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Food & Function

PAPER

Essential oils of three cow parsnips – composition and activity against nosocomial and foodborne pathogens and food contaminants

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Although some widespread, native cow parsnips (*Heracleum* L. spp., Apiaceae) had broad medicinal and culinary application throughout history, the knowledge about their volatile constituents is insufficient. This work investigates the composition and bioactivities of *H. sphondylium* L. (HSPH), *H. sibiricum* L. (HSIB) and *H. montanum* Schleich. ex Gaudin (HMON) essential oils. The composition was tested by GC and GC-MS. (Z)- β -Ocimene was the most abundant in HSPH (28.9%) and HMON (20.4%) root oils, while in HSIB root oil, β -pinene (26.2%), methyl eugenol (22.3%) and elemicin (25.6%) prevailed. Leaf and flower oils were dominated by various sesquiterpenes (germacrene D, β -sesquiphellandrene, (E)- β -farnesene and/or (E)-caryophyllene) and/or phenylpropanoids (apiole, methyl eugenol, elemicin and/or (Z)-isoelemicin). Octyl acetate (57.5–67.1%) was the main constituent of all fruit oils. Antimicrobial activity was screened by microdilution method against eight bacteria and eight fungi. The strongest antimicrobial effect, in several cases better than the activity of antibiotics, was shown by HSPH (MICs=0.12–3.30 mg mL⁻¹) and HMON (MICs=0.10–1.30 mg mL⁻¹) flower oils against bacteria, and HSIB fruit oil against fungi (MICs=0.15–0.40 mg mL⁻¹). MTT test revealed that the oils were not or weakly cytotoxic against human malignant HeLa, LS174 and/or A549 cells (except HSPH root oil; IC₅₀=5.72–24.31 μ g mL⁻¹) and that tested oils were not toxic against human normal MRC-5 cells (at 200.00 μ g mL⁻¹). Significant activity observed against microorganisms that are the common cause of foodborne diseases, food contamination and/or hospital-acquired infections justifies certain traditional uses of investigated plants and represents a good basis for further research of these *Heracleum* oils.

1. Introduction

Many essential oils isolated from various edible plants are added to food as flavors and some of them possess prominent biological activities. The sources of such essential oils are, among others, the plants from Apiaceae (parsley, celery or carrot) family. They are widely distributed in the temperate climate regions where they are often used as vegetables, spices or drugs, mostly due to the presence of these volatile secondary metabolites.¹ This family includes many edible *Heracleum* L. species (commonly known as cow parsnips or hogweeds), which are applied in traditional medicine in the different cultures of the world. In the focus of the present study are three widespread and morphologically related species of this genus, belonging to group *H. sphondylium* L. s.l.²

*Heracleum sphondylium*³ (common cow parsnip, common hogweed) is usually a lowland plant, mainly native in the northern and western Europe, but extending to Scandinavia, eastern and central Europe and the Mediterranean region. Its larger cauline leaves are composed of 3–7 segments, petals are white, rarely pink, and the outer flowers are radiate.³ French pharmacopoeia (2007)⁴ includes monograph “*Heracleum sphondylium* for homeopathic preparations” (its other latin name used in homoeopathy is *Branca ursina*), which is defined as the whole, fresh, blooming parts of this plant. Moreover, homeopathic preparation containing *H. sphondylium* herb and *Prunus spinosa* L. (Rosaceae) is produced in Germany, and it is intended for various respiratory and CNS disorders, as well as for genital and dermatomycoses.⁵ Furthermore, the ethnomedicinal use of this plant is well documented. In some regions of the Balkans, various preparations of the roots and the aerial parts of this plant were used to treat stomach disorders, digestion problems and diarrhea.⁶ Additionally, in Romania and Morocco, the herbal tea of its aerial parts was reputed to be aphrodisiac and to treat hypertension.⁷ In Italy, the root decoction was used as digestive and aperitif,⁸ while the tinctures of aerial parts and fruits were applied as sedative for CNS and against nervous depression.⁹ In Switzerland, this

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plant was used against bronchitis.¹⁰ It is also found in several Renaissance herbals in central Europe as a cure for epilepsy.¹¹ *Heracleum sphondylium* has also interesting history of use as the food ingredient. It was the original constituent of “borsch” or “barszcz”, traditional soup in Russia, Poland, Ukraine and other Eastern European countries, but it was eventually replaced by the more-palatable cultivated beet, *Beta vulgaris* L. (Amaranthaceae). The term “barszcz” is actually common name for *H. sphondylium* in Poland.^{12,13} Additionally, its young stems were added to food for gustatory or decorative purposes, while the buds of flowers were cooked as vegetables in Switzerland.¹⁰ Slavs used the cooked leaves and fruits to prepare alcoholic beverage “barč”, which was consumed as a substitute for beer, and in northern France, the liqueur drink was prepared from *H. sphondylium*.¹⁴ Today, the consumption of the roots, as well as young leaves and stems of this plant is suggested only in survival handbooks.¹⁵ Considering the wide application of *H. sphondylium* throughout history, the knowledge about the chemical composition and bioactivity of this plant is incomplete. Previously, the chemical composition of the essential oils of *H. sphondylium* leaves, stems, flower petals and fruits collected in Trento (Italy) was analyzed.¹⁶ According to available literature survey, the composition of its root oil was not investigated till now. Furthermore, some fatty acids and phytosterols were identified in the fatty oil of its fruits.^{17,18} Besides essential oil, the secondary metabolites that are characteristic for this genus are coumarins. The main simple coumarins and furanocoumarins of the roots, fruits and flowers of *H. sphondylium* were previously investigated.^{19,20} Additionally, the dry dichloromethane extract of *H. sphondylium* aerial parts exhibited vasorelaxant effect in isolated rat thoracic aorta.⁷

Heracleum sibiricum L.^{21,22} (Siberian cow parsnip, Siberian hogweed) inhabits wet places along mountain streams, as well as meadows, grasslands and forests. The plant is native in Eastern Europe, Siberia, Scandinavia and some parts of Central Europe. Like in *H. sphondylium*, its larger cauline leaves are composed of 3-7 segments, but the flowers are greenish and the outer ones are not or only slightly radiate.^{3,21-23} This plant has also interesting ethnomedicinal and ethnoculinary uses that are similar to those of *H. sphondylium*. In Bulgaria, the macerate prepared from *H. sibiricum* roots or fruits, as well as the infusion obtained from the aerial plant parts were traditionally used as appetizers and for the treatment of diarrhea and other gastrointestinal diseases. The macerates were also known as hypotensive and spasmolytic remedies.^{9,24} Different plant parts of *H. sibiricum* were also used as food in Serbia and Bulgaria. The young shoots were consumed raw, fried or stewed. They were also added to salads and soups, while the leaf peduncles were eaten pickled. Its essential oil gave food a specific flavor and acted as a natural preservative.^{25,26} The essential oils of *H. sibiricum* were partially investigated till now, *i.e.* Miladinović *et al.*²⁷ analyzed the chemical composition and antibacterial activity of the aerial parts essential oil (originating from Mt. Vidlič, Serbia). Additionally, principal furanocoumarins were previously isolated from the petroleum ether extract of the roots and

fruits of this plant.^{28,29} Furthermore, the ethanol extract of the fruits exhibited apoptotic effect, *in vitro*, on human leukemia cells,³⁰ while the mixture of furanocoumarins isolated from the roots of *H. sibiricum* and *H. verticillatum* Pančić exhibited anticonvulsive activity, *in vivo*, in rats and mice.³¹

Heracleum montanum Schleich. ex Gaudin³² (mountain cow parsnip, mountain hogweed) is distributed in the mountain areas of central Europe, extending locally southward to Sicilia and southern Spain, as well as in eastern Russia. In contrast to *H. sphondylium* and *H. sibiricum*, its larger cauline leaves are almost always ternate (*i.e.* composed of three segments), and the flowers are white and the outer ones are radiate, similarly to *H. sphondylium*.³ Moreover, it was traditionally used for similar purposes as two aforementioned plants.³³ Regarding its metabolites, only few furanocoumarins were identified in the various parts of this plant previously.³⁴

Considering their wide ethnobotanical usage, as well as insufficient chemical and pharmacological investigations, the aim of this work was to analyze the composition, and antimicrobial and cytotoxic effects of the essential oils isolated from the different plant parts of *H. sphondylium*, *H. sibiricum* and *H. montanum*.

2. Materials and methods

2.1. Plant material

Heracleum sphondylium (HSPH) and *H. montanum* (HMON) were collected in Slovenia, HSPH beside roads near Ljubljana, while HMON on the Kamnik-Savinja Alps; leaves and flowers in July 2015, and roots and fruits in September 2015. *Heracleum sibiricum* (HSIB) was collected beside roads near Niš (Serbia); roots in November 2011, leaves in June 2014, flowers in July 2014, and fruits in September 2014. Voucher specimens are deposited in the Herbarium of the Natural History Museum, Belgrade (BEO) under collector numbers 20150704/01 (HSPH), 20150707/01 (HMON) and 20140717/01 (HSIB). The plants were identified by Dr. Marjan Niketić, curator/botanist of the BEO.

2.2. Isolation of the essential oils

Air-dried plant material was powdered (roots and fruits) or crashed (leaves and flowers), and hydrodistilled using Clevenger-type apparatus for 2.5 h. Collecting solvent was *n*-hexane. The essential oils were dried over anhydrous sodium sulfate and kept at 4 °C until analysis. HSPH roots, leaves, flowers and fruits yielded 0.09, 0.14, 0.12 and 0.99% w/w, HSIB roots, leaves, flowers and fruits 0.41, 0.13, 0.49 and 1.38% w/w, and HMON roots, leaves, flowers and fruits 0.08, 0.13, 0.10 and 1.42% w/w of the oils, respectively.

2.3. Essential oils analysis

The GC and GC-MS analyses were performed on an Agilent 6890N Gas Chromatograph equipped with a split/splitless injector (200 °C), a FID detector and a capillary column (HP-5MS, 30 m × 0.25 mm, 0.25 μm film thickness), and coupled

with an Agilent 5975C MS Detector operating in the EI mode at 70 eV. The carrier gas was He, flow 1.0 mL min⁻¹. The oven temperature was programmed linearly, increasing from 60 to 280 °C at 3 °C min⁻¹. The FID and MSD transfer line temperatures were 300 and 250 °C, respectively. Split ratio was 1:10 and the injected volume was 1 µL of 3% solution of essential oil in 99.9% (v/v) ethanol. The linear retention indices (RIs) of the essential oil constituents were determined in relation to the homologue series of *n*-alkanes (C₈-C₄₀) ran under the same operating conditions. The identification of the compounds was based on the comparison of their RIs, retention times (Rt) and mass spectra to those from the NIST/NBS 05 and Wiley (8th edition) libraries, and the literature.^{35,36} Relative percentages of the compounds were calculated based on the peak areas from the FID data.

2.4. Antimicrobial activity

2.4.1. Microbial strains. The Gram-positive bacteria *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (clinical isolate), *Listeria monocytogenes* (NCTC 7973) and *Micrococcus flavus* (ATCC 10240), and the Gram-negative bacteria *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 35210), *Salmonella typhimurium* (ATCC 13311) and *Enterobacter cloacae* (human isolate) were used. The fungi *Aspergillus fumigatus* (human isolate), *A. versicolor* (ATCC 11730), *A. ochraceus* (ATCC 12066), *A. niger* (ATCC 6275), *Trichoderma viride* (IAM 5061), *Penicillium funiculosum* (ATCC 36839), *P. ochrochloron* (ATCC 9112) and *P. verrucosum* var. *cyclopium* (food isolate) were tested. The micromycetes were maintained on malt agar, the cultures stored at 4 °C and sub-cultured once a month.

2.4.2. Antibacterial activity. Minimum inhibitory and minimum bactericidal concentrations (MICs and MBCs) were determined by the microdilution method in 96-well microtitre plates.³⁷⁻³⁹ Bacterial suspensions were adjusted with sterile saline to a concentration of 1.00 × 10⁵ CFU mL⁻¹. The essential oils were dissolved in 5% dimethylsulfoxide (DMSO) solution that contained 0.10% Tween 80 (v/v) (10 mg mL⁻¹) and added to Tryptic Soy broth (TSB) medium (100 µL) with bacterial inoculum (1.00 × 10⁴ CFU per well), to achieve concentrations from 0.06 to 16.00 mg mL⁻¹. The MICs were defined as the lowest concentrations without visible bacterial growth (determined at binocular microscope). The MICs were also determined by the colorimetric microbial viability assay that is based on the reduction of *p*-iodonitrotetrazolium violet (INT) color. Results were compared to the positive controls. The MBCs were determined by serial sub-cultivations of 2 µL of tested oils (dissolved in medium and inoculated for 24 h) into microtitre plates that contained 100 µL of broth per well, after further incubation for 24 h. The lowest concentration without visible bacterial growth was defined as the MBC, indicating that 99.5% of the original inoculum was killed. The optical density of each well was measured by Microplate manager 4.0 (Bio-Rad Laboratories, USA) at the wavelength of 655 nm and

compared to the blank and positive controls. Streptomycin, Sigma P 7794 (0.04-0.52 mg mL⁻¹) and ampicillin, Panfarma, Serbia (0.25-1.24 mg mL⁻¹) were used as the positive controls. 5% DMSO was used as the negative control.

2.4.3. Antifungal activity. In order to investigate the antifungal activity of the essential oils, modified microdilution technique was used.³⁹⁻⁴¹ Fungal spores were washed off from the surface of agar plates with 0.85% sterile saline that contained 0.10% Tween 80 (v/v). Spore suspensions were adjusted with sterile saline to a concentration of 1.00 × 10⁵ in the final volume of 100 µL per well. The oils were dissolved in 5% DMSO solution that contained 0.10% Tween 80 (v/v) (10 mg mL⁻¹) and added to broth Malt medium with the inoculum (to achieve concentrations 0.15-8.00 mg mL⁻¹). The lowest concentrations without visible growth (at the binocular microscope) were defined as MICs. The minimum fungicidal concentrations (MFCs) were determined by serial sub-cultivations of a 2 µL of the tested oils (dissolved in medium and inoculated for 72 h) into microtitre plates that contained 100 µL of broth per well, after further incubation for 72 h at 28 °C. The MFC was defined as the lowest concentration without visible growth, indicating that 99.5% of the original inoculum was killed. Commercial fungicides bifonazole, Srbolek, Serbia (0.10-0.25 mg mL⁻¹) and ketoconazole, Zorkapharma, Serbia (0.20-3.50 mg mL⁻¹) were used as the positive controls. 5 % DMSO was used as the negative control.

2.4.4. Statistical analysis. All the tests were carried out in triplicate. The results were expressed as mean values ± standard deviation (SD), and analyzed by one-way analysis of variance (ANOVA), followed by Tukey's HSD test with α=0.05, to determine whether there is a statistically significant difference between them. The analysis was carried out by Statistical Package for the Social Sciences (SPSS) version 18.0.

2.5. Cytotoxic activity

2.5.1. Cell cultures. Cervix adenocarcinoma HeLa, human colon carcinoma LS174, non-small cell lung carcinoma A549, as well as human normal fetal lung fibroblast MRC-5 cell lines (ATCC) were cultured as a monolayer in the RPMI 1640 nutrient medium, supplemented with heat inactivated (at 56 °C) 10% fetal bovine serum (FBS), 3 mmol L⁻¹ of L-glutamine and antibiotics, at 37 °C, in a humidified air atmosphere with 5% CO₂.³⁹

2.5.2. Treatment of cell lines. *In vitro* assay for the cytotoxic activity of the essential oils was performed when the cells reached 70-80% of confluence. The stock solution (100 mg mL⁻¹) of each oil was dissolved in RPMI 1640 medium to obtain required concentrations. Neoplastic HeLa (2000 cells per well), LS174 (7000 cells per well), A549 (5000 cells per well) and normal MRC-5 cells (5000 cells per well) were seeded into 96-well microtitre plates and 24 h later, after the cell adhesion, five different, double diluted concentrations of the oils were added to the wells. The final concentrations of the

oils were 12.5, 25, 50, 100 and 200 $\mu\text{g mL}^{-1}$. Control wells contained only nutrient medium that was made of RPMI 1640 medium, supplemented with 3 mmol L^{-1} L-glutamine, 100 mg mL^{-1} streptomycin, 100 IU mL^{-1} penicillin, 10% heat inactivated (56 °C) FBS and 25 mmol L^{-1} HEPES (2-[4-(2-hydroxyethyl)piperazinyl] ethanesulfonic acid). The pH of the medium was adjusted to 7.2 with bicarbonate solution. The cultures were incubated for 72 h.³⁹

2.5.3. Determination of cell survival (MTT test). The effect of the essential oils on cell survival was determined by the MTT test (microculture tetrazolium test), according to Mosmann,⁴² with modification by Ohno and Abe,⁴³ 72 h after the addition of the oils. Briefly, 20 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg mL^{-1} phosphate-buffered saline, PBS) was added to each well. The samples were incubated for further 4 h, at 37 °C, in 5% CO_2 humidified air atmosphere. During this period MTT dye was converted to insoluble product, formazan by viable cells. This precipitate was then dissolved by adding 100 μL of 10% sodium dodecyl sulfate (SDS). The number of viable cells in each well was proportional to the intensity of the light absorbance (A) that was measured 24 h later by an ELISA plate reader (Thermo Fisher Scientific Inc., Australia) at 570 nm. To calculate the inhibition of cell survival (%), the A of a sample with cells grown in the presence of various concentrations of the oils were divided with control optical density (the A of control cells grown only in nutrient medium) and multiplied by 100. The A of the blank was always subtracted from the A of the corresponding sample with target cells. The IC_{50} value was defined as the concentration of an agent that inhibits the survival of 50 % cells, compared to the vehicle treated control. Cisplatin was used as the positive control. The IC_{50} values were expressed as mean values \pm SD that were determined on the basis of the results of two independent experiments.

3. Results and discussion

3.1. Chemical composition of essential oils

The chemical composition of the investigated *H. sphondylium* (HSPH), *H. sibiricum* (HSIB) and *H. montanum* (HMON) root, leaf, flower and fruit essential oils is presented in Table 1. HMON, HSPH and HSIB root oils were characterized by monoterpenes (23.2–47.2%). Additionally, sesquiterpenes were the prominent constituents of HSPH (28.5%) and HMON (35.9%) root oils, whilst the notable amount of phenylpropanoids (48.3%) was present in HSIB root oil. (*Z*)- β -Ocimene was the most abundant in HSPH (28.9%) and HMON (20.4%) root oils, while HSIB root oil contained only 5.2% of this component. In HSIB oil, β -pinene (26.2%), methyl eugenol (22.3%) and elemicin (25.6%) were predominant. The lower amounts of β -pinene were present in HSPH (6.0%) and HMON (0.7%) root oils, and only the traces of elemicin (in both these oils) and methyl eugenol (in HSPH oil) were detected.

Regarding previously tested *Heracleum* root essential oils, Tkachenko⁴⁴ showed that the oils of nine *Heracleum* species, grown at experimental station in Leningrad Oblast' (Russia), contained the significant amounts of ocimene (10.9–24.0%), but the exact isomer was not specified. Like in HSIB oil, β -pinene was the most abundant in the root oils of the taxa collected in south-eastern Europe, *i.e.* *H. verticillatum* Pančić, *H. pyrenaicum* subsp. *pollinianum* (Bertol.) F. Pedrotti & Pignatti and *H. ternatum* Velen. (23.5–47.3%). On the other hand, the root oil of *H. orphanidis* Boiss. was mainly composed of (*Z*)-falcariol (80.0%), a polyacetylene present in smaller amounts in the tested HSIB, HSPH and HMON root oils (0.6–4.6%).^{39,45}

The analyzed *H. sibiricum*, *H. montanum* and *H. sphondylium* leaf and flower essential oils were characterized by sesquiterpene fractions (14.9–81.2%), with germacrene D (11.0%) and β -sesquiphellandrene (10.6%) being the most abundant in HSPH leaf oil, α -acorenol (9.0%) in HSPH flower oil, (*E*)- β -farnesene (18.4%) and (*E*)-caryophyllene (12.4%) in HMON leaf oil, (*E*)- β -farnesene (11.4%) in HMON flower oil, and (*E*)-caryophyllene (9.5%) in HSIB leaf oil. Additionally, HMON leaf and flower oils also contained notable amounts of monoterpene fractions (29.1 and 35.1%), with sabinene being the dominant (6.2 and 8.0%). Furthermore, several phenylpropanoids were characteristic for HSPH flower oil, and HSIB leaf and flower oils. Predominant were apiole (16.8%) in HSPH flower oil, and methyl eugenol, elemicin and (*Z*)-isoelemicin (14.1–22.9%) in HSIB leaf and flower oils.

Bicchi *et al.*¹⁶ investigated the composition of the essential oils obtained by the microdissections of the secretory structures of the leaves and the flower petals of *H. sphondylium* collected in Italy. The leaf oil obtained by this technique contained six sesquiterpenes, four monoterpenes and hexadecanoic acid, while in the petals oil, two sesquiterpenes and one monoterpene were identified. The predominant constituent of these oils was (*E*)-caryophyllene (28.0 and 19.5%), followed by α -bergamotene (14.3 and 4.4%). In the present study, in HSPH leaf and flower oils obtained by hydrodistillation, 82 and 95 components were identified, respectively. (*E*)-Caryophyllene and α -*trans*-bergamotene were also identified, but only in traces or in much smaller quantities (2.8–4.7%) compared to the previously investigated oils. These findings confirmed that geographical origin, *i.e.* ecological conditions, as well as the method of isolation significantly influence the oils composition.

The prevalence of sesquiterpenes and/or phenylpropanoids in the essential oils isolated from several other *Heracleum* species aerial parts was previously shown. For example, regarding sesquiterpenes, *H. verticillatum* leaf oil contained the significant amount of (*E*)-caryophyllene (19.1%), while *H. candicans* Wall. leaf oil of germacrene D (29.5%). Regarding phenylpropanoids, *H. ternatum* leaf oil was mostly composed of (*Z*)-isoelemicin (35.1%), *H. rechingeri* Manden. flower oil and *H. transcaucasicum* Manden. aerial parts oil mostly of elemicin (39.5 and 41.1%), and *H. moellendorffii* Hance aerial parts oil mostly of apiole (11.0%).^{39,46}

Table 1 Chemical composition of investigated *Heracleum* essential oils (%)

RI _{exp} ^a	RI _{lit} ^b	Compound	Class ^c	<i>H. sphondylium</i>				<i>H. sibiricum</i>				<i>H. montanum</i>			
				root	leaf	flower	fruit	root	leaf	flower	fruit	root	leaf	flower	fruit
875	863	<i>n</i> -Hexanol	AC	tr ^e	tr	tr	tr	-	-	-	tr	tr	tr	tr	tr
888	880	Isopropyl 2-methyl butanoate	AE	-	-	-	0.1 ^d	-	-	-	tr	-	-	-	0.1
894	-	Isopropyl isovalerate	AE	-	-	-	tr	-	-	-	tr	-	-	-	0.1
899	900	<i>n</i> -Nonane	AH	tr	-	-	tr	tr	-	-	tr	tr	-	tr	tr
904	901	Heptanal	AL	tr	tr	tr	tr	tr	tr	tr	tr	0.4	tr	0.1	tr
916	908	Isobutyl isobutanoate	AE	-	-	-	tr	-	-	-	tr	-	-	-	tr
931	924	α -Thujene	MH	tr	tr	-	-	0.7	tr	tr	-	tr	0.4	tr	-
940	932	α -Pinene	MH	tr	1.4	0.6	0.1	3.9	tr	tr	tr	tr	1.7	3.1	0.2
955	946	Camphene	MH	tr	0.3	tr	tr	0.5	tr	tr	tr	tr	0.3	0.5	tr
961	953	Thuja-2,4(10)-diene	MH	tr	-	-	-	tr	-	-	-	-	-	-	-
979	969	Sabinene	MH	tr	tr	tr	-	tr	tr	tr	-	tr	6.2	8.0	tr
987	974	β-Pinene	MH	6.0	tr	tr	-	26.2	tr	tr	-	0.7	2.1	0.8	tr
990	981	6-Methyl-5-hepten-2-one	AK	tr	0.5	tr	-	-	tr	-	-	tr	tr	tr	-
994	988	Myrcene	MH	1.8	1.5	2.2	-	2.3	tr	0.6	-	1.0	4.7	5.1	-
995	988	Dehydro-1,8-cineole	MH	0.8	tr	-	-	-	-	-	-	0.5	tr	tr	-
1004	998	<i>n</i> -Octanal	AL	1.8	0.2	1.4	1.6	0.2	tr	0.3	0.6	1.5	tr	1.0	0.8
1006	-	Isobutyl isovalerate	AE	-	-	-	tr	-	-	-	tr	-	-	-	0.2
1017	-	2-Methyl butyl isobutanoate	AE	-	-	-	tr	-	-	-	tr	-	-	-	tr
1021	1014	α -Terpinene	MH	tr	tr	-	-	tr	tr	-	-	tr	0.4	0.3	-
1028	1020	<i>p</i> -Cymene	MH	tr	tr	tr	tr	tr	0.2	tr	tr	tr	0.3	0.2	tr
1036	1024	Limonene	MH	0.9	2.8	4.2	0.1	5.3	0.1	0.1	tr	tr	2.7	3.3	0.1
1043	1032	(Z)-β-Ocimene	MH	28.9	0.9	0.6	-	5.2	0.3	0.6	0.2	20.4	2.6	2.4	-
1044	-	Butyl 2-methyl butanoate	AE	-	-	-	tr	-	-	-	tr	-	-	-	tr
1049	-	Butyl isovalerate	AE	-	-	-	tr	-	-	-	tr	-	-	-	tr
1052	1044	(<i>E</i>)- β -Ocimene	MH	0.9	1.3	0.4	-	0.1	tr	0.1	-	0.5	2.8	5.3	-
1062	1049	(2 <i>E</i>)-Octen-1-al	AL	-	-	tr	-	-	-	-	-	tr	-	-	-
1063	1054	γ -Terpinene	MH	tr	tr	tr	-	0.3	0.3	tr	-	tr	1.1	1.0	-
1065	1047	(3 <i>Z</i>)-Octen-1-ol	AC	-	-	-	0.2	-	-	-	0.9	-	-	-	0.6
1066	-	2-Methyl decane	AH	tr	-	-	-	-	-	-	-	0.8	-	-	-
1072	1063	<i>n</i>-Octanol	AC	tr	tr	1.5	16.6	-	-	1.2	21.1	tr	-	2.0	15.7
1082	-	Isobutyl 3-methyl 2-butenate	AE	tr	-	-	-	tr	-	-	-	tr	-	-	tr
1092	1086	Terpinolene	MH	tr	tr	-	-	1.2	tr	tr	-	tr	0.2	0.2	-
1093	1087	2-Nonanone	AK	tr	tr	0.1	-	-	-	-	-	tr	-	tr	-
1100	1098	<i>trans</i> -Sabinene hydrate (IPP vs. OH)	OM	-	-	-	-	-	-	-	-	-	0.2	0.4	-
1100	1100	<i>n</i> -Undecane	AH	tr	-	-	-	-	-	-	-	tr	-	-	-
1100	1100	Isopentyl 2-methyl butanoate	AE	-	-	-	tr	-	-	tr	tr	-	-	-	tr
1104	1100	2-Methyl butyl 2-methyl butanoate	AE	-	-	-	0.1	-	-	tr	tr	-	tr	-	0.2
1106	1100	<i>n</i> -Nonanal	AL	tr	tr	0.7	-	tr	-	-	-	tr	-	0.9	-
1106	1102	Isopentyl isovalerate	AE	-	-	-	tr	tr	-	tr	tr	-	-	tr	tr
1109	1103	2-Methyl butyl isovalerate	AE	tr	-	-	tr	-	-	tr	tr	-	0.1	tr	0.1
1124	1118	<i>cis-p</i> -Menth-2-en-1-ol	OM	tr	-	-	-	tr	-	-	-	-	0.2	0.2	-
1131	1128	<i>allo</i> -Ocimene	MH	tr	-	-	-	tr	-	-	-	tr	tr	tr	-
1134	1128	(<i>Z</i>)-Epoxy-ocimene	OM	tr	tr	-	-	tr	-	-	-	tr	tr	tr	-
1143	1136	<i>trans-p</i> -Menth-2-en-1-ol	OM	-	-	-	-	tr	-	-	-	-	0.2	tr	-
1151	1147	Hexyl isobutanoate	AE	-	-	-	0.1	-	-	tr	0.2	-	-	-	tr
1160	1160	(<i>Z</i>)-Isocitral	OM	-	tr	0.3	-	-	-	-	-	-	tr	tr	-
1170	1165	Lavandulol	OM	-	-	-	-	-	-	-	-	-	tr	0.5	-
1181	1174	Terpinen-4-ol	OM	tr	tr	tr	-	0.3	tr	-	-	tr	2.5	1.4	-
1186	-	1-Methyl butyl 3-methyl 2-butenate	AE	tr	-	-	tr	tr	-	tr	tr	tr	tr	tr	tr
1188	1179	<i>p</i> -Cymen-8-ol	OM	-	-	-	-	tr	-	-	-	-	-	-	-
1192	1187	1-Dodecene	AH	tr	-	-	-	-	-	-	-	tr	-	-	-
1193	1191	Hexyl butanoate	AE	-	-	-	tr	-	-	-	-	-	-	-	-

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1193	1186	α -Terpineol	OM	-	-	-	-	0.2	-	-	-	-	tr	tr	-
1196	1193	(4Z)-Decenal	AL	-	-	-	tr	-	-	-	tr	-	-	-	tr
1198	1194	Myrtenol	OM	tr	-	-	-	tr	-	-	-	tr	-	-	-
1200	1195	Methyl chavicol	PH	tr	-	-	-	0.3	-	-	-	tr	-	-	-
1205	1197	2-Methyl 4-methyl pentyl butanoate	AE	0.7	-	-	-	-	-	-	-	tr	tr	tr	-
1206	1201	<i>n</i> -Decanal	AL	-	-	tr	tr	-	-	-	tr	-	-	-	0.6
1215	1210	(2E,4E)-Nonadienal	AL	tr	-	-	-	-	-	-	-	tr	-	-	-
1216	1211	Octyl acetate	AE	-	-	4.4	67.1	tr	-	13.4	64.3	-	-	3.2	57.5
1221	1215	<i>trans</i> -Carveol	OM	-	tr	tr	-	-	-	-	-	-	tr	-	-
1239	1233	Hexyl 2-methyl butanoate	AE	-	-	tr	0.4	-	-	0.1	0.4	-	-	tr	0.2
1244	1241	Hexyl isovalerate	AE	-	-	tr	0.3	-	-	0.1	0.3	-	-	tr	0.1
1260	1255	(4Z)-Decen-1-ol	AC	-	-	-	tr	-	-	-	tr	-	-	-	tr
1264	1260	(2E)-Decenal	AL	0.5	tr	0.5	-	tr	-	-	-	tr	-	tr	-
1264	-	2-Methyl dodecane	AH	0.6	-	-	-	-	-	-	-	tr	-	-	-
1274	1266	<i>n</i> -Decanol	AC	-	-	-	0.1	-	-	-	tr	-	-	-	0.2
1287	1282	(E)-Anethol	PH	tr	-	-	-	-	-	-	-	tr	0.2	-	-
1289	1287	Bornyl acetate	OM	-	tr	tr	tr	0.7	-	-	-	-	0.2	0.7	tr
1290	1288	Lavandulyl acetate	OM	-	-	tr	tr	-	-	-	-	-	tr	1.8	tr
1293	1289	<i>trans</i> -Sabinyl acetate (IPP vs. Acetyl)	OM	-	-	-	-	-	-	-	-	-	tr	-	-
1293	1292	(2E,4Z)-Decadienal	AL	tr	tr	tr	-	tr	-	-	-	tr	tr	-	-
1298	1300	<i>n</i> -Tridecane	AH	tr	-	-	-	tr	-	-	-	tr	-	tr	-
1303	-	Octyl propanoate	AE	-	-	-	tr	-	-	tr	tr	-	-	-	tr
1307	1305	Undecanal	AL	-	-	tr	tr	-	-	-	tr	-	-	tr	tr
1311	1311	Nonanyl acetate	AE	-	-	-	tr	-	-	tr	tr	-	-	-	tr
1316	1315	(2E,4E)-Decadienal	AL	tr	tr	tr	-	tr	-	-	-	tr	tr	tr	-
1346	-	Octyl isobutanoate	AE	-	-	tr	0.1	-	-	tr	0.2	-	-	tr	tr
1353	1346	α -Terpinyl acetate	OM	-	-	-	-	0.1	-	-	-	-	-	-	-
1362	1356	Eugenol	PH	-	-	-	-	tr	0.1	0.7	tr	-	-	-	-
1378	1374	α -Copaene	SH	tr	0.7	0.5	tr	-	0.2	tr	-	-	0.3	tr	-
1381	1380	Daucene	SH	tr	tr	tr	-	tr	tr	tr	-	tr	-	-	-
1385	1382	Hexyl hexanoate	AE	-	-	-	tr	-	-	-	-	-	-	-	0.1
1387	1387	β -Bourbonene	SH	-	0.4	tr	-	-	0.2	tr	tr	-	0.5	0.3	-
1389	-	Octyl butanoate	AE	-	-	1.0	1.0	-	-	0.7	2.8	-	-	1.1	1.6
1391	1387	β -Cubebene	SH	tr	tr	-	-	-	0.2	-	-	tr	tr	tr	-
1391	1390	7- <i>epi</i> -Sesquithujene	SH	tr	-	-	-	-	-	-	-	0.6	-	-	-
1394	-	1-Butenylidene-cyclohexane	AH	-	-	-	0.2	-	-	-	0.2	-	-	-	0.4
1394	1389	β -Elemene	SH	-	4.2	0.8	-	tr	2.6	0.6	-	-	4.5	0.6	-
1397	1397	(Z)-Trimenal	AL	-	-	tr	tr	-	-	-	0.2	-	-	-	0.2
1404	1403	Methyl eugenol	PH	tr	-	-	-	22.3	14.1	22.9	0.4	-	tr	tr	-
1410	1407	Decyl acetate	AE	-	-	0.3	0.8	-	-	-	0.8	-	-	tr	1.1
1411	1409	α -Gurjunene	SH	-	tr	-	-	-	-	-	-	-	tr	-	-
1416	1411	α - <i>cis</i> -Bergamotene	SH	-	tr	-	-	-	-	-	-	tr	-	-	-
1421	1417	(E)-Caryophyllene	SH	-	2.8	4.7	tr	-	9.5	3.1	0.2	tr	12.4	2.8	-
1424	1424	2,5-Dimethoxy- <i>p</i> -cymene	OM	tr	-	-	-	-	-	-	-	tr	-	-	-
1431	1430	β -Copaene	SH	tr	tr	tr	-	-	tr	tr	-	tr	tr	tr	-
1435	-	Octyl 2-methyl butanoate	AE	-	-	tr	0.1	-	-	0.1	0.4	-	-	tr	tr
1437	1432	α - <i>trans</i> -Bergamotene	SH	3.2	3.3	tr	-	0.4	8.1	2.6	0.1	3.2	tr	tr	-
1439	-	Octyl isovalerate	AE	-	-	tr	0.1	-	-	-	0.5	-	-	tr	0.1
1444	1440	(Z)- β -Farnesene	SH	tr	-	-	-	-	-	-	-	1.3	-	-	-
1447	1444	Acora-2,4(15)-diene	SH	tr	-	-	-	-	-	-	-	tr	-	-	-
1459	1452	α -Humulene	SH	-	tr	0.8	-	-	2.8	0.9	tr	-	1.7	0.3	-
1461	1454	(E)-β-Farnesene	SH	0.9	6.3	6.2	-	0.1	tr	3.3	tr	0.8	18.4	11.4	-
1463	1464	α -Acoradiene	SH	-	-	tr	-	-	-	0.1	-	-	-	-	-
1466	1461	<i>cis</i> -Cadina-1(6),4-diene	SH	-	-	-	-	-	-	-	-	-	1.1	1.6	-
1469	1469	β -Acoradiene	SH	tr	-	-	-	tr	-	-	-	tr	-	tr	-
1480	1478	γ -Muurolole	SH	-	-	tr	-	-	0.2	tr	-	-	-	tr	-
1483	1479	<i>ar</i> -Curcumene	SH	tr	-	-	-	tr	-	-	-	tr	-	-	-

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1486	1484	Germacrene D	SH	-	11.0	6.3	-	-	5.6	0.9	-	-	8.7	3.6	-
1488	1487	(<i>E</i>)- β -Ionone	AK	-	tr	tr	-	-	-	-	-	-	tr	tr	-
1489	1489	β -Selinene	SH	-	tr	tr	-	-	0.2	tr	-	tr	0.2	-	-
1493	1491	10,11-Epoxy-calamenene	OS	-	tr	-	-	-	-	-	-	-	-	-	-
1498	1493	α -Zingiberene	SH	tr	-	-	-	tr	-	tr	-	tr	3.1	1.8	-
1498	1500	Bicyclogermacrene	SH	2.1	4.1	4.9	-	0.2	0.8	0.1	-	tr	tr	tr	-
1500	1500	Isodaucene	SH	1.3	tr	tr	-	0.5	0.3	tr	-	tr	tr	tr	-
1510	1505	(<i>E,E</i>)- α -Farnesene	SH	-	2.7	2.4	-	0.2	-	0.6	-	-	4.9	tr	-
1512	1505	β -Bisabolene	SH	2.8	4.3	tr	tr	0.4	3.9	0.3	tr	2.0	tr	7.1	-
1518	1513	γ -Cadinene	SH	-	-	tr	-	-	0.1	tr	tr	-	tr	-	-
1518	1514	(<i>Z</i>)- γ -Bisabolene	SH	5.0	6.5	-	-	-	-	-	-	7.8	-	-	-
1521	-	Bornyl isovalerate	OM	-	-	-	-	0.2	-	-	-	-	-	-	-
1524	1517	Myristicin	PH	tr	-	tr	-	-	-	1.2	-	-	tr	tr	-
1526	1521	β-Sesquiphellandrene	SH	tr	10.6	2.8	-	tr	1.9	0.1	-	tr	2.0	2.2	-
1532	1529	Kessane	OS	tr	-	-	-	0.3	-	-	-	1.7	tr	tr	tr
1535	1529	(<i>E</i>)- γ -Bisabolene	SH	2.1	1.4	-	-	0.4	-	-	-	1.5	0.5	0.3	-
1536	1531	(<i>Z</i>)-Nerolidol	OS	-	-	0.3	-	-	-	-	-	-	-	-	-
1545	-	(<i>E</i>)- α -Bisabolene	SH	1.7	tr	tr	-	tr	-	-	-	1.9	tr	tr	-
1549	-	Not identified ^f	-	6.9	-	-	-	tr	-	-	-	7.7	-	-	-
1559	1555	Elemicin	PH	tr	-	tr	tr	25.6	14.9	22.7	0.3	tr	0.9	7.9	-
1560	1559	Germacrene B	SH	-	tr	-	-	-	-	-	-	tr	-	-	-
1564	1561	(<i>E</i>)-Nerolidol	OS	tr	0.5	0.7	-	-	-	-	-	1.2	0.3	0.3	-
1575	1568	(<i>Z</i>)-Isoelemicin	PH	tr	-	-	-	0.1	16.6	18.5	0.2	tr	-	-	-
1580	1577	Spathulenol	OS	1.3	2.3	1.2	-	-	-	-	-	1.3	0.7	tr	-
1583	-	Octyl hexanoate	AE	-	-	3.2	8.4	-	-	-	-	2.5	-	3.4	15.0
1590	1582	Caryophyllene oxide	OS	-	2.9	2.5	-	-	4.9	0.8	-	-	3.2	0.5	-
1590	1577	<i>trans</i> -Sesquisabinene hydrate (IPP vs. OH)	OS	1.3	-	-	-	-	-	-	-	0.8	-	-	-
1596	1594	Salvial-4(14)-en-1-one	OS	-	0.3	0.3	-	-	tr	-	-	-	tr	tr	-
1600	1595	6-Methoxy elemicin	PH	-	-	-	-	tr	tr	tr	-	-	-	-	-
1614	1608	Humulene epoxide II	OS	-	1.6	0.4	-	-	0.9	0.1	-	0.9	0.2	-	-
1633	1631	(<i>E</i>)-Sesquilandulol	OS	2.5	-	-	-	-	-	-	-	2.8	-	-	-
1633	1632	α-Acorenol	OS	tr	3.1	9.0	0.1	tr	0.4	1.3	-	tr	-	tr	-
1635	1639	Caryophylla-4(14),8(15)-dien-5 β -ol	OS	-	-	-	-	-	tr	-	-	-	tr	tr	-
1640	1636	Gossonorol	OS	tr	3.5	-	-	tr	-	-	-	tr	-	-	-
1641	1639	Caryophylla-4(14),8(15)-dien-5 α -ol	OS	-	-	0.9	-	-	0.1	tr	-	-	0.3	tr	-
1648	-	Isospathulenol	OS	0.9	-	-	-	-	-	-	-	0.7	-	-	-
1651	-	Caryophylla-3,8(13)-dien- α -ol	OS	-	0.4	tr	-	-	-	-	-	-	tr	tr	-
1657	1658	<i>neo</i> -Intermedeol	OS	1.8	0.9	0.7	-	-	-	tr	-	3.8	0.6	-	-
1658	1658	Selin-11-en-4 α -ol	OS	-	-	-	-	-	-	0.1	-	-	-	-	-
1658	1652	α -Cadinol	OS	-	-	-	-	-	0.5	-	-	-	-	-	-
1659	1666	14-Hydroxy-(<i>Z</i>)-caryophyllene	OS	-	-	-	-	-	-	-	-	-	0.3	tr	-
1662	1660	<i>cis</i> -Calamenen-10-ol	OS	-	-	-	-	-	0.2	tr	-	-	-	-	-
1671	1668	<i>trans</i> -Calamenen-10-ol	OS	-	0.4	0.7	-	-	0.2	tr	-	-	-	tr	-
1672	1668	14-Hydroxy-9- <i>epi</i> -(<i>E</i>)-caryophyllene	OS	-	1.0	-	-	-	-	-	-	-	0.2	tr	-
1674	1674	β -Bisabolol	OS	1.4	1.0	0.8	-	tr	-	-	-	2.8	0.4	tr	-
1684	1685	α -Bisabolol	OS	tr	0.5	-	-	-	-	-	-	tr	-	-	-
1685	1677	Apiole	PH	-	-	16.8	-	-	0.5	-	-	-	-	tr	-
1689	1687	Eudesma-4(15),7-dien-1- β -ol	OS	-	2.1	1.0	-	-	0.1	-	-	-	0.9	0.9	-
1699	-	Not identified ^g	-	4.1	-	-	-	tr	-	-	-	5.0	-	-	-
1706	1690	(<i>Z</i>)- α - <i>trans</i> -Bergamotol	OS	tr	-	0.3	-	-	-	-	-	tr	-	tr	-
1710	1700	Amorpha-4,9-dien-2-ol	OS	-	0.4	tr	-	-	tr	tr	-	-	0.3	tr	-
1725	-	Eudesma-4,11-dien-2-ol	OS	-	0.8	tr	-	-	-	-	-	-	0.5	tr	-
1747	1714	Nootkatol	OS	-	0.4	tr	-	-	tr	tr	-	-	tr	tr	-
1759	1755	7,14-Anhydro-amorpha-4,9-diene	OS	-	0.5	tr	-	-	-	-	-	-	-	-	-
1766	-	Tetradecanoic acid	FA	tr	-	-	-	-	-	-	-	tr	-	0.8	-
1768	1775	2- α -Hydroxi-amorpha-4,7(11)-diene	OS	-	tr	tr	-	-	0.3	tr	-	-	tr	-	-
1769	-	Not identified ^h	-	5.0	-	-	-	tr	-	-	-	4.4	-	-	-

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1777	-	Octyl octanoate	AE	-	-	0.5	0.8	-	-	tr	0.8	-	-	0.5	1.7
1778	1766	12-Hydroxy-(Z)-sesquicineole	OS	tr	-	-	-	-	-	-	-	0.7	-	-	-
1818	-	1,13-Tetradecadiene	AH	2.3	-	-	-	-	-	-	-	1.1	-	-	-
1837	-	Neophytadiene	D	-	0.6	-	-	-	0.1	-	-	-	0.4	tr	-
1844	-	Hexahydrofarnesyl acetone	AK	-	tr	0.2	-	-	tr	tr	-	-	0.2	tr	-
1968	1959	Hexadecanoic acid	FA	3.3	0.4	1.2	-	tr	1.5	-	-	6.3	-	1.8	-
2030	2033	Isobergapten	C	tr	-	-	-	0.2	-	-	-	tr	-	-	-
2038	2035	(Z)-Falcarinol	PA	1.8	-	0.5	-	0.6	tr	-	-	4.6	-	tr	-
2059	2056	Bergapten	C	tr	-	-	tr	tr	-	-	-	tr	-	-	tr
2080	2077	<i>n</i> -Octadecanol	AC	-	0.3	-	-	-	-	-	-	-	-	-	-
2090	-	Falcarinol isomer	PA	1.5	-	-	-	-	-	-	-	2.5	-	-	-
2095	2100	<i>n</i> -Heneicosane	AH	-	-	tr	-	-	-	tr	-	-	tr	0.5	-
2099	-	γ -Palmitolactone	FD	-	-	tr	-	-	-	-	-	-	-	0.5	-
2112	-	Phytol isomer	D	-	0.8	0.3	-	-	2.0	0.1	-	-	1.1	tr	-
2128	-	Pimpinellin	C	tr	-	-	-	0.3	-	-	-	tr	-	tr	-
2137	2095	Methyl linoleate	FD	-	-	-	-	-	tr	-	-	1.0	-	-	-
2141	2140	Osthole	C	tr	-	-	-	0.3	-	-	-	-	-	-	-
2194	2200	<i>n</i> -Docosane	AH	-	-	tr	-	-	-	tr	-	-	tr	0.3	-
2194	-	Falcarindiol	PA	2.3	-	-	-	tr	-	-	-	1.6	-	-	-
2236	2237	Isopimpinellin	C	tr	-	-	-	tr	-	-	-	tr	-	-	-
2294	2300	<i>n</i> -Tricosane	AH	-	tr	1.4	-	-	tr	0.2	-	-	tr	3.1	-
2394	2400	<i>n</i> -Tetracosane	AH	-	-	tr	-	-	-	tr	-	-	tr	0.3	-
2494	2500	<i>n</i> -Pentacosane	AH	-	tr	1.6	-	-	tr	0.2	-	-	tr	1.4	-
2693	2700	<i>n</i> -Heptacosane	AH	-	tr	1.0	-	-	tr	0.1	-	-	tr	0.5	-
2793	2800	<i>n</i> -Octacosane	AH	-	-	tr	-	-	-	-	-	-	-	tr	-
2892	2900	<i>n</i> -Nonacosane	AH	-	tr	0.8	-	-	0.1	0.1	-	-	tr	0.2	-
		Monoterpene hydrocarbons (MH)		39.3	8.2	7.8	0.2	45.7	0.9	1.3	0.2	23.2	25.7	30.0	0.3
		Oxygenated monoterpenes (OM)		tr	tr	0.3	tr	1.5	tr	-	-	tr	3.4	5.1	tr
		Sesquiterpene hydrocarbons (SH)		19.2	58.3	29.3	tr	2.2	36.6	12.6	0.3	19.2	58.4	32.0	-
		Oxygenated sesquiterpenes (OS)		9.3	22.9	18.9	0.1	0.3	7.6	2.3	-	16.7	8.0	1.7	tr
		Phenylpropanoids (PH)		tr	-	16.8	tr	48.3	46.2	66.0	0.9	tr	1.0	7.9	-
		Aliphatic esters (AE)		0.7	-	9.5	79.5	tr	-	14.4	73.2	tr	0.1	8.2	78.2
		Aliphatic hydrocarbons (AH)		2.9	tr	4.8	0.2	tr	0.1	0.6	0.2	1.8	tr	6.5	0.4
		Aliphatic alcohols (AC)		tr	0.3	1.5	17.0	-	-	1.2	22.0	tr	tr	2.0	16.5
		Aliphatic aldehydes (AL)		2.3	0.2	2.6	1.6	0.2	tr	0.3	0.8	2.0	tr	2.0	1.6
		Aliphatic ketones (AK)		tr	0.5	0.4	-	-	tr	tr	-	tr	0.2	tr	-
		Diterpenes (D)		-	1.4	0.3	-	-	2.1	0.1	-	-	1.5	tr	-
		Coumarins (C)		tr	-	-	tr	0.8	-	-	-	tr	-	tr	tr
		Polyacetylenes (PA)		5.5	-	0.5	-	0.6	tr	-	-	8.7	-	tr	-
		Fatty acids (FA) and their derivatives (FD)		3.3	0.4	1.2	-	tr	1.5	-	-	7.3	-	3.0	-
		Total identified		82.5	92.2	93.8	98.5	99.6	95.1	98.9	97.6	78.9	98.3	98.3	97.1
		Number of identified compounds		91	82	95	52	69	64	76	54	91	92	111	50

^a RI_{exp} - Retention indices on HP-5MS column relative to C₈-C₄₀ *n*-alkanes. ^b RI_{lit} - Retention indices obtained from the literature.³⁵ ^c Class of compound. ^d Relative area percentage of the compounds obtained from FID area percent data. ^e tr - trace (< 0.1%). ^f MS data, 70 eV, *m/z* (rel. int.): 41 (29), 55 (23), 69 (30), 84 (16), 109 (17), 122 (15), 123 (20), 125 (80), 140 (100), 222 (19). ^g MS data, 70 eV, *m/z* (rel. int.): 41 (53), 68 (42), 79 (39), 81 (79), 93 (69), 107 (87), 109 (52), 121 (100), 163 (82), 222 (2). ^h MS data, 70 eV, *m/z* (rel. int.): 41 (54), 81 (48), 91 (56), 95 (58), 107 (100), 109 (56), 119 (49), 121 (75), 123 (84), 222 (4).

In contrast to the investigated *H. sphondylium*, *H. sibiricum* and *H. montanum* root, leaf and flower essential oils, the chemical composition of their fruit oils was significantly different. The tested fruit oils contained the lower amounts of terpenes and were dominated by aliphatic esters (73.2-79.5%), with octyl acetate (57.5-67.1%) being the most prominent, followed by octyl hexanoate in HSPH and HMON oils (8.4 and 15.0%), and octyl butanoate (2.8%) in HSIB oil. The significant

amounts of *n*-octanol (15.7-21.1%) were also present in the investigated fruit oils.

Other previously tested fruit essential oils of *Heracleum* taxa had similar composition as well.^{39,45} In the oil isolated by the microdissection of the vittae of *H. sphondylium* fruits collected in Italy in different development stages, the main components were also octyl acetate (18.1-34.6%) and octyl hexanoate (24.5-30.5%). Besides aliphatic esters, this oil contained the notable quantity of furanocoumarins, mostly

bergapten and byakangelicol (2.8-8.7%),¹⁶ in contrast to the analyzed HSPH oil in which only the trace of bergapten was identified. Considering that furanocoumarins are less volatile constituents, these results indicate that the method of isolation significantly affect the oils furanocoumarin profile.

Miladinović *et al.*²⁷ investigated the composition of the essential oil isolated from the aerial parts of *H. sibiricum* collected on Mt. Vidlič (Serbia), but plant organs included in the analyzed aerial parts were not specified. This oil was similar to the tested HSIB fruit oil, through the domination of aliphatic esters, but with significant differences in the content of individual compounds (predominant was octyl butanoate with 36.8%).

3.2. Bioactivity of essential oils

The antimicrobial activities of the investigated *H. sphondylium*, *H. sibiricum* and *H. montanum* root, leaf, flower and fruit essential oils were tested against eight bacteria (Table 2) and

eight fungi (Table 3). Besides standard strains, some clinical and food isolates were also used. The tested bacteria are the cause of foodborne diseases (*Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella typhimurium* and *Escherichia coli*) and hospital-acquired infections (*S. aureus*, *Pseudomonas aeruginosa*, *E. coli* and *Enterobacter cloacae*).⁴⁷ The tested fungi of *Aspergillus* and *Penicillium* genera are the common food contaminants and the producers of potentially carcinogenic mycotoxins. *Aspergillus fumigatus* on the other hand, can cause invasive aspergillosis, particularly in immunocompromised patients.⁴⁸⁻⁵⁰ All the microorganisms were susceptible to all the tested oils.

Overall, the best antibacterial activity was shown by the flower essential oils of HMON and HSPH. HMON flower oil showed pronounced activity against the broad spectrum of bacteria. Namely, against all the tested bacteria, except *L. monocytogenes* and *P. aeruginosa* (MICs 0.10-0.40 mg mL⁻¹, MBCs 0.13-1.00 mg mL⁻¹), this oil exhibited similar effect to ampicillin. The activity of HSPH flower oil against all the tested

Table 2 Antibacterial activity of investigated *Heracleum* essential oils and antibiotics (mg mL⁻¹)

	Bacteria	<i>Staphylococcus aureus</i>	<i>Bacillus cereus</i>	<i>Micrococcus flavus</i>	<i>Listeria monocytogenes</i>	<i>Pseudomonas aeruginosa</i>	<i>Salmonella typhimurium</i>	<i>Escherichia coli</i>	<i>Enterobacter cloacae</i>
		MIC MBC	MIC MBC	MIC MBC	MIC MBC	MIC MBC	MIC MBC	MIC MBC	MIC MBC
<i>Heracleum sphondylium</i>	root	0.50±0.01 ^e	0.50±0.00 ^e	0.80±0.01 ^e	1.30±0.10 ^c	1.00±0.01 ^e	0.80±0.01 ^c	3.30±0.20 ^e	0.06±0.00 ^a
		1.00±0.02 ^d	1.00±0.03 ^d	2.00±0.10 ^e	4.00±0.30 ^c	4.00±0.00 ^d	1.30±0.02 ^c	4.00±0.60 ^e	0.12±0.02 ^a
	leaf	0.40±0.05 ^d	0.40±0.04 ^{de}	3.30±0.03 ^f	4.00±0.20 ^f	3.30±0.20 ^f	2.00±0.30 ^{de}	3.30±0.20 ^e	2.00±0.20 ^f
		0.50±0.06 ^c	0.50±0.00 ^c	4.00±0.10 ^f	6.50±0.30 ^d	4.00±0.10 ^d	4.00±0.50 ^e	4.00±0.50 ^e	4.00±0.30 ^f
	flower	1.00±0.02 ^f	0.50±0.02 ^e	0.80±0.03 ^e	3.30±0.06 ^e	0.40±0.00 ^c	0.12±0.05 ^a	0.15±0.00 ^a	0.15±0.08 ^b
		2.00±0.10 ^e	1.00±0.06 ^d	1.00±0.02 ^d	4.00±0.08 ^d	1.00±0.08 ^b	0.15±0.06 ^a	0.30±0.04 ^a	0.30±0.06 ^b
fruit	6.00±0.60 ^j	0.50±0.03 ^e	0.35±0.00 ^d	8.00±1.20 ^j	6.00±0.80 ^j	6.00±0.90 ^f	2.00±0.50 ^d	3.00±0.80 ^e	
	8.00±0.30 ^h	1.00±0.04 ^d	0.50±0.05 ^c	16.00±1.50 ^h	12.00±1.00 ^h	8.00±1.00 ^h	4.00±0.60 ^e	4.00±0.90 ^f	
<i>Heracleum sibiricum</i>	root	0.20±0.01 ^c	0.15±0.04 ^b	0.80±0.00 ^e	2.00±0.10 ^d	4.00±0.40 ^h	1.75±0.90 ^{de}	0.50±0.05 ^b	3.50±0.80 ^h
		0.30±0.00 ^b	0.30±0.02 ^b	1.00±0.03 ^d	4.00±0.30 ^c	7.00±0.60 ^e	2.00±0.80 ^d	1.00±0.06 ^c	4.00±0.90 ^f
	leaf	2.35±0.60 ^{ph}	4.70±0.60 ^h	9.40±1.20 ^j	4.70±0.80 ^g	9.40±1.20 ^j	1.20±0.30 ^d	7.00±0.80 ^h	1.90±0.08 ^f
		4.70±0.80 ^{fg}	9.40±1.00 ⁱ	14.00±2.10 ⁱ	14.00±1.00 ^g	14.00±2.30 ^j	4.70±0.20 ^f	14.00±1.20 ^g	4.70±0.30 ^e
	flower	1.90±0.05 ^e	1.90±0.08 ^e	7.35±0.20 ^j	4.90±0.20 ^f	4.90±0.50 ^j	1.90±0.20 ^{de}	4.90±0.80 ^f	4.90±0.60 ^j
		7.35±1.00 ^g	3.70±0.06 ^c	9.80±0.50 ^h	9.80±0.60 ^f	9.80±0.90 ^g	3.70±0.60 ^e	7.35±0.50 ^{ef}	9.80±1.00 ^j
fruit	2.00±0.30 ^g	0.80±0.00 ^f	1.00±0.06 ^e	3.00±0.30 ^e	2.00±0.20 ^f	4.00±0.40 ^e	0.80±0.05 ^c	2.00±0.06 ^f	
	4.00±0.80 ^f	1.00±0.08 ^d	2.00±0.08 ^e	4.00±0.60 ^c	4.00±0.30 ^d	6.20±0.80 ^g	1.00±0.06 ^c	4.00±0.20 ^f	
<i>Heracleum montanum</i>	root	0.50±0.01 ^e	0.50±0.04 ^e	0.12±0.08 ^a	1.00±0.08 ^c	0.30±0.00 ^b	1.50±0.10 ^d	1.00±0.10 ^c	0.09±0.00 ^a
		1.00±0.02 ^d	1.00±0.08 ^d	0.25±0.06 ^a	4.50±1.00 ^c	0.50±0.08 ^a	2.25±0.30 ^d	2.25±0.30 ^d	0.30±0.01 ^b
	leaf	0.25±0.00 ^{cd}	0.35±0.03 ^d	6.00±0.80 ^h	6.00±0.60 ^h	4.00±0.60 ^h	6.00±0.50 ^f	6.00±0.90 ^g	1.50±0.90 ^e
		0.50±0.03 ^c	0.50±0.06 ^c	8.00±0.90 ^g	8.00±0.50 ^e	8.00±0.30 ^f	8.00±0.80 ^h	8.00±1.00 ^f	2.00±0.80 ^e
	flower	0.10±0.06 ^b	0.25±0.06 ^c	0.20±0.03 ^b	1.00±0.30 ^c	1.30±0.08 ^e	0.30±0.08 ^b	0.40±0.06 ^b	0.25±0.05 ^c
		0.13±0.00 ^a	0.50±0.04 ^c	0.30±0.05 ^a	2.00±0.06 ^b	2.00±0.06 ^c	0.50±0.00 ^b	0.50±0.04 ^b	1.00±0.05 ^d
fruit	3.00±0.60 ^h	0.35±0.00 ^d	1.50±0.80 ^f	8.00±1.00 ^j	8.00±1.00 ^k	4.00±0.90 ^e	3.00±0.30 ^e	6.00±0.80 ^j	
	4.00±0.50 ^f	0.50±0.06 ^c	2.00±0.90 ^e	16.00±1.20 ^h	12.00±1.30 ^h	8.00±0.60 ^h	4.00±0.20 ^e	8.00±0.30 ^h	
Amp	0.04±0.00 ^a	0.09±0.00 ^a	0.17±0.04 ^b	0.17±0.06 ^a	0.17±0.08 ^a	0.17±0.08 ^a	0.17±0.03 ^a	0.26±0.08 ^c	
	0.09±0.00 ^a	0.17±0.00 ^a	0.34±0.06 ^b	0.34±0.08 ^a	0.34±0.06 ^a	0.34±0.00 ^a	0.34±0.04 ^a	0.52±0.00 ^c	
Str	0.25±0.01 ^{cd}	0.25±0.06 ^c	0.25±0.06 ^c	0.37±0.00 ^b	0.74±0.04 ^d	0.37±0.00 ^b	0.25±0.06 ^{ab}	0.37±0.03 ^d	
	0.37±0.00 ^b	0.37±0.05 ^b	0.37±0.06 ^b	0.49±0.03 ^a	1.24±0.06 ^b	0.49±0.06 ^b	0.49±0.05 ^b	0.74±0.04 ^c	

MICs and MBCs are expressed as the mean ± SD determined from the results obtained in three independent experiments. Amp - ampicillin. Str - streptomycin. ^{a-1} Significant differences between the MICs (or the MBCs) of the tested oils obtained against one bacterium are indicated by the different letters in superscript ($p < 0.05$).

Table 3 Antifungal activity of investigated *Heracleum* essential oils and antibiotics (mg mL⁻¹)

Fungi	<i>Aspergillus fumigatus</i>	<i>Aspergillus versicolor</i>	<i>Aspergillus ochraceus</i>	<i>Aspergillus niger</i>	<i>Trichoderma viride</i>	<i>Penicillium funiculosum</i>	<i>Penicillium ochrochloron</i>	<i>Penicillium verucosum</i>
	MIC MFC	MIC MFC	MIC MFC	MIC MFC	MIC MFC	MIC MFC	MIC MFC	MIC MFC
<i>Heracleum sphondylium</i> root	1.00±0.03 ^e	0.50±0.03 ^c	1.00±0.08 ^d	3.30±0.90 ^e	0.50±0.08 ^b	1.50±0.06 ^{cd}	0.50±0.04 ^c	2.00±0.06 ^f
	2.00±0.06 ^d	1.00±0.08 ^c	2.00±0.06 ^c	4.00±0.80 ^e	1.00±0.06 ^c	2.00±0.06 ^c	1.00±0.06 ^c	4.00±0.20 ^f
	2.00±0.06 ^f	1.00±0.08 ^d	3.30±0.30 ^e	4.00±0.90 ^f	1.50±0.06 ^e	3.30±0.08 ^e	2.00±0.08 ^e	2.00±0.08 ^f
	4.00±0.90 ^e	4.00±0.06 ^e	4.00±0.50 ^d	8.00±1.00 ^g	2.00±0.08 ^d	4.00±0.06 ^d	4.00±0.04 ^e	4.00±0.05 ^f
<i>Heracleum sphondylium</i> leaf	0.50±0.05 ^c	0.50±0.08 ^c	0.50±0.03 ^{bc}	4.00±0.05 ^f	0.80±0.04 ^c	2.00±0.10 ^d	4.00±0.30 ^f	4.00±0.10 ^h
	1.00±0.03 ^c	2.00±0.04 ^d	1.00±0.02 ^b	8.00±0.08 ^g	2.00±0.20 ^d	4.00±0.20 ^d	8.00±0.20 ^g	8.00±0.20 ^h
	1.50±0.20 ^{ef}	1.00±0.02 ^d	1.50±0.06 ^{de}	1.50±0.08 ^d	0.50±0.08 ^b	1.50±0.10 ^{cd}	1.00±0.08 ^d	1.50±0.05 ^e
	4.00±0.50 ^e	2.00±0.05 ^d	2.00±0.06 ^c	4.00±0.20 ^e	2.00±0.20 ^d	2.00±0.20 ^c	2.00±0.10 ^d	2.00±0.06 ^e
<i>Heracleum sibiricum</i> root	0.30±0.05 ^b	1.15±0.10 ^d	0.60±0.03 ^c	0.60±0.05 ^c	0.60±0.08 ^c	1.15±0.10 ^c	0.60±0.08 ^c	1.15±0.30 ^e
	0.60±0.08 ^b	2.30±0.20 ^d	1.15±0.10 ^b	1.15±0.20 ^c	1.15±0.20 ^c	2.30±0.20 ^c	2.30±0.30 ^d	2.30±0.40 ^e
	0.60±0.08 ^d	2.35±0.10 ^e	4.70±0.30 ^e	4.70±0.40 ^g	3.50±0.10 ^f	2.35±0.20 ^d	1.20±0.09 ^d	1.80±0.08 ^{ef}
	2.35±0.09 ^d	4.70±0.20 ^f	7.00±0.40 ^e	7.00±0.40 ^f	4.70±0.30 ^e	4.70±0.30 ^e	4.70±0.10 ^f	4.70±0.10 ^g
<i>Heracleum sibiricum</i> leaf	0.30±0.08 ^b	0.30±0.06 ^c	0.30±0.05 ^b	0.60±0.08 ^c	0.50±0.04 ^b	0.30±0.04 ^b	0.30±0.05 ^b	0.30±0.08 ^c
	0.60±0.09 ^b	0.60±0.08 ^b	1.20±0.08 ^b	1.20±0.09 ^c	0.60±0.08 ^b	0.60±0.08 ^b	0.60±0.06 ^b	1.20±0.09 ^d
	0.15±0.02 ^a	0.25±0.03 ^{bc}	0.15±0.03 ^a	0.40±0.04 ^b	0.20±0.05 ^{ab}	0.15±0.06 ^a	0.15±0.03 ^a	0.25±0.06 ^{bc}
	0.25±0.03 ^a	0.50±0.04 ^b	0.25±0.05 ^a	0.50±0.06 ^b	0.25±0.08 ^a	0.25±0.08 ^b	0.25±0.04 ^a	0.50±0.08 ^c
<i>Heracleum montanum</i> root	0.50±0.00 ^c	0.80±0.08 ^d	0.40±0.05 ^{bc}	1.50±0.20 ^d	0.80±0.08 ^c	1.00±0.10 ^c	0.60±0.06 ^c	1.00±0.08 ^e
	1.00±0.10 ^c	2.25±0.20 ^d	1.00±0.04 ^b	2.25±0.10 ^d	2.25±0.10 ^d	2.25±0.20 ^c	2.25±0.20 ^d	2.25±0.20 ^e
	2.00±0.20 ^f	1.00±0.08 ^d	1.00±0.08 ^d	4.00±0.20 ^f	1.00±0.10 ^d	2.00±0.20 ^d	0.50±0.08 ^c	1.50±0.08 ^e
	4.00±0.40 ^e	4.00±0.20 ^e	4.00±0.10 ^d	8.00±0.30 ^g	2.00±0.30 ^d	4.00±0.30 ^d	1.00±0.09 ^c	4.00±0.20 ^f
<i>Heracleum montanum</i> leaf	0.50±0.05 ^c	0.25±0.05 ^{bc}	0.25±0.03 ^{ab}	1.60±0.20 ^d	0.25±0.08 ^{ab}	1.00±0.06 ^c	0.50±0.08 ^c	0.50±0.04 ^d
	1.00±0.08 ^c	0.50±0.04 ^b	1.00±0.06 ^b	2.00±0.30 ^d	1.00±0.08 ^c	2.00±0.20 ^c	1.00±0.09 ^c	2.00±0.20 ^e
	3.00±0.08 ^g	2.00±0.20 ^e	1.00±0.02 ^d	3.00±0.20 ^e	0.50±0.06 ^b	2.00±0.10 ^d	0.50±0.06 ^c	3.00±0.20 ^f
	4.00±0.20 ^e	4.00±0.30 ^e	2.00±0.06 ^c	4.00±0.40 ^e	2.00±0.10 ^d	4.00±0.20 ^d	1.20±0.08 ^c	4.00±0.40 ^f
Ket	0.15±0.03 ^a	0.10±0.02 ^a	0.15±0.03 ^a	0.15±0.04 ^a	0.15±0.03 ^a	0.20±0.04 ^{ab}	0.20±0.03 ^{ab}	0.10±0.06 ^a
	0.20±0.04 ^a	0.20±0.03 ^a	0.20±0.06 ^a	0.20±0.06 ^a	0.20±0.04 ^a	0.25±0.06 ^b	0.25±0.06 ^a	0.20±0.08 ^a
Bif	0.20±0.02 ^{ab}	0.20±0.08 ^b	1.50±0.90 ^{de}	0.20±0.08 ^{ab}	1.00±0.08 ^d	0.20±0.04 ^{ab}	2.50±0.10 ^e	0.20±0.06 ^b
	0.50±0.03 ^b	0.50±0.06 ^b	2.00±0.90 ^c	0.50±0.09 ^b	1.00±0.08 ^c	0.50±0.06 ^b	3.50±0.20 ^{de}	0.30±0.08 ^{ab}

MICs and MFCs are expressed as the mean ± SD determined from the results obtained in three independent experiments. Bif - bifonazole. Ket - ketoconazole. ^{a-h} Significant differences between the MICs (or the MFCs) of the tested oils obtained against one fungus are indicated by the different letters in superscript ($p < 0.05$).

Gram-negative bacteria (MICs 0.12–0.40 mg mL⁻¹, MBCs 0.15–1.00 mg mL⁻¹) was stronger than the activity of ampicillin, while the activity against *S. typhimurium*, *E. coli* and human isolate of *E. cloacae* was better even than the activity of streptomycin. Regarding other tested oils several results can be considered as significant. For example, the effects, better than the effects of both antibiotics, were exhibited by HSPH and HMON root oils on *E. cloacae* (MICs 0.06 and 0.09 mg mL⁻¹, MBCs 0.12 and 0.30 mg mL⁻¹), as well as by HMON root oil on *Micrococcus flavus* (MIC 0.12 mg mL⁻¹, MBC 0.25 mg mL⁻¹). HMON root oil also showed better effect than ampicillin against *P. aeruginosa* (MIC 0.30 mg mL⁻¹, MBC 0.50 mg mL⁻¹). On the other hand, HSIB oils were the least active, but several observed results are interesting. Among these oils, the most active was the root oil. Its activity against *S. aureus* and clinical isolate of *B. cereus* (MICs 0.20 and 0.15 mg mL⁻¹, MBCs 0.30 mg mL⁻¹) was more pronounced than the activity of ampicillin.

HSIB essential oils, which were the weakest antibacterials, on the contrary, were the strongest antifungal agents. Among these oils, the fruit oil showed the strongest activity (MICs 0.15–0.40 mg mL⁻¹, MFCs 0.25–0.50 mg mL⁻¹). Namely, against

all the tested fungi, including human isolate of *A. fumigatus* and food isolate of *P. verrucosum* var. *cyclopium*, the activity of this oil was similar to the activity of bifonazole and similar or even better when compared to the activity of ketoconazole. Additionally, the effects of the root oil (MICs 0.30–0.60 mg mL⁻¹, MFCs 0.60–2.30 mg mL⁻¹) and the flower oil (MICs 0.30–0.50 mg mL⁻¹, MFCs 0.60–1.20 mg mL⁻¹) of HSIB on *A. fumigatus*, *A. ochraceus*, *Trichoderma viride* and *P. ochrochloron*, as well as the effects of the flower oil of HSIB on *A. versicolor* and *P. funiculosum* (MICs 0.30 mg mL⁻¹, MFCs 0.60 mg mL⁻¹) were similar or more pronounced when compared to the effects of ketoconazole. Regarding HSPH and HMON oils, their antifungal activity was in several cases stronger than the activity of ketoconazole, *i.e.* HSPH flower oil and HMON root and flower oils on *A. ochraceus* (MICs 0.50, 0.40 and 0.25 mg mL⁻¹, MFCs 1.00 mg mL⁻¹), as well as HSPH root and fruit oils (MICs 0.50 and 1.00 mg mL⁻¹, MFCs 1.00 and 2.00 mg mL⁻¹) and all HMON oils (MICs 0.50–0.60 mg mL⁻¹, MFCs 1.00–2.25 mg mL⁻¹) against *P. ochrochloron*.

Previous studies confirm that *Heracleum* essential oils are promising antimicrobial agents.^{39,45,51} Namely, *H. ternatum*, *H.*

verticillatum, *H. pyrenaicum* subsp. *pollinianum* and *H. orphanidis* root, leaf and fruit oils exhibited antimicrobial activity that was in some cases stronger than the activity of reference antibiotics.^{39,45} Additionally, *H. orphanidis* aerial parts oil exhibited prominent antibacterial activity, and moreover, it inhibited *P. aeruginosa* PAO1 biofilm formation and synthesis of toxic pyocyanin, as well as reduced its twitching and flagella mobility.⁵¹

The cytotoxic activity of the analyzed *H. sphondylium*, *H. sibiricum* and *H. montanum* root, leaf, flower and fruit essential oils was tested against the cell lines of common cancer types, i.e. on human malignant cervix adenocarcinoma HeLa, colon carcinoma LS174 and/or non-small cell lung carcinoma A549 cell lines (Table 4). Except from HSPH root oil ($IC_{50}=5.72-24.31 \mu\text{g mL}^{-1}$), the activity of the tested oils against these cells was insignificant. In addition, to test their selectivity, the toxicity of the oils (except HSPH and HMON flower oils) was tested against human normal fetal lung fibroblast MRC-5 cells, and they were not toxic toward these cells at $200.00 \mu\text{g mL}^{-1}$.

Table 4 Cytotoxic activity of investigated *Heracleum* essential oils and cisplatin

Essential oils	IC_{50} ($\mu\text{g mL}^{-1}$)				
	Malignant cells			Normal cells	
	HeLa	LS174	A549	MRC-5	
<i>H. sphondylium</i>	root	5.72±0.11	24.31±0.52	16.23±0.72	>200
	leaf	94.41±1.35	121.46±1.31	102.55±2.41	>200
	flower	39.35±3.00	n.t. ^a	n.t.	n.t.
	fruit	>200	>200	>200	>200
<i>H. sibiricum</i>	root	132.33±0.12	>200	>200	>200
	leaf	155.77±0.22	128.62±5.67	194.94±0.35	>200
	fruit	>200	>200	>200	>200
<i>H. montanum</i>	root	106.24±2.47	138.21±1.58	152.47±1.33	>200
	leaf	80.08±0.59	93.74±1.39	111.43±1.58	>200
	flower	36.34±0.94	n.t.	n.t.	n.t.
	fruit	>200	>200	>200	>200
Cisplatin	0.75±0.05	2.49±0.22	3.11±0.54	14.11±0.74	

IC_{50} values are expressed as the mean ± SD determined from the results of MTT assay in two independent experiments. ^an.t. - not tested.

Conclusions

The present study revealed that the root, leaf and flower essential oils of widespread cow parsnips, *H. sphondylium*, *H. sibiricum* and *H. montanum* were dominated by various monoterpenes, sesquiterpenes and/or phenylpropanoids, while the fruit essential oils were characterized by aliphatic esters. Regarding previously investigated *H. sphondylium* leaf, flower petal and fruit essential oils, we observed some important differences, which confirm that the method of isolation and ecological conditions significantly influence the composition of the essential oils. Considering other tested

Heracleum essential oils, in this work, they were analyzed for the first time.

The tested essential oils showed interesting antimicrobial activity against microorganisms that are the common cause of foodborne diseases, food contamination and/or hospital-acquired infections. In several cases, the oils exhibited similar or even better activity compared to the reference antibiotics. Demonstrated antimicrobial effect of the tested essential oils can explain some of the traditional uses of investigated cow parsnips. Regarding cytotoxic activity, only *H. sphondylium* root oil exhibited pronounced activity against malignant cell lines, and all the tested oils were not toxic against normal cell lines.

Conflict of interest statement

The authors declare no conflict of interest.

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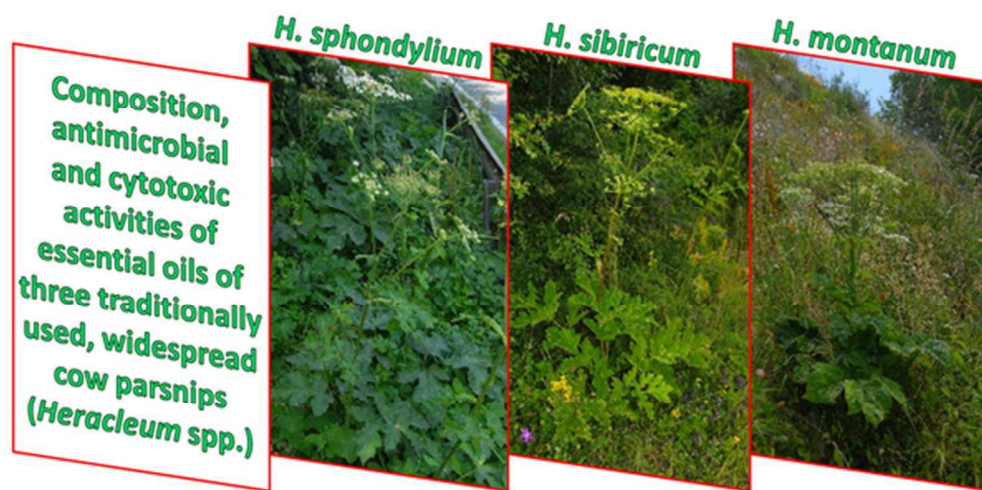
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