Research Article

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The essential oil composition of selected Hemerocallis cultivars and their biological activity

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Abstract: The horticultural cultivars of Hemerocallis (daylily) have been used to treat diseases such as insomnia, inflammation and depression, and also as a vegetable in eastern Asia. Taking into consideration the fact, that the volatile compounds in Hemerocallis cultivars have not been investigated to date, we decided to study the composition of the essential oils (EOs) from the aerial parts of ten varieties collecting in Poland. EOs, obtained by hydrodistillation, were analyzed by GC/MS method that resulted in identification of 23-36 volatile compounds comprising 89.5%–96.3% of the total amount. The essential oils differed in their composition and they can be classified into three groups. The antibacterial and antioxidant activities of EOs were also evaluated. Gram-negative strains were most strongly inhibited by all tested oils. Two model systems have been used for the antioxidant efficacy, 2,2-diphenyl-1-picryl-hydrazyl (DPPH•) and β-carotene bleaching assays. The essential oils with the high presence of oxygenated monoterpenes and monoterpene hydrocarbons showed higher antioxidant activity. The chemical composition of EOs of Hemerocallis cultivars and their biological activity is reported for the first time. Thus, the findings presented here suggest that the aerial parts of Hemerocallis cultivars may be candidates for the development of new phytomedicine.

Keywords: Hemerocallis; essential oils; eucalyptol; antioxidant; antimicrobial

1 Introduction

The essential oil-producing species are extensively arranged among the plant kingdom. The volatile compounds are not only important in plant physiology but also in pharmaceutical, food and cosmetics industries. Numerous studies showed that essential oils possess therapeutic properties and can prevent and cure many diseases [1].

The genus Hemerocallis, belonging to Asphodelaceae family (Hemerocallidoideae subfamily), is mainly of East Asia origin and contains hardy plants surviving from North American Zones [2]. According to the American Daylily Society [3], more than 80 000 Hemerocallis cultivars exist in the world. They have usually been created by interspecific, intraspecific or interploidy cross [4,5]. These perennial plants are cultivated as ornamental species in Europe and America, as well as important ingredients in food and traditional medicine [6-8]. Crude extracts of daylilies have been used in Asia as diuretic, anthelmintic, antiemetic, antispasmodic, sedative and antiphlogistic remedies [9-11]. From the aerial parts and roots of these plants various kinds of constituents including polyphenols [12,13], carotenoids [8], anthraquinones [9], naphthalene glycosides [6], steroidal saponins [16], and lactams [17,18], have been isolated. The pharmacological studies have shown that Hemerocallis species demonstrate the biological activities, such as antioxidant [6,14,19-21], neurological [22], cytoprotection [10], anti-inflammatory [23,24], antidepressant [25,26], and anticancer [27].

To the best of our knowledge, there is only one report on chemical composition of the essential oils of Hemerocallis. Consequently, the purpose of the present research was carried out to investigate the chemical composition, and also antioxidant and antibacterial activities of the essential oils of the aerial parts of ten Hemerocallis cultivars.
Table 1: *Hemerocallis* cultivars used in the experiment for EOs determination. *Year of introduction to cultivation in Botanical Garden in Lublin (Poland).

| Inventory no. | Name                          | Origin                                                                                     | Year* |
|---------------|-------------------------------|-------------------------------------------------------------------------------------------|-------|
| 2427          | *Hemerocallis citrina* Baroni | Botanical Garden of the Adam Mickiewicz University in Poznań, Poland                      | 1967  |
| 75            | *Hemerocallis fulva* (L.) L. (Hemerocallis fulva* (L.) var. kwanso Regal) | Botanical Garden of the Adam Mickiewicz University in Poznań, Poland                      | 1967  |
| 243           | *Hemerocallis* ‘Aten’         | Private collection, Chocznia near/ Andrychów, Poland                                       | 1981  |
| 158/2004      | *Hemerocallis* ‘Bożena’       | Arboretum Wojsławice, Poland                                                              | 2004  |
| 297           | *Hemerocallis* ‘Catherine Woodbury’ | Private collection, Chocznia near/ Andrychów, Poland                                      | 1981  |
| 3/2008        | *Hemerocallis* ‘Chicago Apache’ | Garden Center, Zemborzycka Street, Lublin, Poland                                         | 2008  |
| 156/2004      | *Hemerocallis* ‘Danuta’       | Arboretum Wojsławice, Poland                                                              | 2004  |
| 96/2014       | *Hemerocallis* ‘Jaskółka’     | Arboretum Wojsławice, Poland                                                              | 2014  |
| 396           | *Hemerocallis* ‘Pink Solace’  | Botanical Garden in Powsin, Poland                                                       | 1989  |
| 249           | *Hemerocallis* ‘Rebel Cause’  | Botanical Garden in Powsin, Poland                                                       | 1983  |

2 Experimental

2.1 Plant material and essential oils isolation

Flowers, leaves and stems of ten *Hemerocallis* cultivars, as shown in Table 1, were collected in the Maria Curie-Skłodowska University (UMCS) Botanical Garden in Lublin (Poland), at altitude of 181.2 m a.s.l. (coordinates 51°15’46” N; 22°30’51” E) in August 2017, in their full flowering phase. Taxonomical identification was confirmed by Dr. A. Dąbrowska.

The plants were dried in a drying chamber at 35°C, immediately after the harvest. The essential oils were obtained by 4-h hydrodistillation using the Clevenger apparatus. The ratio of dried material and distilled water was 1:7 (weight/volume). The weight of essential oils was measured after the process and according to the formula described in our previous study [28]. The oil yields are given in Table 2 and 3.

2.2 Chemicals and reagents

2,6-di-tert-butyl-4-methylphenol (BHT), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), β-carotene, linoleic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemical reagents used in the experiment were of analytical grade and were provided by POCH (Gliwice, Poland).

2.3 GC-MS analysis

The composition of essential oils of *Hemerocallis* cultivars was analyzed using GC-MS on a Trace GC Ultra apparatus (Thermo Electron Corporation, Milan, Italy) with FID and the MS DSQ II detector after dilution in diethyl ether (10 μL in 1 mL). More details of chromatographic conditions and quantitation methods can be found in the study of Szewczyk and co-authors [28]. The percentage data shown are mean values of three injections.
2.4 Antioxidant activity

Both assays were performed using 96-well microplates (Nunclon, Nunc, Roskilde, Denmark) and were measured in an Elisa Reader Infinite Pro 200F (Tecan Group Ltd., Männedorf, Switzerland).

2,2-diphenyl-1-picryl-hydrazyl (DPPH•) free radical scavenging activity of the essential oils and BHT was tested using a previously described method [29]. 180 μL of methanolic DPPH• solution (0.07 mg/mL) was mixed with 20 μL of various concentrations of EOs. After shaking and incubation at 28°C for 30 min in the dark, absorbance was measured at 517 nm. To determine EC_{50} values a dose response curves were plotted.

β-carotene bleaching method was carried out according to previously described method [30] and modified by Deba and co-authors [31]. The absorbance was measured at 470 nm.

2.5 Antibacterial assay

Zones of bacterial growth inhibition caused by tested samples were evaluated for the reference microorganisms...
from the American Type Culture Collection (ATCC) including Gram-positive bacteria (Staphylococcus aureus ATCC 25923, Staphylococcus epidermidis ATCC 12228) and Gram-negative bacteria (Escherichia coli ATCC 25992, Pseudomonas aeruginosa ATCC 27853). Clinical strains (Escherichia coli and Pseudomonas aeruginosa isolated from infected urine and wounds, respectively) were obtained from University Hospital No 4, 8 Jaczewskiego Street in Lublin, and stored in the micro banks at the Department of Biochemistry and Biotechnology, Medical University of Lublin, Poland. The strains were maintained at -70°C until the study was performed. Before the experiments, the bacterial strains were passaged onto fresh Mueller-Hinton agar (M-H) (Oxoid, UK) at 37°C for 48 h. Each inoculum was prepared with fresh microbial culture in sterile 0.9% NaCl to match the turbidity of 0.5 McFarland.

The antibacterial activity of samples against bacteria was evaluated by measuring the zones of inhibition in the standard disk diffusion method (Kirby-Bauer Disk Diffusion Susceptibility Test Protocol). Antibacterial disc diffusion assays were carried out on Petri plates with solid medium (M-H agar). Suitable strain culture was separately spread over the agar surface using cotton swab. Next, 10 μL of the undiluted essential oils were brought using sterile disc (disc dispenser BioMaxima, Poland) on Petri plates with agar medium. After 18 h of incubation at 37°C zones of microbial growth produced around the tested samples were measured and recorded as the diameters of inhibition [mm]. All experiments were performed in fivefold.

### 2.6 Statistical analysis

All the results were expressed as means ± standard deviation (SD) of three independent experiments. One-way ANOVA with Tukey’s post hoc test was used for the statistical analysis of significance of differences between means. P values below 0.05 were accepted as statistically significant. Calculations were done in Statistica 10.0 (StatSoft Poland, Cracow, Poland).

Ethical approval: The conducted research is not related to either human or animal use.

### 3 Results and discussion

From ancient time, *Hemerocallis* species have been cultivated in their native regions of Asia where these plants are still an important source of remedies and food. In Chinese and Japanese medicine daylilies have been used to treat ailments such as insomnia, fever, diuretic, inflammation, depression, and anemia [8,23,32]. In Europe, *Hemerocallis* spp. appeared in the late sixteenth century where they were cultivated especially for ornamental purpose [8].

Although phytochemical studies conducted on various organs of *Hemerocallis* have revealed the presence of many kinds of active compounds such as flavonoids [6,14,33,34], antraquinones [9,11], alkaloids [34], triterpenes [11], and caffeoylquinic acid derivatives [14,35], there is only one report about volatile oils in this genus [36].

In the present study, the essential oils (EOs) of ten *Hemerocallis* cultivars were obtained by hydrodistillation from air-dried aerial parts (flowers, leaves and stems). All EOs were collected as a fragrant and pale-yellow liquids. The yield of EOs (expressed in percentage; % v/w relative to dry material weight) was comparable in all samples and ranged from 0.024% (*H. ‘Jaskółka’*) to 0.034% (*H. ‘Pink Solace’*). The chemical composition was analyzed by the GC-MS method, that resulted in identification of 23-36 volatile compounds comprising from 89.5%–96.3% of the total volume in individual oils. All identified compounds in the aerial parts of *Hemerocallis* cultivars oils are given in Table 2 and 3.

The investigated essential oils differed in chemical composition. According to chemical profile they can be classified into three groups. The first group is composed of four EOs, 2 (*H. citrina Baroni*), 7 (*H. ‘Pink Solace’*), 8 (*H. ‘Bożena’*), and 9 (*H. ‘Jaskółka’*) (Table 2). These oils contained mainly oxygenated monoterpenes with 1,8-cineole being the major constituent (73.7-85.5%), followed by α-terpineol, α-terpinyl acetate, and terpinen-4-ol.

EOs classified to the second group [1 (*H. ‘Rebel Cause’*), 6 (*H. ‘Danuta’*), 10 (*H. ‘Aten’*)] and third group [3 (*H. ‘Catherine Woodbuery’*), 4 (*H. fulva*), 5 (*H. ‘Chicago Apache’*)] (Table 3) had a lot of common constituents such as C13 ketones ((E)-β-damascenone, β-ionone, and geranylacetone), C18 ketones (farnesylacetone and hexahydrofarnesylacetone) as well as long chain aliphatic hydrocarbons, both saturated and unsaturated. All these constituents are present in EOs of the third group in significantly higher amounts than in the second group. To the contrary, the second group EOs were characterized by pronounced amounts of very volatile C5 aliphatic aldehydes with the same skeleton, 2- and 3-methylbutanal, 2-methylenebutanal (2-ethylcrolein), and trans-2-methylbut-2-enal (tiglic aldehyde) as well as furfural and furfuryl alcohol. EO 3 contained elemicin (13.0%) and methylheugenol (2.6%).
Table 2: Composition of essentials oils of *Hemerocallis* cultivars aerial parts: 2 – *H. citrina* Baroni; 7 – *H. ‘Pink Solace’*; 8 – *H. ‘Bożena’*; 9 – *H. ‘Jaskółka’*. RI<sub>exp</sub>, experimental retention index; RI<sub>lit</sub>, literature retention index; t, trace – percentage value less than 0.05%; n.i., not identified.

| No. | Constituent                | RI<sub>exp</sub> | RI<sub>lit</sub> | 2   | 7   | 8   | 9   | Class of compound |
|-----|---------------------------|------------------|------------------|-----|-----|-----|-----|-------------------|
|     |                           |                  |                  |     |     |     |     |                   |
| 1   | furfuryl alcohol          | 842              | 844              | 0.1 | -   | -   | -   | O                 |
| 2   | α-thujene                 | 930              | 932              | 0.1 | -   | 0.1 | 0.1 | MTH               |
| 3   | α-pinene                  | 931              | 934              | 1.4 | 0.5 | 1.9 | 1.9 | MTH               |
| 4   | camphene                  | 947              | 950              | t   | t   | 0.1 | t   | MTH               |
| 5   | sabinene                  | 968              | 973              | 0.5 | 0.2 | 0.4 | 0.5 | MTH               |
| 6   | β-pinene                  | 970              | 978              | 0.6 | 0.2 | 0.6 | 0.7 | MTH               |
| 7   | myrcene                   | 983              | 982              | 0.2 | t   | 0.1 | 0.1 | MTH               |
| 8   | p-cymene                  | 915              | 1015             | 0.1 | 0.7 | t   | 1.4 | MTH               |
| 9   | 1,8-cineole               | 1025             | 1024             | **83.4** | **73.7** | **85.5** | **83.7** | MTO           |
| 10  | limonene                  | 1026             | 1025             | t   | 2.2 | t   | t   | MTH               |
| 11  | trans-linalool oxide      | 1060             | 1062             | t   | -   | t   | -   | MTO               |
| 12  | cis-linalool oxide        | 1073             | 1072             | t   | -   | t   | -   | MTO               |
| 13  | linalool                  | 1086             | 1087             | 0.2 | 0.3 | 0.2 | 0.1 | MTO               |
| 14  | trans-pinocarveol         | 1135             | 1137             | 0.1 | 0.2 | 0.1 | 0.1 | MTO               |
| 15  | δ-terpinol               | 1152             | 1155             | 0.1 | 0.2 | -   | 0.1 | MTO               |
| 16  | terpinen-4-ol            | 1162             | 1164             | 0.9 | 1.5 | 0.8 | 0.8 | MTO               |
| 17  | α-terpinol               | 1178             | 1178             | 4.2 | **11.3** | 3.7 | **3.4** | MTO           |
| 18  | carvone                  | 1215             | 1214             | 0.1 | 0.1 | t   | t   | MTO               |
| 19  | geranial                 | 1242             | 1245             | 0.2 | 0.2 | t   | MTO               |
| 20  | α-terpinyl acetate       | 1333             | 1333             | 1.4 | **3.5** | 1.1 | 1.1 | MTO               |
| 21  | n.i. 79, 109, 91,135     | 1336             | -                | 1.8 | 1.0 | t   | 0.3 |                   |
| 22  | β-caryophyllene          | 1416             | 1418             | t   | 0.1 | t   | t   | STH               |
| 23  | geranylacetone           | 1428             | 1430             | t   | -   | -   | STH               |
| 24  | aromadendrene            | 1439             | 1443             | t   | 0.1 | 0.1 | 0.1 | STH               |
| 25  | bicyclogermacrene       | 1492             | 1494             | t   | 0.2 | t   | 0.1 | STH               |
| 26  | spathulenol              | 1568             | 1572             | t   | 0.3 | 0.1 | t   | STO               |
| 27  | caryophyllene oxide      | 1575             | 1578             | t   | t   | t   | t   | STO               |
| 28  | tricosane                | 2300             | 2300             | 0.1 | t   | t   | t   | AH                |
| 29  | pentacosane              | 2500             | 2500             | 0.1 | t   | t   | 0.1 | AH                |
| 30  | heptacosane              | 2700             | 2700             | 0.1 | t   | t   | t   | AH                |
| 31  | Total identified         |                  |                  | 95.7 | 96.3 | 95.0 | 94.6 |                   |
| 32  | Monoterpane hydrocarbons MTH |                |                  | 2.9  | 3.8  | 3.2  | 4.7  |                   |
| 33  | Oxygenated monoterpenes MTO |                |                  | 90.6 | 90.8 | 91.6 | 89.3 |                   |
| 34  | Sesquiterpane hydrocarbons STH |            |                  | -    | 0.4  | 0.1  | 0.2  |                   |
| 35  | Oxygenated sesquiterpenes STO |               |                  | -    | 0.3  | 0.1  | -    |                   |
| 36  | Aliphatic hydrocarbons AH |                  |                  | 0.3  | -    | -    | 0.1  |                   |
| 37  | Other O                  |                  |                  | 0.1  | -    | -    | -    |                   |
| 38  | Oil yield                |                  |                  | 0.034 | 0.034 | 0.027 | 0.024 |                   |
Table 3: Composition of essential oils of *Hemerocallis* cultivars aerial parts: 1 – *H.* 'Rebel Cause'; 3 – *H.* 'Catherine Woodbuery'; 4 – *H.* *fulva*; 5 – *H.* 'Chicago Apache'; 6 – *H.* 'Danuta'; 10 – *H.* 'Aten'. RI<sub>exp</sub>, experimental retention index; RI<sub>lit</sub>, literature retention index; t, trace – percentage value less than 0.05%; n.i., not identified; *tentatively identified according to MS.

| No. | Constituent | RI<sub>exp</sub> | RI<sub>lit</sub> | 1 | 3 | 4 | 5 | 6 | 10 | Class of compound |
|-----|-------------|------------------|-----------------|---|---|---|---|---|----|------------------|
|     |             |                  |                 |   |   |   |   |   |    |                  |
| 1   | 2-methybutanal | 634              | 643             | 3.6 | - | - | - | 3.0 | 2.0 | AO               |
| 2   | 2-methybutanal | 644              | 640             | 2.7 | - | - | - | 3.5 | 2.0 | AO               |
| 3   | 2-methylenbutanal* | 651             | -               | 7.2 | - | - | - | 39.0 | 7.3 | AO               |
| 4   | trans-2-methylbut-2-enal | 724          | 724             | 4.9 | - | - | - | 0.7 | 1.1 | AO               |
| 5   | 2-methylbutanol | 726              | 726             | 4.5 | - | - | - | 1.1 | 1.4 | AO               |
| 6   | hexanal      | 775              | 770             | - | - | - | - | - | 0.3 | AO               |
| 7   | furfural     | 790              | 794             | 0.5 | - | - | t | - | 0.4 | O                |
| 8   | furfuryl alcohol | 846             | 846             | 5.6 | - | - | - | 0.7 | 3.0 | O                |
| 9   | 6-methylhept-5-en-2-one | 966         | 965             | - | 0.3 | - | - | - | 0.5 | AO               |
| 10  | 2-pentylfuran | 979              | 981             | 0.3 | - | - | - | 0.1 | t | O                |
|     | phenylacetaldehyde | 1010            | 1012            | 0.6 | - | - | - | - | - | O                |
|     | p-cymene     | 1012             | 1015            | - | 0.2 | - | - | t | 0.5 | MTH              |
|     | 1,8-cineole  | 1022             | 1024            | - | 4.9 | - | 1.0 | 0.3 | 13.6 | MTO              |
|     | limonene     | 1023             | 1025            | - | 0.3 | - | - | - | 0.2 | MTH              |
|     | linalool     | 1083             | 1086            | 0.9 | 4.2 | - | 2.5 | 5.8 | 2.5 | 0.6 | MTO              |
|     | terpinen-4-ol| 1162             | 1164            | - | - | 0.2 | t | - | 0.5 | 0.1 | MTO              |
|     | α-terpineol  | 1174             | 1176            | 0.2 | 0.5 | 0.7 | 0.6 | t | 0.7 | MTO              |
|     | n.i. 79, 109, 91,135 | 1336         | -               | - | 55.9 | 31.4 | 39.8 | 21.6 | 34.2 | 49.9 |                  |
|     | (E)-β-damascenone | 1361           | 1363            | - | 1.9 | 0.9 | t | 0.1 | t | O                |
|     | methyleugenol | 1371             | 1369            | - | 2.6 | - | - | - | 0.3 | O                |
|     | dihydrodehydro-β-ionone* | 1397          | -               | 0.1 | 1.1 | 0.9 | 0.4 | 0.7 | t | O                |
|     | geranylacetone | 1429             | 1430            | 0.3 | 1.7 | 1.1 | 0.9 | 0.5 | 0.5 | O                |
|     | β-ionone     | 1465             | 1468            | t | 0.8 | 0.4 | 0.4 | t | t | O                |
|     | elemicin     | 1519             | 1522            | 0.1 | 13.0 | - | 0.5 | - | 0.3 | O                |
|     | hexadec-1-ene| 1587             | 1588            | 0.3 | 1.0 | 0.5 | 1.5 | 0.2 | - | AH               |
|     | octadec-1-ene| 1787             | 1788            | 0.3 | 0.8 | 0.6 | 1.3 | 0.3 | - | AH               |
|     | hexahydrofarnesylacetone | 1826        | 1827            | 0.9 | 5.6 | 3.0 | 7.5 | 1.2 | 1.0 | O                |
|     | farnesylacetone | 1890           | 1890            | 0.3 | 1.2 | 1.2 | 1.2 | 0.5 | 0.3 | O                |
|     | isophytol    | 1935             | 1936            | 0.2 | 2.0 | 0.5 | 0.5 | 0.3 | 0.3 | DT               |
|     | palmitic acid | 1943             | 1942            | 0.6 | - | 0.3 | - | - | 0.3 | AO               |
|     | eicos-1-ene  | 1987             | 1988            | 0.1 | t | 0.2 | 0.3 | t | t | AH               |
|     | eicosane     | 2000             | 2000            | t | 0.5 | 0.1 | 0.9 | 0.3 | t | AH               |
|     | phytol       | 2104             | 2105            | 0.5 | 2.2 | 5.0 | 0.7 | 0.3 | 0.4 | DT               |
|     | tricosane    | 2300             | 2300            | 0.5 | 2.9 | 7.7 | 6.9 | 0.9 | 1.0 | AH               |
|     | tetracosane  | 2400             | 2400            | 0.2 | 0.9 | 1.8 | 1.8 | 0.2 | 0.2 | AH               |
In all EOs the same unidentified constituent was found (RI 1336), its mass spectrum is presented in Figure 2. This compound was the main constituent of EOs from the second (34.2-55.9%) and third group (21.6-39.8%) and minor component of the first group EOs (traces to 1.8%).

Only one report on essential oil composition of daylily Hemerocallis flava from China was found. The essential oil obtained by simultaneous distillation-extraction (SDE) contained 3-furfuryl alcohol (47.9%) and 2-furfural (10.4%) as main out of 51 constituents [36]. Considering the fact that composition of volatile oils depends on many factors, such as botanical traits, cultivation and climatic factors, as well as plant materials storage and/or treatments applied during the processing of raw material [37], it is hard to make a reliable comparison with only one published work on the essential oils in Hemerocallis species. Further investigation of composition and bioactivity of EOs in relation to other populations of Hemerocallis cultivars are needed.

Table 3: Composition of essentials oils of Hemerocallis cultivars aerial parts: 1 – H. ‘Rebel Cause’; 3 – H. ‘Catherine Woodbuery’; 4 – H. fulva; 5 – H. ‘Chicago Apache’; 6 – H. ‘Danuta’; 10 – H. ‘Aten’. RI<sub>exp</sub>, experimental retention index; RI<sub>lit</sub>, literature retention index; t, trace – percentage value less than 0.05%; n.i., not identified; *tentatively identified according to MS.

| No. | Constituent               | RI<sub>exp</sub> | RI<sub>lit</sub> | 1  | 3  | 4  | 5  | 6  | 10 | Class of compound |
|-----|--------------------------|-----------------|-----------------|----|----|----|----|----|----|------------------|
|     | pentacosane              | 2500            | 2500            | 1.7| 8.3|16.3|21.5| 1.7| 3.0 | AH               |
|     | hexacosane               | 2600            | 2600            | t  | 0.5| 0.8| 1.1| 0.1| 0.2 | AH               |
|     | heptacosane              | 2700            | 2700            | 0.6| 3.4| 5.5|10.2| 0.8| 1.0 | AH               |
|     | nonacosane               | 2900            | 2900            | t  | 1.1| 1.9| 2.8| 0.2| 0.3 | AH               |
|     | Total identified         | 93.3            | 93.8            | 91.7|89.5|93.8|92.7|    |     |                  |
|     | Monoterpene hydrocarbons |                |                |  - | 0.5| -  | -  | -  | -  | 0.7  | MTH              |
|     | Oxygenated monoterpenes  |                |                |  1.1| 9.8| 3.2| 7.4| 3.3|15.0 | MTO             |
|     | Diterpenes               |                |                |  0.7| 4.2| 5.5| 1.2| 0.6| 0.7  | DT               |
|     | Aliphatic hydrocarbons   |                |                |  3.7|19.4|35.4|48.3| 4.7| 5.7  | AH               |
|     | Oxygenated aliphatic     |                |                | 23.5| 0.3| 0.3| -  | 47.3|14.9 | compounds AO     |
|     | Other O                  |                |                |  8.4|28.2| 7.5|11.0| 3.7| 5.8  |                  |
|     | Oil yield                | 0.033           | 0.032           | 0.029|0.028|0.028|0.033|0.028|      |                  |

Figure 2: GC-MS chromatogram of unidentified constituent (RI 1336).
It is known, as a result of bacterial resistance, that the efficacy of antibiotic therapy decreases, which needs new and safe drug strategies [38,39]. There it was favourable to examine safe therapies based on plants materials, which can prevent bacterial resistance.

Essential oils samples were determined for activity against medically relevant microorganisms, not only reference, but also clinical strains: Gram-negative (E. coli and P. aeruginosa) and Gram- positive (S. aureus and S. epidermidis). Favourable, big zones of inhibition, on solid M-H medium around all tested EOs (1-10) were against Gram-negative strains. Gram-negative strains were most strongly inhibited by samples 8 (H. ‘Bożena’), 6 (H. ‘Danuta’) (33 mm-25 mm), and also 3 (H. ‘Catherine Woodbuery’), and 2 (H. citrina Baroni) (29 mm -18 mm). The high content of 1,8-cineole that is well-known compound with pronounced antibacterial potential [40] may be responsible for strong activity of studied EOs.

Importantly, this significant activity was directed against the Gram-negative strains of both reference and troublesome clinical pathogens derived from patients’ urine or infected wounds (Figure 3).

None of the tested strains, in tested concentration, showed significant activity against Gram-positive bacteria. This means that Hemerocallis oils have a narrow spectrum of action directed against Gram-negative pathogens (Figure 4).

Recently, attention is focused on the protective function of naturally occurring antioxidants [40]. The present research was also undertaken to investigate the antioxidant activities of essential oils from ten Hemerocallis cultivars using two protocols with 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radicals and β-carotene/linoleic acid. The results are summarized in Table 4. The essential oils with the high presence of oxygenated monoterpenes and monoterpane hydrocarbons [2 (H. citrina), 7 (H. ‘Pink Solace’), 8 (H. ‘Bożena’), 9 (H. ‘Jaskółka’, 10 (H. ‘Aten’)] showed higher scavenging ability and they had IC₅₀ values from 4.49±0.28 μg/mL to 19.62±0.11 μg/mL, whereas those of the synthetic antioxidant (BHT) activity was 18.32±0.92 μg/mL. Our results are in agreement with those obtained by Ruberto and Baratta [41], who showed that some monoterpane hydrocarbons and oxygenated compounds like allylic alcohols have an appreciable antioxidant activity. The essential oils of H. fulva (4) and H. ‘Rebel Cause’ (1) were found to be less efficient in the DPPH assay with IC₅₀ values of 60.72±1.10 and 51.59±1.13 μg/mL, respectively.

In the second method that measures the ability to inhibit lipid peroxidation, the essential oils of 8 (H. ‘Bożena’), 4 (H. fulva), 2 (H. citrina), 5 (H. ‘Chicago Apache’), and also 1 (H. ‘Rebel Cause’) had a great activity with IC₅₀ values even twenty one times lower than BHT used as lipophilic antioxidant reference.

From the obtained results, it can be concluded that the essential oil of Hemerocallis ‘Bożena’ (8) and H. citrina (2) had the best antioxidant activity in both performed assays.
Conclusions

The present study reported the composition, antibacterial and antioxidant activity of essential oils from *Hemerocallis* cultivars. The examined essential oils contained mainly oxygenated monoterpenes with 1,8-cineole being the major constituent. In all EOs the same unidentified constituent (RI 1336) was found in a great quantity. The obtained EOs, especially from *H. Bożena*, *H. Danuta*, and also *H. ‘Catherine Woodbuery’*, and *H. citrina* demonstrated strong antimicrobial activity against Gram-negative bacteria. Moreover, our observations suggest that essential oils from the aerial parts of *Hemerocallis* ‘Bożena’ and *H. citrina* possess strong antioxidative activity and they might be a good potential source of preservatives used in cosmetics, food and pharmaceutical industries.

Conflicts of Interest: The authors declare no conflict of interest.

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