66	-
4	17.251	*n*-decanal	1206.2	0.84	-
5	18.236	nerol	1229.3	4.77	0.09
6	20.660	bornyl acetate	1286.0	0.47	-
7	20.852	dihidroedulan I	1290.4	2.48	-
8	22.325	silphiperfol-5-ene	1325.1	-	0.21
9	23.124	7-*epi*-silphiperfol-5-ene	1343.7	-	0.36
10	23.704	eugenol	1357.4	0.44	-
11	24.496	silphiperfol-6-ene	1376.4	-	0.12
12	24.682	modheph-2-ene	1380.9	-	0.14
13	24.947	*α*-isocomene	1387.2	-	0.12
14	25.199	*β*-elemene	1393.2	-	0.21
15	25.250	phenyl ethyl isobutanoate	1394.8	0.41	-
16	25.758	*β*-isocomene	1406.7	0.38	0.2
17	26.383	*β*-caryophyllene	1422.6	4.9	0.44
18	26.398	2,5-dimethoxy-*p*-cymene	1422.8	0.28	0.1
19	27.387	*epi*-*β*-santalene	1447.5	-	0.22
20	27.639	geranyl acetone	1454.0	0.91	-
21	27.710	*α*-humulene	1456.0	0.46	-
22	28.584	*β*-chamigrene	1477.7	-	0.16
23	28.893	thymol isobutyrate	1485.7	0.76	0.24
24	29.019	(*E*)-*β*-jonone	1488.8	1.11	-
25	29.099	*β*-selinene	1490.7	-	0.46
26	29.153	neryl isobutanoate	1492.1	3.07	0.77
27	29.397	*α*-selinene	1498.1	0.67	0.31
28	29.809	*β*-bisabolene	1508.6	-	0.18
29	30.009	modhephen-8-*β*-ol	1514.3	0.61	0.35
30	30.235	(*E*)-dihydro-apofarnesal	1519.3	0.39	-
31	30.479	*δ*-cadinene	1526.0	0.82	-
32	31.240	*α*-calacorene	1545.7	0.54	-
33	31.589	isocaryophyllene oxide	1554.8	1.33	-
34	31.815	*epi*-longipinanol	1560.6	0.57	-
35	32.053	(*E*)-nerolidol	1566.4	11.54	-
36	32.410	neryl (*S*)-2-metilbutyrate	1575.8	-	0.48
37	32.425	prenopsan-8-ol	1576.2	4.7	-
38	32.945	caryophyllene oxide	1589.7	10.54	0.98
39	33.795	humulene epoxide II	1612.4	1.71	0.14
40	34.488	murola-4,10(14)-diene-1-*β*-ol	1631.2	0.33	-
41	34.582	*α*-acorenol	1634.0	-	0.22
42	34.676	caryophylla-4(12),8(13)-dien-5-*α*-ol	1636.3	2.04	-
43	35.320	*β*-eudesmol	1653.8	2	0.66
44	35.565	selin-11-en-4-*α*-ol	1660.8	0.88	0.44
45	35.753	14-hydroxy-9-*epi*-*β*-caryophyllene	1665.8	-	0.2
46	35.923	14-hydroxy-(Z)- caryophyllene	1670.5	-	0.14
47	36.107	14-hydroxy-9-epi-(E)- caryophyllene	1675.6	0.89	0.53
48	36.418	epi-*α*-bisabolol	1683.7	1.31	-
49	36.655	eudesma-3,11-(13)-dien-12-al	1690.6	0.55	0.21
50	36.954	*n*-heptadecane	1698.6	0.49	-
51	37.218	*δ*-dodecalactone	1705.8	0.29	-
52	37.510	(2*E*,6*Z*)-farnesal	1714.6	0.66	-
53	37.678	3-methoxy-cuminil isobutyrate	1719.1	-	0.22
54	37.795	(2*Z*,6*E*)-farnesol	1722.8	4.52	-
55	37.887	naphthalen-2-ol	1725.3	-	0.49
56	38.523	(2*E*,6*E*)-farnesal	1743.4	0.47	-
57	38.867	ciclocolorenone	1753.7	0.39	-
58	39.224	(*Z*)-lanceol	1763.7	0.46	-
59	40.435	*n*-octadecane	1798.6	0.29	-
60	43.894	alantolactone	1902.7	-	2.56
61	44.280	10-isobutyryloxi thymol isobutyrate	1914.6	-	0.33
62	44.410	(5*E*,9*E*)-farnesyl acetone	1919.0	0.33	-
63	44.416	10-isobutyryloxy-8.9-dehydrothymyl isobutyrate	1919.2	0.34	-
64	45.229	isoalantolactone	1944.7	3.64	83.41
65	47.940	10-isobutyryloxy-8,9-epoxithymyl isobutyrate	2031.6	1.31	1.42
66	50.427	(*E*)-phytol	2114.3	4.14	-
67	60.949	*n*-pentacosane	2498.9	0.69	-
Total identified				85.05	97.26
Oxygenated monoterpenes				15.77	3.8
Sesquiterpene hydrocarbons				7.77	3.13
Oxygenated sesquiterpenes				49.14	90.33
Others				12.37	-

[a] RI, retention indices as determined on HP-5 column using homologous series of C_8-C_{30} alkanes.
[b] AEO, aerial parts essential oil.
[c] UEO, underground parts essential oil

Sesquiterpene hydrocarbons constituted 3.13 %, while oxygenated monoterpenes constituted 3.80 % of the oil, with thymol derivative 10-isobutyryloxi-8,9-epoxithymol isobutyrate (1.42 %), found in appreciable amount.

In the previous investigations, aerial parts collected during the flowering period of *T. speciosa* from Bosnia and Herzegovina and Serbia contained slightly higher amounts of essential oil than in the current study, i.e. 0.04 % and 0.06 % (v/w), respectively. The major components of those oils were oxygenated sesquiterpenes (*E,Z*)-farnesol (12.0 % in the essential oil from Serbia), (*E*)-nerolidol (10.2-10.3 %), caryophyllene oxide (4.5-8.2 %), (*Z,E*)-farnesol (7.7 % in the essential oil from Bosnia and Herzegovina), *β*-caryophyllene (5.4 %), similar to the currently analyzed AEO (Cilović et al., 2019; Radulović et al., 2010).

It is worth noting that *T. speciosa* leaf essential oil, originating from Poland, as dominant constituents also contained a farnesol isomer (i.e. (*E,E*)-farnesol, 21.2 %) and (*E*)-nerolidol (17.9 %), while flower essential oil was abundant with isoalantolactone (23.0 %) and 10-isobutyryloxy-8,9-epoxythymol isobutyrate (20.5 %) along with other several thymol derivatives (Wajs-Bonikowska et al., 2012). These two compounds were present in several times lower amounts in the currently analyzed oil, which could be explained by the fact that it was obtained from the complete aerial parts in which flowers represented only one minor part.

Based on the previously and currently analyzed essential oil from *T. speciosa* aerial parts it could be concluded that the characteristic compounds for analyzed oil are (*E*)-nerolidol, caryophyllene oxide, different stereoisomers [(*E,Z*)-, (*Z,E*)-, (*E,E*)-] of farnesol, *β*-caryophyllene, and other compounds present in lower amounts.

In the previous investigations, underground parts collected during the flowering period of *T. speciosa* from Bosnia and Herzegovina and Poland contained almost equal or somewhat higher amounts of essential oil than in the current study, i.e. 0.29 % and 0.41 % (v/w), respectively. Only underground parts of *T. speciosa* collected during the flowering period from Montenegro contained higher amounts of essential oil than in the current study, i.e. 1.7 %. The dominant compound in all those samples was isoalantolactone (62.3 % - 95 %). Beside isoalantolactone, other oxygenated sesquiterpene alantolactone (2.4 % in the essential oil from Bosnia and Herzegovina), and thymol derivatives 10-isobutyryloxi-8,9-epoxithymol isobutyrate (2.9-3.4 %), 9-isobutyryloxythymol isobutyrate (2.1 % in the essential oil from Poland) were also present in the previously analyzed essential oils (Chalchat et al., 2004; Cilović et al., 2019; Wajs-Bonikowska et al., 2012). Results about the chemical composition of *T. speciosa* underground parts essential oil obtained in this study are in accordance with the published literature data. Taking into account previous data and the currently analyzed oil underground parts of *T. speciosa* may be considered as a potential raw material for isoalantolactone isolation.

3.2. Antimicrobial activity of essential oils

Both investigated essential oils AEO and UEO exerted antimicrobial activity against all tested microorganisms (Tables 2 and 3).

As shown in Table 2, bacterio- and fungistatic effects of UEO were more prominent then those of AEO, but lower then the control antibiotics streptomycin and ampicillin and antifungal ketoconazole. Inhibition of growth of tested microorganisms by *T. speciosa* UEO was achieved with MICs ranging from 1.0 to 11.0 mg mL^{-1}, while MICs of AEO varied from 4.0 to 30.0 mg mL^{-1}. Similarly, bactericide and fungicide effects were observed in the presence of lower concentrations of UEO (MBC/MFC 4.0-15.0 mg mL^{-1}) in comparison to AEO (MBC/MFC 7.0-90.0 mg mL^{-1}) (Table 3). However, both essential oils were less effective than the antimicrobial drugs that were used as positive controls.

Among tested bacteria, ATCC strains of *S. aureus* and *E. coli* were the most susceptible strains to *T. speciosa* UEO (MIC 1.1, and 1.0 mg mL^{-1}, respectively; MBC 7.0 mg mL^{-1} for both microorganisms), while both standard and clinical strains of *P. aeruginosa* were the most (and equally) resistant in the presence of this oil (MIC and MBC were 7.0 mg mL^{-1}, and 15.0 mg mL^{-1}, respectively). There was no apparent difference in susceptibility toward UEO between Gram-positive and Gram-negative bacteria. On the other hand, AEO exhibited stronger effects on the tested Gram-negative bacteria than on Gram-positive ones. In addition, even though AEO showed generally lower activity in comparison to UEO, the standard strain of *P. aeruginosa* (the only one among the tested strains) was more susceptible to AEO (MIC 4.0 mg mL^{-1}) than to UEO (MIC 7.0 mg mL^{-1}). The most resistant strain to *T. speciosa* AEO was a clinical isolate of *B. cereus* (MIC 30.0 mg mL^{-1}; MBC 90.0 mg mL^{-1}). Clinical isolate of *C. albicans* was highly susceptible to UEO (MIC 1.0 mg mL^{-1}), but not to AEO (MIC 11.0 mg mL^{-1}). Both EOs showed similar and relatively low anticandidal activity on standard strain of this fungus.

The analyzed essential oils did not show a large difference in AMA against clinical and ATCC strains of microorganisms, in contrast, to control antibiotics (streptomycin, ampicillin) and antifungal ketoconazole which showed a lower effect on clinical than on standard strains, except in the case of *C. albicans*. Clinical isolate of this fungus is more than 10 times susceptible to UEO compared to the standard strain.

The potential of major constituents in the currently analyzed oils to act as antimicrobials or to contribute to the antimicrobial effects of essential oils was confirmed in several previous studies. Qiu et al. (2011) investigated the activity of IAL on *S. aureus* and *α*-toxin. *α*-Toxin is a product of most *S. aureus* microorganisms and is essential for the pathogenesis of pneumonia. MIC value of IAL for *S. aureus* was more than 1.024 mg mL^{-1} and IAL inhibited the expression of *α*-toxin, in *S. aureus* at very low concentrations. Results were confirmed *in vitro* and *in vivo*. Also IAL, in combination with penicillin G, exhibited significant synergism against 21 *β*-lactamase-positive *S. aureus* strains (including MRSA). MIC values of the penicillin G alone and in combination with IAL against tested bacterial *S. aureus* and MRSA strains were reduced three to even twenty-six times (Zhou et al., 2020).

Nerolidol, the major compound in the currently analyzed *T. speciosa* AEO, is common component found in the essential oil of various medicinal plants. Many studies showed its antimicrobial activity. The mixture of (*Z,E*)-nerolidol exhibited potent antimicrobial activity against *S. aureus* and 20 strains of MRSA (Hada et al., 2003). (*E*)-Nerolidol also exhibited antimicrobial activity against *S. aureus* with MIC values ranging from 125 to 500 *μ*g mL^{-1} (Braca et al., 2008). In another study MIC values for nerolidol against *Streptococcus mutans*, *Salmonela enterica*, and *Aspergillus niger* were 25.0, from 3.9 to 15.6, and 62.5 *μ*g mL^{-1}, respectively (Chan et al., 2016). For (*E,E*)-farnesol, alicyclic sesquiterpene alcohol present in many essential oils as well as in *T. speciosa* AEO, good antimicrobial and fungistatic activity is reported (Krist et al., 2015).

Even though MIC values for caryophyllene oxide and *β*-caryophyllene, to the best of knowledge, were not yet published, the AMA of essential oil from leaves of *Croton heliotropifolius* which contained *β*-caryophyllene as dominant compound inhibited the growth of several Gram-positive and Gram-negative bacteria. Its antibacterial activity was characterized as weak to moderate against the analyzed strains (de

Table 2. Minimum inhibitory concentrations (MIC) of *Telekia speciosa* aerial and underground parts essential oils and streptomycin, ampicillin and ketoconazole controls

Microorganism	AEO[a] [mg mL^{-1}]	UEO[b] [mg mL^{-1}]	Streptomycin [mg mL^{-1}]	Ampicillin [mg mL^{-1}]	Ketoconazole [mg mL^{-1}]
Staphylococcus aureus ATCC 6538	15.0 ± 2.0	1.1 ± 0.1	0.006 ± 0.001	0.012 ± 0.001	n.d.
Staphylococcus aureus clinical isolate	15.0 ± 1.0	4.0 ± 0.3	0.100 ± 0.010	0.100 ± 0.008	n.d.
Bacillus cereus clinical isolate	30.0 ± 3.0	3.0 ± 0.1	0.025 ± 0.003	0.100 ± 0.010	n.d.
Pseudomonas aeruginosa ATCC 27853	4.0 ± 0.3	7.0 ± 0.5	0.025 ± 0.002	0.050 ± 0.002	n.d.
Pseudomonas aeruginosa clinical isolate	11.0 ± 1.0	7.0 ± 0.6	0.100 ± 0.015	0.300 ± 0.009	n.d.
Escherichia coli ATCC 35210	7.0 ± 0.5	1.0 ± 0.1	0.100 ± 0.002	0.150 ± 0.008	n.d.
Candida albicans ATCC 10231	15.0 ± 1.0	11.0 ± 1.0	n.d.	n.d.	0.0016 ± 0.0001
Candida albicans clinical isolate	11.0 ± 2.0	1.0 ± 0.1	n.d.	n.d.	0.0031 ± 0.0001

[a] AEO, aerial parts essential oil.
[b] UEO, underground parts essential oil.
[c] n.d., not detected.

Table 3. Minimum bactericidal/fungicidal concentrations (MBC/MFC) of *Telekia speciosa* aerial and underground parts essential oils and streptomycin, ampicillin, and ketoconazole controls.

Microorganism	AEO[a] [mg mL^{-1}]	UEO[b] [mg mL^{-1}]	Streptomycin[c] [mg mL^{-1}]	Ampicillin [mg mL^{-1}]	Ketoconazole [mg mL^{-1}]
Staphylococcus aureus ATCC 6538	30.0 ± 2.0	7.0 ± 0.5	0.012 ± 0.001	0.025 ± 0.002	n.d.
Staphylococcus aureus clinical isolate	30.0 ± 1.2	7.0 ± 0.3	0.200 ± 0.01	0.150 ± 0.009	n.d.
Bacillus cereus clinical isolate	90.0 ± 5.0	4.0 ± 0.1	0.050 ± 0.006	0.150 ± 0.01	n.d.
Pseudomonas aeruginosa ATCC 27853	7.0 ± 1.0	15.0 ± 1.0	0.050 ± 0.009	0.100 ± 0.007	n.d.
Pseudomonas aeruginosa clinical isolate	15.0 ± 1.0	15.0 ± 1.5	0.200 ± 0.01	0.500 ± 0.03	n.d.
Escherichia coli ATCC 35210	15.0 ± 2.0	7.0 ± 0.6	0.200 ± 0.008	0.200 ± 0.02	n.d.
Candida albicans ATCC 10231	30.0 ± 2.0	15.0 ± 1.0	n.d.	n.d.	0.0062 ± 0.001
Candida albicans clinical isolate	15.0 ± 1.5	7.0 ± 0.5	n.d.	n.d.	0.0058 ± 0.300

[a] AEO, aerial parts essential oil.
[b] UEO, underground parts essential oil.
[c] n.d., not detected.

Alencar Filho et al., 2017).

In the context of the current investigation, it is worth noting that there is a great similarity in the chemical composition of the essential oil of *T. speciosa* underground parts with previously analyzed essential oil of *Inula helenium* L. root which exhibited substantial antimicrobial activity against several bacterial and fungal strains. The dominant principles of *I. helenium* root essential oil were three isomeric eudesmane-type sesquiterpene lactones: alantolactone (51.3-55.8 %), isoalantolactone (26.1-36.9 %), and diplophyllin (5.1 %). It was experimentally confirmed that essential structural parts responsible for antimicrobial activity of *I. helenium* root essential oil are eudesmane core olefinic bonds, alongside the α,β-methylene-lactone ring of sesquiterpene lactones. Bacterial and fungal strains used in this research were similar to those in the current study. Clinical isolate of *B. cereus* and standard strain of *S. aureus* were among the most susceptible strains to analyzed *I. helenium* L. root essential oil (MIC 0.017 and 0.6 mg mL^{-1}, respectively). The most resistant strains were clinical isolate of *P. aeruginosa* and standard strain of *E. coli* (MIC 14.8 mg mL^{-1} for both). Analyzed essential oil showed generally better AMA against Gram-positive strains regarding Gram-negative ones. *Candida* strains were among the most susceptible with MIC values ranging from 0.009 mg mL^{-1} to 0.12 mg mL^{-1} (Deriu et al., 2008; Stojanović-Radić et al., 2012).

T. speciosa UEO obtained best results of antibacterial activity also for the standard strain of *S. aureus*, clinical isolate of *B. cereus*, and unlike *I. helenium* root essential oil, for the standard strain of *E. coli*. Clinical isolate of *P. aeruginosa* was among the most resistant strains to this essential oil, too. The oil showed also good fungicidal activity against clinical isolate of *C. albicans*, while standard strain was among the most resistant ones.

CONCLUSION

In the current study, *Telekia speciosa* (Schreb.) Baumg. was characterized by the presence of a high concentration of oxygenated sesquiterpenes with isoalantolactone as the predominant constituent of essential oil underground parts, and (*E*)-nerolidol and caryophyllene oxide as the major constituents of essential oil aerial parts. According to this, underground parts may be a potential raw material for isoalantolactone isolation.

T. speciosa essential oil was, to the best of our knowledge, investigated for antimicrobial activity against pathogens for the first time in this study. Essential oil of underground parts achieved better antimicrobial activity compared to the essential oil of aerial parts with the most susceptible standard strains of *S. aureus* and *E. coli*, and clinical isolate of *C. albicans*.

The obtained results are important from the aspect of *T. speciosa* application as an antimicrobial agent. Traditionally its underground parts are used in bronchial asthma therapy and have a great potential to be used as a new therapeutic drug with recommendation for further pharmacological and toxicological investigations.

ACKNOWLEDGMENTS

This research is funded by the Serbian Ministry of Education, Science and Technological Development (Contract No. 451-03-68/2020-14/200007)

REFERENCES

Braca, A., Siciliano, T., D'Arrigo, M. and Germanò, M. P. (2008). Chemical composition and antimicrobial activity of *Momordica charantia* seed essential oil, *Fitoterapia* **79**(2): 123–125.

Cazella, L. N., Glamočlija, J., Soković, M., Gonçalves, J. E., Linde, G. A., Colauto, N. B. and Gazim, Z. C. (2019). Antimicrobial activity of essential oil of *Baccharis dracunculifolia* DC (Asteraceae) aerial parts at flowering period, *Frontiers in Plant Science* **10**: Article 27.

Chalchat, J.-C., Maksimović, Z. and Petrović, S. (2004). Isoalantolactone, the principal constituent of the essential oil of *Telekia speciosa* (schreb.) Baumg., Asteraceae underground parts, *Arhiv za farmaciju* **54**(1-2): 15–23.

Chan, W.-K., Tan, L., Chan, K.-G., Lee, L.-H. and Goh, B.-H. (2016). Nerolidol: A sesquiterpene alcohol with multi-faceted pharmacological and biological activities, *Molecules* **21**(5): 529.

Cilović, E., Brantner, A., Tran, H. T., Arsenijević, J. and Maksimović, Z. (2019). Methanol extracts and volatiles of *Telekia speciosa* (Schreb.) Baumg. from Bosnia and Herzegovina, *Technologica Acta* **12**(1): 9–13.

de Alencar Filho, J. M., da Araújo, L. C., Oliveira, A. P., Guimarães, A. L., Pacheco, A. G., Silva, F. S., Cavalcanti, L. S., Lucchese, A. M., Almeida, J. R. d. S. and Araújo, E. C. d. C. (2017). Chemical composition and antibacterial activity of essential oil from leaves of *Croton heliotropiifolius* in different seasons of the year, *Revista Brasileira de Farmacognosia* **27**(4): 440–444.

Deliorman, D., Ergun, F. and Koyuncu, M. (2002). Sterols of *Telekia speciosa*, *Chemistry of Natural Compounds* **38**(2): 201–201.

Deriu, A., Zanetti, S., Sechi, L. A., Marongiu, B., Piras, A., Porcedda, S. and Tuveri, E. (2008). Antimicrobial activity of *Inula helenium* L. essential oil against Gram-positive and Gram-negative bacteria and *Candida* spp., *International Journal of Antimicrobial Agents* **31**(6): 588–590.

Domac, R. (1994). *Flora Hrvatske: priručnik za određivanje bilja*, Školska knjiga.

Hada, T., Shiraishi, A., Furuse, S., Inoue, Y., Hamashima, H., Matsumoto, Y., Masuda, K., Shiojima, K. and Shimada, J. (2003). Inhibitory effects of terpenes on the growth of *Staphylococcus aureus*, *Natural medicines* **57**(2): 64–67.

Krist, S., Banovac, D., Tabanca, N., Wedge, D. E., Gochev, V. K., Wanner, J., Schmidt, E. and Jirovetz, L. (2015). Antimicrobial activity of nerolidol and its derivatives against airborne microbes and further biological activities, *Natural Product Communications* **10**(1): 143–148.

Marković, M., Matović, M., Pavlović, D., Zlatković, B., Marković, A., Jotić, B. and Stankov-Jovanović, V. (2010). Resources of medicinal plants and herbs collector's calendar of Pirot County (Serbia), *Biologica Nyssana* **1**(1-2): 9–21.

Orhan, I. and Sener, B. (2003). Comparative fatty acid analysis of *Telekia speciosa*, *Chemistry of Natural Compounds* **39**(3): 244–245.

Qiu, J., Luo, M., Wang, J., Dong, J., Li, H., Leng, B., Zhang, Q., Dai, X., Zhang, Y., Niu, X. and Deng, X. (2011). Isoalantolactone protects against *Staphylococcus aureus* pneumonia, *FEMS Microbiology Letters* **324**(2): 147–155.

Radulović, N., Blagojević, P., Palić, R. and Zlatković, B. (2010). Volatiles of *Telekia speciosa* (Schreb.) Baumg. (Asteraceae) from Serbia, *Journal of Essential Oil Research* **22**(3): 250–254.

Rasul, A., Khan, M., Ali, M., Li, J. and Li, X. (2013). Targeting apoptosis pathways in cancer with alantolactone and isoalantolactone, *The Scientific World Journal* **2013**: 1–9.

Redzić, S. S. (2007). The ecological aspect of ethnobotany and ethnopharmacology of population in Bosnia and Herzegovina, *Collegium Antropologicum* **31**(3): 869–890.

Stojakowska, A., Malarz, J. and Kisiel, W. (2011). Terpenoids from a multiple shoot culture of *Telekia speciosa*, *Acta Societatis Botanicorum Poloniae* **80**(3): 253–256.

Stojakowska, A., Malarz, J. and Kisiel, W. (2015b). Quantitative analysis of sesquiterpene lactones and thymol derivatives in extracts from *Telekia speciosa*, *Phytochemistry Letters* **11**: 378–383.

Stojakowska, A., Malarz, J., Żylewski, M. and Kisiel, W. (2015a). Acylated hydroxycinnamic acid glucosides from flowers of *Telekia speciosa*, *Phytochemistry Letters* **12**: 257–261.

Stojanović-Radić, Z., Čomić, L., Radulović, N., Blagojević, P., Denić, M., Miltojević, A., Rajković, J. and Mihajilov-Krstev, T. (2012). Antistaphylococcal activity of *Inula helenium* L. root essential oil: eudesmane sesquiterpene lactones induce cell membrane damage, *European Journal of Clinical Microbiology & Infectious Diseases* **31**(6): 1015–1025.

Swamy, M. K., Akhtar, M. S. and Sinniah, U. R. (2016). Antimicrobial properties of plant essential oils against human pathogens and their mode of action: An updated review, *Evidence-Based Complementary and Alternative Medicine* **2016**: 1–21.

Tsukatani, T., Suenaga, H., Shiga, M., Noguchi, K., Ishiyama, M., Ezoe, T. and Matsumoto, K. (2012). Comparison of the WST-8 colorimetric method and the CLSI broth microdilution method for susceptibility testing against drug-resistant bacteria, *Journal of Microbiological Methods* **90**(3): 160–166.

Wajs-Bonikowska, A., Stojakowska, A. and Kalemba, D. (2012). Chemical composition of essential oils from a multiple shoot culture of *Telekia speciosa* and different plant organs, *Natural Product Communications* **7**(5): 625–628.

Yuan, C.-B., Tian, L., Yang, B. and Zhou, H.-Y. (2018). Isoalantolactone protects LPS-induced acute lung injury through Nrf2 activation, *Microbial Pathogenesis* **123**: 213–218.

Zhou, Y., Guo, Y., Wen, Z., Ci, X., Xia, L., Wang, Y., Deng, X. and Wang, J. (2020). Isoalantolactone enhances the antimicrobial activity of penicillin G against *Staphylococcus aureus* by inactivating β-lactamase during protein translation, *Pathogens* **9**(3): 161.