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Bioprospecting of the *Telekia speciosa*: Uncovering the Composition and Biological Properties of Its Essential Oils

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Abstract: The essential oils (EOs) of *Telekia speciosa*, a perennial plant native to southeastern Europe and Asia Minor, were analyzed for their composition and biological properties. *T. speciosa* is an invasive plant in Poland; however, its beauty prompts gardeners to cultivate the plants. *T. speciosa* serves as a valuable source of nectar and pollen for honey bees. Our results revealed more than 150 compounds in the flower, leaf, and root EOs. Major constituents found in the essential oils from the roots included isoalantolacton (46.2%) and from the flowers nerol (11.9%), while from the leaves, they included (*E*)-nerolidol (10.1%). *T. speciosa* flower EO showed significant cytotoxicity against A375 cells, with IC_{50} values of 7.2, 5.1, and 3.4 µg/mL referring to 24, 48, and 72 h, respectively, indicating its potential as a natural cytotoxic agent. The antimicrobial activity of the essential oils against *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 was also investigated. The essential oils from the flowers and leaves of *T. speciosa* demonstrated higher inhibitory activity against *S. aureus* (MIC: 5.9–7.8 µL/mL) and *E. coli* (MIC: 7.8–11.7 µL/mL) than the essential oil isolated from the roots of the plant (MICs 31.3 and 62.5 µL/mL against *S. aureus* and *E. coli*, respectively).

Keywords: *Telekia speciosa*; essential oil; antimicrobial properties; cytotoxic activity; melliferous plant

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1. Introduction

Heartleaf oxeye (*Telekia speciosa* (Schreb.) Baumg.) is a perennial, herbaceous, and melliferous plant that is native to mountainous areas in southeast Europe: Carpathians, the mountains of the Balkan Peninsula; northern Anatolia; and Caucasus. Climate change, however, favors colonization of new areas in Europe by the plant. In most European countries, as well as in North America and Japan, *T. speciosa* is an introduced species. One of the largest colonies of this plant, with an area of about 2 ha, can be found in Poland on the bank of the Jasiołka River near Tylawa. Moreover, in Poland, the plant is found in the Bieszczady and Mazury regions. In Czech Republic, *T. speciosa* has been classified as an invasive neophyte. It colonizes many areas almost throughout Poland, Czech Republic, Ukraine, Balkans, and other European countries, mainly at middle and high elevations. Heartleaf oxeye became naturalized and, in many cases, spread along streams, rivers, and forest tracks, and to forest clearings and abandoned montane meadows. The species was named after the 19th-century Hungarian aristocrat Teleki von Szek, who valued its beauty and financed the botanical research of German scientist J. Ch. G. Baumgarten. *T. speciosa* typically grows in clusters. The plant may reach 150–200 cm in height and blooms with multi-flowered, yellow capitula [1–3].

T. speciosa is an essential-oil-bearing plant well-known for its richness of biologically active constituents, both volatiles and non-volatiles [4]. Essential oils (EOs) are complex mixtures of volatile compounds that are biosynthesized inside intracellular compartments in diverse plant organs. These volatiles include many biologically active compounds, mainly terpenes, formed from isopentyl diphosphate (IPP) or 3,3-dimethylallyl diphosphate (DPP) units [2]. The roots of *T. speciosa* contain EO characterized by the dominance of volatile sesquiterpene lactone: isoalantolactone. Aerial parts of the plant have been shown to contain isoalantolactone in their EOs as well, albeit in smaller quantities. This biologically active molecule has been a subject of numerous studies. For example, isoalantolactone, in combination with penicillin G, exhibited synergism against β-lactamase-positive *Staphylococus aureus* strains, even including methicillin-resistant *S. aureus* [5,6]. Aerial parts of *T. speciosa* contain sesquiterpene lactones of the eudesmane, guaiane, xanthane, and pseudoguaiane types [4,7]. Flowers of the plant were identified as a source of many different terpenoids, including those of high molecular mass, such as calenduladiol esters. The esters and a sesquiterpene lactone (asperilin) isolated from the flower extract were tested against human cancer cells. Flower extracts and sesquiterpene lactones isolated thereof showed high antiproliferative activity against the cancer cell lines tested [7–9]. Previous analysis of *Telekia speciosa* flower EO, distilled from plants grown in Poland, revealed the presence of isoalantolactone (23.0%) and 10-isobutyryloxy-8,9-epoxythymol isobutyrate (20.5%) as dominant constituents [10]. These two compounds were present in much lower amounts in a recently studied *T. speciosa* EO [11], probably due to the fact that the EO was obtained from aerial parts, in which flowers represented only a minor part. In total, EOs from aerial and unground parts of *T. speciosa* from Bosnia and Herzegovina contained 67 compounds. The major volatiles identified in green parts were (*E*)-nerolidol (11.5%) and caryophyllene oxide (10.5%), while isoalantolactone predominated in roots (83.4%) [11]. EO from the aerial parts of *T. speciosa* collected in Serbia contained (*E*,*Z*)-farnesol (12.0%), (*E*)-nerolidol (10.2%), and β-caryophyllene (5.4%) as major constituents [5]. Such variability in the composition of EOs obtained from the same species is not unusual [12].

Several studies linked the chemicals present in EOs to the biological properties of the oils. EOs from *T. speciosa* demonstrated antibacterial activities against *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albicans* strains [11]. Terpenoids based on thymol skeleton, as well as isoalantolactone, might be, at least in part, responsible for this activity [5,11,13,14]. Isoalantolactone, a compound found in EOs, has been connected to a number of biological activities, including anti-inflammatory and anti-cancer effects. *T. speciosa* root extract was used to treat bronchial asthma in countries such as Bosnia and Herzegovina [15,16].

The purpose of this study was to investigate the composition of essential oils isolated from both the aerial (leaves and flowers separately) and subterranean parts of *T. speciosa* plants growing in Poland. Following that, the cytotoxicity and antibacterial activities of these EOs against chosen human cancer cell lines and bacterial strains were investigated. Based on the present research and the literature data available, we aimed to answer the question whether or not this melliferous plant may be of benefit to honey bees and bee product consumers.

2. Materials and Methods

2.1. Sample Collection and Identification

Roots and aerial parts of *T. speciosa* were collected in August 2017 from plants grown in the Garden of the Medicinal Plants, Institute of Pharmacology, Polish Academy of Sciences, Kraków, where voucher specimens were deposited (voucher No. 1/2017). The plants were obtained from the seeds delivered from the French National Museum of Natural History and the Alpine Botanic Garden: La Jaysinia (Samoens).

2.2. Isolation of Essential Oils

Essential oils from the aerial and underground parts were isolated by hydrodistillation for 4 h using a Clevenger-type apparatus. Each portion of plant material used for hydrodistillation was weighed at 100–150 g. Hydrodistillation of roots (0.491 kg), leaves (2.129 kg), and flowers (0.491 kg) was performed several times in parallel, until the volume of EOs did not increase in the glass tube of the Clevenger equipment.

2.3. GC-MS Analysis of Essential Oils

The identification and quantification of the components of the EOs were performed by GC-FID-MS with a quadrupole MS detector and an advanced ion source for the electron impact (EI). GC Ultra was coupled with a DSQII mass spectrometer (Thermo Electron, Waltham, MA, USA). Simultaneous GC-FID and GC-MS analyses were performed using a MS-FID splitter (SGE Analytical Science, Ringwood, Australia). Mass spectra in the positive ion mode were recorded in the range of m/z 50–550 after EI ionization at 70 eV. We used the capillary non-polar column Rtx–1MS (60 m × 0.25 mm × 0.25 µm). An injection of 1 µL of the EO was applied in 1:100 split modes. GC measurements were performed under the following conditions: Temperature program: 60 °C (0.5 min)–300 °C (30 min) at 4 °C/min injector; the detector temperatures were 320 °C and 310 °C, respectively. Helium, with a constant flow rate of 1.5 mL/min, was used as a carrier gas.

2.4. Identification of Compounds

The identification of compounds was based on a comparison of MS with the computer mass library NIST98.1, Wiley Registry of Mass Spectral Data, 10th edition, along with relative retention indices (RI, Rtx-1). The value of RI was based on Kovat's index formula. The experimental retention indices of the volatiles were compared to the RIs of the alkane standard mixture (C5-C-26: Sigma Aldrich).

2.5. Strains and Cultivation Conditions

The following reference strains were used in the study: *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922. Bacteria were inoculated on Columbia agar with 5% sheep blood (bioMeriéux, Warsaw, Poland) and then allowed to grow for 24 h at 37 °C in an aerobic environment. EOs from all parts of *T. speciosa*, including leaves, flowers, and roots, were used for the antimicrobial activity, and we used pure thymol compound (1 µg/mL) as a standard antibacterial drug.

Determination of the Minimum Inhibitory Concentration (MIC) of Essential Oils against Bacteria

Determination of the minimum inhibitory concentration (MIC) of EOs against bacteria. The MIC of the EOs against bacteria was determined by serial dilution in Mueller–Hinton broth (MHB, Sigma-Aldrich, Darmstadt, Germany), according to the recommendations of the Clinical and Laboratory Standards Institute (protocol M07-A9) [17]. The stock solution of the tested oils was prepared with the addition of Tween 80 (1%) (Sigma-Aldrich, Darmstadt, Germany), obtaining a concentration range of 1.0 to 125 µL/mL. A total of 50 µL of sequential concentrations of the oils were added to a 96-well microplate. Then, 50 µL of a bacterial suspension with a concentration of 10^6 CFU/mL was added to each well of the microplate. After a 24-h incubation at 37 °C, the MIC for individual oils was determined by adding 20 µL of a 0.02% resazurin solution (Sigma-Aldrich, Darmstadt, Germany) to the wells [18]. A color change from navy blue to pink after 3 h of incubation with resazurin at 37 °C indicated the presence of bacteria. The first well in which the navy blue color persisted determined the MIC value. In addition, a positive control (MHB + bacterial suspension of *S. aureus* or *E. coli*) and control of the purity of the medium (MHB) were performed. For each oil, the test was carried out in duplicate.

2.6. Cell Culture and Treatment

Melanoma cells A375 were obtained from Sigma-Aldrich (St. Louis, MO, USA). Melanoma cells C32 and normal human skin fibroblasts CCD25Sk were purchased from the American Type Culture Collection (Manassas, VA, USA). HaCaT keratinocytes were purchased from AddexBio (San Diego, CA, USA). The cells were maintained at 37 °C in a 5% CO_2 incubator in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (Gibco, Waltham, MA, USA). The EO was dissolved in dimethyl sulfoxide (DMSO) and stored at −20 °C for up to 1 month. The final concentration of DMSO in the culture medium never exceeded 0.1%, and the same concentration of DMSO was used as the control. Cisplatin (Sigma-Aldrich) was dissolved in the culture medium just before it was added to the cells.

2.7. Cell Viability Assay

Cells were seeded at a density of 1×10^4 cells per well in 96-well plates and allowed to adhere for 24 h. Next, cells were treated with EO or cisplatin at concentrations of 1.5, 3.1, 6.2, 12.5, and 25 μg/mL for 24 h, 48 h, and 72 h. 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT, Sigma-Aldrich) solution was added to each well, and the cells were incubated at 37 °C for 4 h in the dark. The medium was then removed, and formazan crystals were dissolved in 100 μL of DMSO and 12.5 μL of Sorensen's glycine buffer on a plate shaker. The optical density was measured at 570 nm. The half-maximal inhibitory concentration (IC_{50}) was calculated using GraphPad Prism 7 software.

2.8. Apoptosis Assay

A375 and C32 cells were plated in 6-well plates at 1×10^5 cells per well and allowed to adhere for 24 h. Cells were treated with EO at concentrations of 1.5, 3.1, 6.2, 12.5, and 25 μg/mL for another 24 h. Floating and adherent cells were collected and assayed using a Dead Cell Apoptosis Kit with annexin V-fluorescein isothiocyanate (FITC) and propidiumiodide (PI) for flow cytometry (#V13242, Thermo Fisher Scientific, Waltham, MA, USA), according to the manufacturer's protocol. Briefly, cells were dispersed in 100 μL annexin-binding buffers containing 5 μL annexin V-FITC conjugate solution, 1 μg/mL PI, and 1 μg/mL Hoechst 33342 and incubated for 15 min at room temperature. Then, 400 μL of annexin-binding buffer was added, and the cell suspension was transferred to 96-well plates and visualized using a fluorescence microscope. Early apoptotic cells showed green and blue fluorescence, while late apoptotic cells showed green, red, and blue fluorescence.

2.9. Western Immunoblot

A375 cells were treated with 1.5, 3.1, 6.2, and 12.5 μg/mL of EO for 24 h. Adherent and floating cells were harvested, and total protein was extracted from the cells using RIPA buffer supplemented with a 1% protease and phosphatase inhibitor cocktail (#78440, Thermo Fisher Scientific, Waltham, MA, USA). A Lowry assay was performed to quantify the protein content in the homogenates. Proteins (20–40 μg) were resolved on 10% or 12% SDS-PAGE gels. Proteins were transferred to a nitrocellulose membrane (Bio-Rad, Hercules, CA, USA). The membranes were blocked with 5% skim milk for 1 h at room temperature and incubated with primary antibodies overnight at 4 °C. The following antibodies were used: anti-PARP (#9542, 1:1000 dilution), anti-cleaved PARP antibody (#5625, 1:1000 dilution), anti-caspase-8 antibody (#9746, 1:1000 dilution), anti-caspase-9 antibody (#9508, 1:1000 dilution), anti-caspase-3 antibody (#9662, 1:1000 dilution), anti-cleaved caspase-3 antibody (#9664, 1:500 dilution) (all purchased from Cell Signaling Technology, Danvers, MA, USA), and anti-actin antibody (#A2066, 1:2000 dilution; purchased from Sigma-Aldrich). Next, the membranes were incubated with secondary antibodies (anti-mouse IgG-HRP, Sigma-Aldrich, #A9044, 1:5000 dilution, and anti-rabbit IgG-HRP, Sigma-Aldrich, #A9169, 1:5000 dilution) for 1 h at room temperature. The membranes were treated with ECL-HRP substrate (GE Healthcare, Chicago, IL, USA), and the signal was detected using the BioSpectrum Imaging System (Ultra-Violet Products, Ltd., Cambridge, UK).

2.10. Statistical Analysis

The results were analyzed in GraphPad Prism 7 software using one-way ANOVA, followed by Tukey's test, accepting $p < 0.05$ as significant.

3. Results and Discussion

3.1. Composition of Telekia speciosa Essential Oils

After four hours of hydrodistillation, light-yellow EOs with a strong fragrant scent were isolated. The yields of EO from *T. speciosa* leaves and flowers were 0.12% and 0.16% (*w*/*w*), respectively. Roots produced more volatile compounds (0.37%). EO yields from aerial parts and roots were modest and similar, as previously reported (leaf: 0.17%, flower: 0.19%, root: 0.41%) [10]. Over time, crystals were discovered in the root EO. This was most likely due to a high concentration of thymol derivatives. Radulovic et al. discovered seven thymol derivatives in *T. speciosa* oil, but two of them were not identified [6]. Nevertheless, in a prior work, we were able to identify these chemicals as 9-isobutyryloxythymol isobutyrate and 9-(2-methybutyryloxy)-thymol isobutyrate [10]. Radulovic et al. studied the composition of EOs of *T. speciosa* from Serbia and found the terpenoid fraction of the predominant group within the EO, with acyclic-farnesane sesquiterpenoids being the dominant compound class [5]. Scientific research covering the composition and biological properties of *T. speciosa* EO has been going on for several decades as the plant colonizes new areas of Europe. The first wide and screened analysis based on the composition of EOs from Serbia was published in 2010 [5], while the EO composition from different parts of plants harvested in Poland was published in 2012 [10]. This article is a continuation of the research on "Polish" *T. speciosa* oils, which, despite being an invasive plant in Poland, have benefits for honey bees. The knowledge based on the composition and biological properties of EOs from Bosnia and Herzegovina was expanded in 2021 [11]. It is worth mentioning that regardless of the region of origin of the *T. speciosa* leaf EO, dominant volatiles are oxygenated derivatives of sesquiterpene, e.g., (*E*)-nerolidol (10%) and (*E*,*E*)-farnesol (6.7%). Both volatiles occurred in our previous results in reverse, but at simultaneously higher concentrations: (*E*,*E*)-farnesol (21.2%) and (*E*)-nerolidol (17.9%) [10]. Surprisingly, the composition of the flower EO differs strongly from our previous results [10]. The main flower volatiles are nerol (11.9%), linallol (6.9%), (*Z*)-nerolidol (6.1%), and β-caryophyllene oxide (6.1%), which are opposite to the isoalantolactone and thymol derivatives, which were predominant in our previous study [10]. This may be due to genetic factors. The seeds of the plants studied in our previous study originated from west and southeast Europe, while the present study is focused on plants that grew from seeds collected in western Europe (France). Based on the previously and currently analyzed EO from *T. speciosa* roots, we could conclude that the characteristic compound for the analyzed root oils is isoalantolactone, which constituted 62.3% and 46.2% of the oils, respectively. One of the major differences between this study and those previously reported [5,10,11] is the greater number of identified components. However, according to GC-FID-MS analysis, a few, still unidentified, significant compounds were detected in the examined oils. These volatiles were detected in quite high amounts in the leaf EO (2.8%) and flower EO (5.5%) of heartleaf oxeye. Among them, 5 compounds (numbers referring to Table 1: 111, 113, 115, 116, 117) exhibited very similar mass spectra, with molecular peaks [M]+ at *m*/*z* 206. Four of the five unidentified volatiles in this study were also found in our previous study [10]. The unidentified compounds have very similar fragmentation patterns and molar masses, as mentioned in the literature for macrophyllilactone F. Due to the lack of a literature retention index for macrophyllilactone, it has not been possible for us to confirm the structure of unidentified volatiles. The skeleton of macrophyllilactone F is similar to isoalantolactone, the main volatile in EO root. Thymol derivatives, which are characteristic compounds for *Telekia speciosa*, affect the strong biological properties of natural products. These volatiles occurred in all plants and were found in the leaf, flower, and root EOs at 3.3%, 5.5%, and 5.3%, respectively.

Table 1. Composition of *Telekia speciosa* essential oils, hydrodistilled from leaves, flowers, roots.

No.	Compounds	Leaf	Flower	Root	RI Exp. [1]	RI Lit. [2]
		[%]	[%]	[%]		
1	Isobutyric acid	tr			752	752
2	Hexanal		tr	0.4	773	771
3	(*E*)-Hex-2-en-1-al	tr		0.1	828	822
4	(*E*)-Hex-3-en-1-ol	tr	0.1		838	838
5	Hexan-1-ol	tr			852	852
6	α-Pinene	0.2	0.7		926	935
7	Benzaldehyde	tr	0.2		957	941
8	Oct-1-en-3-ol	0.3	0.2		962	962
9	2-Pentylfuran	0.2	0.9	0.1	977	981
10 + 11	Octanal + β-Myrcene (1:1)	0.2	0.2		980	982
12	(*E*)-2-(2-Pentenyl)furan	0.1			984	984
13	β-Pinene	0.1	tr	tr	993	990
14	δ-Car-3-ene	tr			1006	1005
15	*m*-Cymene	tr	0.1	tr	1009	1013
16	*p*-Cymene	0.1	0.1	tr	1010	1016
17	Limonene	0.5			1018	1025
18	(*E*,*E*)-Hepta-2,4-dien-1-ol	0.4			1027	–
19	2,4,4-trimethyl-Cyclohex-2-en-1-ol	tr	0.1		1034	–
20	*cis*-Linalool oxide (furanoid)	1.1	0.2	tr	1064	1064
21	Nonanal	0.0			1075	1076
22	Terpinolene	0.2	tr	tr	1080	1082
23	Linalool	6.4	6.9	1.1	1086	1087
24	*cis*-*p*-Menth-2-en-1-ol	tr	tr	tr	1104	1106
25	Camphor	tr	0.1	tr	1114	1123
26	2-hydroxy-3-methylBenzaldehyde	tr	0.4		1129	1125
27	(*E*)-Non-2-enal	0.2			1134	1133
28	Nerol oxide	1.4	1.7		1137	1137
29	Borneol	0.4		4.5	1147	1150
30	Albene	0.2			1160	1154
31	*p*-Cymen-8-ol		0.1	0.1	1162	1159
32	Methyl salicylate	tr	0.1		1169	1171
33	α-Terpineol	2.2	1.9	0.3	1172	1176
34	Myrtenol		0.2		1179	1178
35	Decanal	1.6	0.7		1185	1187
36	β-Cyclocitral	0.3			1196	1196

Table 1. *Cont.*

No.	Compounds	Leaf	Flower	Root	RI Exp. [1]	RI Lit. [2]
		[%]	[%]	[%]		
37	Cumic aldehyde	tr	0.1		1198	1215
38	Nerol	2.3	11.9	1.4	1213	1213
39	Thymol methyl ether	tr		0.4	1220	1215
40	Geraniol	0.9	0.5	tr	1236	1238
41	*cis*-Edulan (Edulan II)	0.1			1246	1247
42	Thymol	0.1			1251	1267
43	*p-tert*-Butylphenol	0.1	tr		1252	1266
44	Bornyl acetate	0.6	1.1	0.7	1267	1270
45	Carvacrol	0.1	0.3	tr	1272	1278
46	Dihydroedulan I	0.1	0.1		1277	_
47	Dihydroedulan II	6.2	0.5		1284	1290
48	Theaspirane isomer1	0.2			1288	1299
49	*trans*-Edulan (Edulan I)	0.1			1294	1313
50	Theaspirane isomer 2	0.2		tr	1301	1313
51	7αH-Silphiperfol-5-ene	0.5	0.3	1.6	1322	1329
52	Presilphiperfol-7-ene	0.4	0.2	0.2	1330	1342
53	Silphiphin-1-ene	0.5			1340	1350
54	α-Longipinene	tr	tr	0.1	1347	1358
55	3-Hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	0.1		tr	1355	1360
56	(*E*)-β-Damascenone	0.1	tr		1358	1361
57	(*E*)-Jasmone	0.1	0.1	tr	1362	1362
58	(*Z*)-Jasmone	0.7	0.1	0.2	1365	1371
59	Longicyclene		0.2	0.1	1369	1378
60	Silphiperfol-6-ene	0.7	0.3	0.5	1369	1378
61	Cyclosativene	0.6			1372	1378
62	Modheph-2-ene	0.1		0.6	1374	1382
63	β-Panasinsene	0.2	tr		1376	1385
64	β-Bourbonene	0.2	0.2		1379	1386
65	Dihydro-α-ionone	1.9	tr	0.7	1387	1437
66	(3Z)-3-(6,6-Dimethyl-2-methylene-3-cyclohexen-1-ylidene)-1-methylbutyl acetate	0.3	0.2	0.5	1389	_
67	Helifolenol A	1.7	tr	0.4	1395	_
68	2,5-Dimethoxycymene			0.6	1399	1399
69	2,6-Dimethoxycymene	0.1	1.3		1399	1402
70	Isobornyl isobutyrate	0.1	0.1	1.4	1402	1402
71	Petasitene	0.1	0.7		1404	1402

Table 1. *Cont.*

No.	Compounds	Leaf	Flower	Root	RI Exp. [1]	RI Lit. [2]
		[%]	[%]	[%]		
72	α-Ionone	0.1	0.3		1407	1405
73	7,8-Dihydro-β-ionone	0.3		tr	1412	1421
74	(*E*)-β-Caryophyllene	1.6	2.8	1.3	1415	1421
75	Pacifigorgia-2,10-diene	0.8	2.4		1417	1426
76	Geranylacetone	1.5	0.7	1.2	1428	1428
77	Thujopsene	0.4	1.7	tr	1437	1430
78	(*E*)-β-Farnesene	0.2	tr	0.5	1444	1444
79	α-Humulene	0.3	0.6	0.2	1448	1445
80	*epi*-β-Santalene	0.1		0.1	1452	1446
81	8,9-Didehydrothymol isobutyrate	0.2	0.1	0.1	1455	1458
82	Thymol isobutyrate	0.4	0.7	0.2	1459	1462
83	(E)-β-Ionone	0.6	0.2	tr	1463	1468
84	Nerylisobutyrate	2.2	2.5	2.1	1471	1468
85	123/94/67 M204 n.i. [3]	tr	0.1	0.2	1475	_
86	Selinena-4,11-diene	0.6	0.3	1.4	1481	1473
87	Germacrene D	0.2		tr	1484	1479
88	γ-Humulene	0.7	0.5	0.8	1490	1483
89	β-Selinene	0.3			1492	1486
90	(*E*,*E*)-α-Farnesene	0.3			1495	1498
91	β-Bisabolene	0.3		0.1	1500	1503
92	Cameroonan-7α-ol	0.7	0.5	tr	1504	1510
93	10-epi Italicen ether	0.4			1506	1511
94	Isoshyobunone	tr	0.4	2.1	1509	1518
95	δ-Cadinene	1.9	0.7	0.1	1516	1520
96	*cis*/*trans*-Calamenene		tr	0.2	1520	1521
97	Nopsan-4-ol	0.2	0.1		1520	1523
98	177/121/91/161/148 n.i. [3]	tr	0.1	0.1	1524	_
99	α-Calacorene	0.6	0.2		1530	1527
100	(*E*)-α-Bisabolene	0.1	0.6	0.2	1533	1530
101	Cadina-1(10),7(11)-diene	2.4	0.6	tr	1539	1538
102	β-Caryophyllene epoxide	0.4	0.2	0.2	1540	1544
103	β-Calacorene	0.1		0.1	1544	1545
104	(*Z*)-Nerolidol	0.2	6.1	0.3	1545	1546
105	(E)-Neroliol	10.1	2.5	1.2	1560	1555

Table 1. *Cont.*

No.	Compounds	Leaf	Flower	Root	RI Exp. [1]	RI Lit. [2]
		[%]	[%]	[%]		
106	Neryl 2-methylbutyrate	1.0		0.4	1564	1560
107	Neryl isovalerate	0.9			1566	1560
108	Prenopsan-8-ol	3.5	2.8		1569	1569
109	β-caryophyllene oxide	5.1	6.7	3.2	1579	1578
110	di-epi-Cedrenoxide	tr	0.1	0.8	1582	1630 (HP5)
111	162/147/M206 n.i. [3]	0.1	tr		1584	_
112	Thujopsan-2α-ol	0.1	0.1		1587	1589
113	162/147 M206 n.i. [3]	0.2	0.6	0.1	1592	_
114	Isoaromadendrene epoxide	tr		0.8	1594	1590
115	162/147/M206/120/173 n.i. [3]	1.3	3.9		1600	_
116	162/147/M206/120/173 n.i. [3]	0.5	1.0	0.2	1601	_
117	162/147/M206/120/91 n.i. [3]	0.7	tr	0.2	1603	_
118	b-Himachalene epoxide	0.2	0.3		1606	1594
119	Eudesm-4-en-7-ol		0.1		1606	1604
120	160/145/91/131/M182 n.i. [3]	0.1	0.3		1609	_
121	Isospathulenol	0.1	0.4	0.4	1611	1619
122	allo-Aromadendrene epoxide	0.2		0.1	1616	1623
123	Cubenol	0.1	0.6	0.4	1620	1630
124	Caryophylla-3(15),7(14)-dien-6-ol	0.3	1.3	0.5	1623	1630
125	Caryophylla-4(12),8(13)-dien-5-α-ol	1.2	tr	tr	1627	1631
126	T-Muurolol	0.2	tr		1631	1633
127	α-Cadinol	0.2			1635	1641
128	β-Eudesmol		0.8	1.1	1635	1644
129	162/161/133/105 M232 n.i. [3]	0.2	0.1	0.2	1638	_
130	Intermedeol	0.1	0.3	1.2	1642	1645
131	δ-Cadinol	0.9	0.6	0.4	1646	1645
132	Isorotundenol	0.1	1.4	0.1	1655	1659
133	6-Methoxythymol isobutyrate	1.0	0.5	1.6	1659	1659
134	6-Methoxy-8,9-didehydrothymol isobutyrate	0.1	0.4	0.4	1664	1676
135	3-Hydroxy-β-ionone	tr	0.2	0.3	1667	1678
136	Farnesal (isomer 2)	0.3	tr	0.3	1682	1683
137	10-Isobutyryloxy-8,9-didehydro thymol methyl ether	0.1	0.4	0.2	1686	1684
138	(Z,Z)-Farnesol	0.9	0.1	0.9	1693	1694

Table 1. *Cont.*

No.	Compounds	Leaf	Flower	Root	RI Exp. [1]	RI Lit. [2]
		[%]	[%]	[%]		
139	Farnesal (isomer 3)	0.1	0.4	tr	1696	1707
140	(E,E)-Farnesol	6.7	1.4	0.4	1710	1716
141	cis-Z-α-Bisabolene epoxide		tr	0.9	1718	1717
142	(E,E)-Farnesal	1.3	tr	tr	1721	1717
143	6-Isopropenyl-4,8α-dimethyl-3,5,6,7,8,8α-hexahydro-2(1H)-naphthalenone	0.5	0.1	0.3	1731	1772 (semi-polar column)
144	Hexahydrofarnesylacetone	1.3	3.8	0.3	1832	1834
145	Alantolactone	0.1	0.3	1.5	1866	1873
146	9-Isobutyryloxythymol isobutyrate	0.7	1.0	0.4	1882	1884
147	10-Isobutyryloxy-8,9-dehydrothymol isobutyrate	0.4	0.6	0.6	1885	1887
148	Isoalantolactone	0.9	3.1	46.2	1907	1912
149	Methyl palmitate	0.1	0.6		1910	1915
150	7-Isobutyryloxythymol isobutyrate	0.3	0.4		1920	1922
151	n-Hexadecoic acid	0.4	0.3	0.4	1954	1956
152	9-(2-Methybutyryloxy) thymol isobutyrate	0.1	0.2	0.4	1969	1964
153	10-(2-Methybutyryloxy)-8,9-didehydrothymol		tr	0.1	1972	1967
154	10-Isobutyryloxy-8,9-epoxythymol isobutyrate	0.5	1.7	2.6	1989	1972
155	18-Norabieta-8,11,13-triene	tr	0.2		1998	_
156	177/150/71/135 M290 n.i. [3]	0.1		0.2	2069	_
157	10-(2-Methybutyryloxy)-8,9-epoxythymol isobutyrate	0.1	0.3	0.2	2076	2084
158	(*E*)-phytol	1.0	0.4	tr	2102	2114
159	Tricosane	tr	0.1	0.1	2299	2300
160	Tetracosane	tr	tr		2400	2400
161	Pentacosane	0.1	1.0		2500	2500
162	Heptacosane	tr	0.2		2695	2700
	Total	98.1	97.1	97.1		

[1] experimental retention indices calculated on non-polar columns; [2] literature retention indices found on non-polar column; [3] mass spectra of non-identified volatiles; tr—trace; <0.05%.

3.2. Effects of Telekia speciosa Essential Oils Melanoma Cells

The strongest cytotoxic activity was noticed for the flower EO. However, the leaf and root Eos' biological activities were of little importance to that of the flower EO; thus, the further explanation and results discussion on the effect on melanoma cells were based on the flower EO. These are the first studies focused on the cytotoxic effect of *Telekia*'s oils.

The effect of *T. speciosa* EOs on the viability of malignant melanoma (A375) and amelanotic melanoma (C32), skin keratinocytes HaCaT, and dermal fibroblasts was evaluated using the MTT assay. Cells were treated with a series of EO concentrations (1.5, 3.1, 6.2, 12.5, and 25 μg/mL) for 24, 48, and 72 h. Cisplatin was used as a reference compound. IC_{50} values were calculated and are presented in Table 2. The inhibitory effect of *T. speciosa* EOs on cell viability was time-dependent in all cell lines tested.

Table 2. Half-maximum inhibitory concentration (IC_{50}) values (μg/mL) calculated for the essential oil from *Telekia speciosa* flowers and cisplatin towards the melanoma cell lines (A375, C32), keratinocytes (HaCaT), and human skin fibroblasts.

Time of Treatment	Cell Line	IC_{50} (μg/mL)	
		Flower Essential Oil	Cisplatin
24 h	A375	7.2 ± 1.6	7.4 ± 1.9
	C32	14.2 ± 2.8	10.6 ± 1.6
	HaCaT	10.2 ± 1.9	13.8 ± 1.5
	Fibroblasts	>25	>25
48 h	A375	5.1 ± 1.4	3.7 ± 1.2
	C32	11.3 ± 1.7	2.9 ± 0.8
	HaCaT	8.1 ± 1.2	2.8 ± 1.0
	Fibroblasts	17.1 ± 1.5	>25
72 h	A375	3.4 ± 1.1	2.0 ± 0.8
	C32	6.3 ± 1.5	1.7 ± 0.6
	HaCaT	4.3 ± 1.1	1.4 ± 0.5
	Fibroblasts	11.8 ± 1.6	14.5 ± 1.4

The strongest cytotoxic activity was noticed for flower EO. However, the biological activity of the leaf and root EOs was similar to that of flower EO; thus, the further explanation of the effect on melanoma cells was based on the flower EO.

A375 cells were the most susceptible to the cytotoxic activity of *T. speciosa* EO at all time points, and its IC_{50} values were 7.2, 5.1, and 3.4 μg/mL for 24, 48, and 72 h of treatment, respectively. The IC_{50} values in other cell lines were around 50% higher in keratinocytes, 2 times higher in C32 cells, and 3 times higher in fibroblasts compared with those obtained in A375 cells. Cisplatin also strongly and time-dependently reduced the viability of all cell lines. The IC_{50} values of A375 cells were 7.4, 3.7, and 2.0 μg/mL for 24, 48, and 72 h of treatment, respectively. The IC_{50} values of cisplatin in C32 cells and keratinocytes were similar to those obtained in A375 cells, whereas fibroblasts were a few times more resistant than these cells. The presented MTT assay data show that *T. speciosa* EO effectively reduced the viability of the A375 cell line, but the viability of both normal cell lines was significantly less affected than that of the A375 cells. The above experiments are the first, focused on *T. speciosa* EOs. In a similar study, 20 non-volatile compounds were identified in the polar extract of *T. speciosa* [7]. Calenduladiol esters and asperirlin were the major constituents of the extract. Their cytotoxic effect on human normal prostate epithelial cells, human prostate carcinoma cell lines, human skin fibroblasts, and human melanoma cell lines was examined in vitro. The above-mentioned triterpene esters, which, because of their molecular structure, cannot be constituents of EOs, showed no cytotoxicity against nearly all cell lines tested, except for prostate carcinoma cells (IC_{50}—62.0 μM). Further, asperilin displayed anticancer activity against the melanomas cell lines, including A375 (IC_{50}—17.6 μM), WM793 (IC_{50}—28.2 μM), and Hs 294T (IC_{50}—29.5 μM) [7].

3.3. *Telekia speciosa* Essential Oil Induces Apoptosis in Melanoma Cells

To investigate the induction of apoptosis in melanoma cells treated with *T. speciosa* EO, an annexin V/PI double staining assay was performed, followed by fluorescence microscopy (Figure 1A). Representative images of stained cells are presented in Figure 1B. Melanoma cells were treated with a series of EO concentrations (1.5, 3.1, 6.2, 12.5, and 25 µg/mL) for 24 h. Treatment of A375 cells with 12.5 µg/mL of EO resulted in an increase in the percentage of early apoptotic cells and late apoptotic cells to 54 ± 4% and 33 ± 3%, respectively. C32 cells were less affected by the same concentration of EO, and the percentage of early apoptotic cells and late apoptotic cells increased to 13.4 ± 2% and 42.0 ± 4%, respectively.

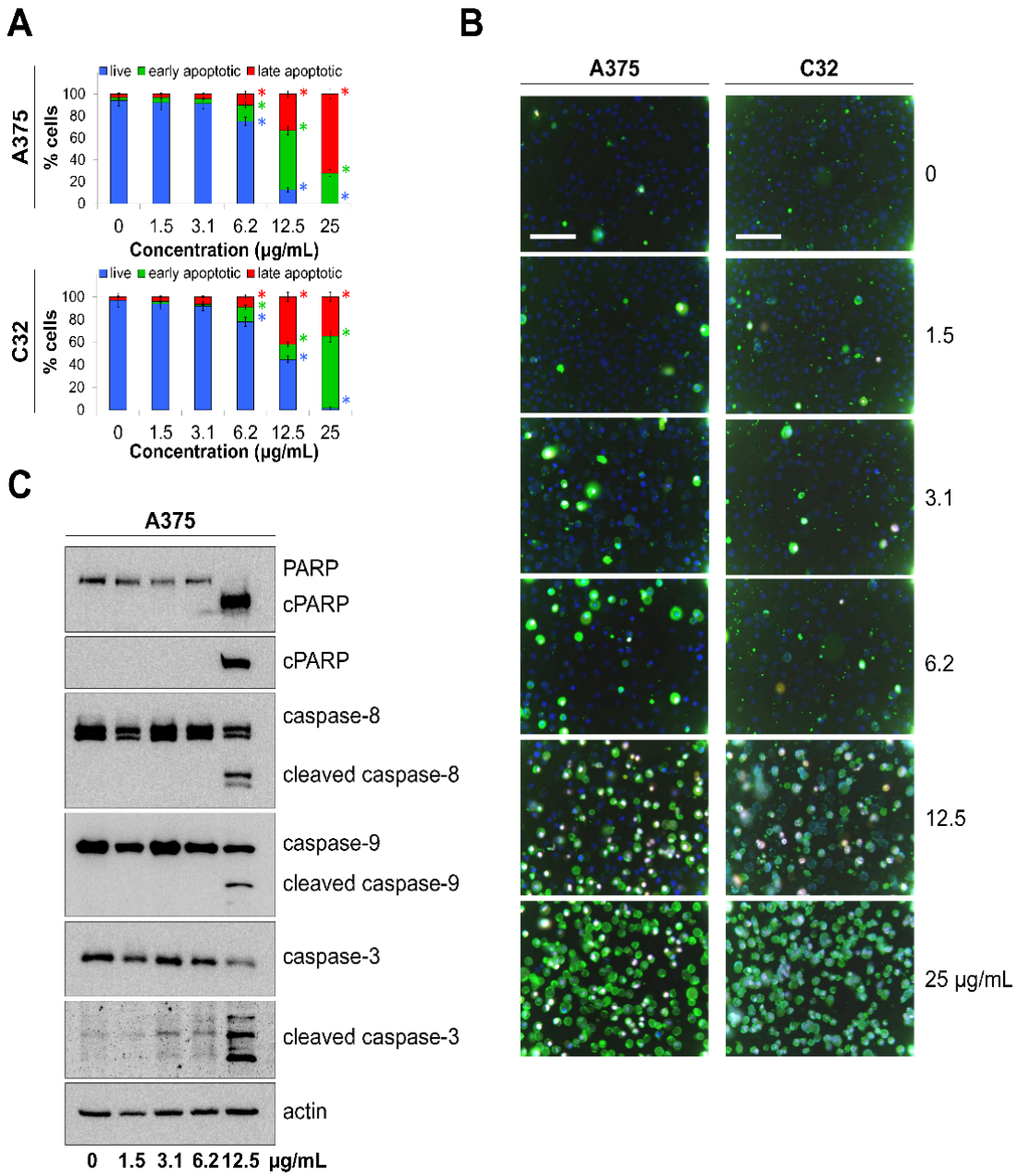

Figure 1. (**A**) Apoptosis rate of A375 and C32 melanoma cells treated with *T. speciosa* flower EO for 24 h determined by annexin V/PI double staining, followed by fluorescence microscopy. Data are presented as the mean ± SD (* $p < 0.05$); (**B**) Representative images of stained cells; (**C**) Expression of PARP, caspase-8, caspase-9 and caspase-3 in A375 cells analyzed by western blotting after 24 h of treatment of A375 cells with *T. speciosa* flower EO.

The data showed that a decrease in the viability of A375 cells was associated with an increase in apoptosis; therefore, we tried to identify the pathway of apoptosis activated by *T. speciosa* EO. A375 cells were treated with a series of EO concentrations (1.5, 3.1, 6.2, and 12.5 µg/mL) for 24 h. Western blot analysis (Figure 1C) revealed cleavage of both initiator caspases, caspase-9 and caspase-8 pro-forms, in cells treated with 12.5 µg/mL of EO. It was accompanied by the activation of caspase-3, a key caspase executor, and the cleavage of PARP, a caspase-3 substrate. The results show that *T. speciosa* EO causes both intrinsic and extrinsic apoptosis in A375 cells.

3.4. Antimicrobial Activities of T. speciosa Essential Oils

Essential oils belonging to all parts of this plant exerted antimicrobial activity against all tested microorganisms (Table 3). *T. speciosa* EOs inhibited the growth of tested microorganisms, with MICs ranging from 5.9 ± 2.8 to 62.0 ± 50.0 µL/mL and EO concentrations ranging from 1.0 to 125 µL/mL. Interestingly, the flower and leaf EOs were more effective against the tested Gram-positive and Gram-negative bacteria than the root oil, which is dominated by isoalantolactone. The tested EOs, on the other hand, were less effective than thymol, an antibacterial chemical and one of the most biologically active components of EOs, employed as a positive control. The antibacterial activity of EOs from leaves was the highest for *S. aureus*, followed by EOs from flowers and roots. Antimicrobial properties of EOs from *T. speciosa* were evaluated in recent research on six different bacterial strains, including *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albicans*. According to the findings, the MICs of the EOs obtained from *T. speciosa* roots ranged from 1.0 to 11.0 mg/mL, whereas the MICs of the EO obtained from the aerial parts varied from 4.0 to 30.0 mg/mL [11]. In a similar study, Zhou et al. evaluated the antimicrobial properties of isoalantolactone extracted from *T. speciosa* against methycillin-resistant *Staphylococcus aureus* (MRSA) [16].

Table 3. Antimicrobial properties of *T. speciosa* essential oils on *Staphylococcus aureus* and *Escherichia coli.*

Essential Oils from:	MIC (µL/mL)	
	Staphylococcus aureus **ATCC 29213**	***Escherichia coli*** **ATCC 25922**
flowers	7.8 ± 0.0	7.8 ± 0.0
roots	31.3 ± 0.0	62.5 ± 0.0
leaves	5.9 ± 2.8	11.7 ± 5.5
thymol (control) [1]	0.9 ± 0.0	7.5 ± 0.0

[1] Weighed at the melting point of thymol, 1 µg/mL of thymol corresponds to c. 0.001 µL/mL.

Overall, the current study on *T. speciosa* reveals differences in the composition and biological properties of the EOs, as compared to previous experiments [5,10,11,16]. The current study allowed identifying the qualitative and quantitative composition of *T. speciosa* EOs more deeply. The composition of the EOs was examined for three separate plant materials: flowers, leaves, and roots. This is further evidence of how the origin of the seed (genetic factor) and the place of plant growing (environmental factor) determine the composition, which in turn determines the specific biological properties of natural products. The current publication links the carefully researched composition (over 150 identified ingredients) with biological properties, both cytotoxicity and antimicrobial.

4. Conclusions

The potential uses of *Telekia speciosa* essential oils are vast and impressive. The composition of its flower, leaf, and root oils boast more than 150 compounds, including the major constituents isoalantolacton, nerol, and (*E*)-nerolidol. The essential oils from *T. speciosa* demonstrated high inhibitory activity against *Staphylococcus aureus* and *Escherichia coli*, highlighting their potential as natural alternatives to synthetic antibiotics and fungicides.

Additionally, the flower oil exhibited potent cytotoxic activity against A375 cells, indicating its potential as a natural cytotoxic agent. As the world faces an ongoing decline in bee populations, natural chemicals like essential oils could be a sustainable and effective alternative to traditional treatments. *T. speciosa*'s high bioactivity, combined with its potential as a melliferous plant, makes it a plant of immense interest for further study and development.

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