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

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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 [12–15], 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	<i>Hemerocallis citrina</i> Baroni	Botanical Garden of the Adam Mickiewicz University in Poznań, Poland 52°25'11" N; 16°52'55" E	1967
75	<i>Hemerocallis fulva</i> (L.) L. (<i>Hemerocallis fulva</i> (L.) L. var. <i>kwanso</i> Regel)	Botanical Garden of the Adam Mickiewicz University in Poznań, Poland 52°25'11" N; 16°52'55" E	1967
243	<i>Hemerocallis</i> 'Aten'	Private collection, Chocznia near/ Andrychów, Poland 49°51'51" N; 19°26'39" E	1981
158/2004	<i>Hemerocallis</i> 'Bożena'	Arboretum Wojśławice, Poland 50°42'40" N; 16°51'18" E	2004
297	<i>Hemerocallis</i> 'Catherine Woodbuery'	Private collection, Chocznia near/ Andrychów, Poland 49°51'51" N; 19°26'39" E	1981
3/2008	<i>Hemerocallis</i> 'Chicago Apache'	Garden Center, Zemborzycka Street, Lublin, Poland	2008
156/2004	<i>Hemerocallis</i> 'Danuta'	Arboretum Wojśławice, Poland 50°42'40" N; 16°51'18" E	2004
96/2014	<i>Hemerocallis</i> 'Jaskółka'	Arboretum Wojśławice, Poland 50°42'40" N; 16°51'18" E	2014
396	<i>Hemerocallis</i> 'Pink Solace'	Botanical Garden in Powsin, Poland 52°06'16" N; 21°05'42" E	1989
249	<i>Hemerocallis</i> 'Rebel Cause'	Botanical Garden in Powsin, Poland 52°06'16" N; 21°05'42" E	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 μ L 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.

Figure 1: Pictures of some *Hemerocallis* cultivars under study: A. *H.* 'Bożena', B. *H.* 'Catherine Woodbuery', C. *H.* 'Danuta', D. *H. fulva*, E. *H.* 'Rebel Cause', F. *H.* 'Jaskółka'.

2.4 Antioxidant activity

Both assays were performed using 96-well microplates (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 μ L of methanolic DPPH[•] solution (0.07 mg/mL) was mixed with 20 μ L 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 EC₅₀ 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 $\pm$ 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], anthraquinones [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-ethylacrolein), and *trans*-2-methylbut-2-enal (tiglic aldehyde) as well as furfural and furfuryl alcohol. EO **3** contained elemicin (13.0%) and methyleugenol (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_{exp}, experimental retention index; RI_{lit}, literature retention index; t, trace – percentage value less than 0.05%; n.i., not identified.

No.	Constituent	RI _{exp}	RI _{lit}	2	7	8	9	Class of compound
Content [%]								
	furfuryl alcohol	842	844	0.1	-	-	-	O
	α-thujene	930	932	0.1	-	0.1	0.1	MTH
	α-pinene	931	934	1.4	0.5	1.9	1.9	MTH
	camphene	947	950	t	t	0.1	t	MTH
	sabinene	968	973	0.5	0.2	0.4	0.5	MTH
	β-pinene	970	978	0.6	0.2	0.6	0.7	MTH
	myrcene	983	982	0.2	t	0.1	0.1	MTH
	p-cymene	915	1015	0.1	0.7	t	1.4	MTH
	1,8-cineole	1025	1024	83.4	73.7	85.5	83.7	MTO
	limonene	1026	1025	t	2.2	t	t	MTH
	trans-linalool oxide	1060	1062	t	-	t	-	MTO
	cis-linalool oxide	1073	1072	t	-	t	-	
	linalool	1086	1087	0.2	0.3	0.2	0.1	MTO
	trans-pinocarveol	1135	1137	0.1	0.2	0.1	0.1	MTO
	δ-terpineol	1152	1155	0.1	0.2	-	0.1	MTO
	terpinen-4-ol	1162	1164	0.9	1.5	0.8	0.8	MTO
	α-terpineol	1178	1178	4.2	11.3	3.7	3.4	MTO
	carvone	1215	1214	0.1	0.1	t	t	MTO
	geranial	1242	1245	0.2	t	0.2	t	MTO
	α-terpinyl acetate	1333	1333	1.4	3.5	1.1	1.1	MTO
	n.i. 79, 109, 91,135	1336	-	1.8	1.0	t	0.3	
	β-caryophyllene	1416	1418	t	0.1	t	t	STH
	geranylacetone	1428	1430	t	-	-	-	STH
	aromadendrene	1439	1443	t	0.1	0.1	0.1	STH
	bicyclogermacrene	1492	1494	t	0.2	t	0.1	STH
	spathulenol	1568	1572	t	0.3	0.1	t	STO
	caryophyllene oxide	1575	1578	t	t	t	t	STO
	tricosane	2300	2300	0.1	t	t	t	AH
	pentacosane	2500	2500	0.1	t	t	0.1	AH
	heptacosane	2700	2700	0.1	t	t	t	AH
	Total identified			95.7	96.3	95.0	94.6	
	Monoterpene hydrocarbons MTH			2.9	3.8	3.2	4.7	
	Oxygenated monoterpenes MTO			90.6	90.8	91.6	89.3	
	Sesquiterpene hydrocarbons STH			-	0.4	0.1	0.2	
	Oxygenated sesquiterpenes STO			-	0.3	0.1	-	
	Aliphatic hydrocarbons AH			0.3	-	-	0.1	
	Other O			0.1	-	-	-	
	Oil yield			0.034	0.034	0.027	0.024	

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_{exp} , experimental retention index; RI_{lit} , literature retention index; t, trace – percentage value less than 0.05%; n.i., not identified; *tentatively identified according to MS.

No.	Constituent	RI_{exp}	RI_{lit}	1	3	4	5	6	10	Class of compound
Content [%]										
	2-methybutanal	634	643	3.6	-	-	-	3.0	2.0	AO
	2-methybutanal	644	640	2.7	-	-	-	3.5	2.0	AO
	2-methylenebutanal*	651	-	7.2	-	-	-	39.0	7.3	AO
	<i>trans</i> -2-methylbut-2-enal	724	724	4.9	-	-	-	0.7	1.1	AO
	2-methylbutanol	726	726	4.5	-	-	-	1.1	1.4	AO
	hexanal	775	770	-	-	-	-	-	0.3	AO
	furfural	790	794	0.5	-	-	-	t	0.4	O
	furfuryl alcohol	846	846	5.6	-	-	-	0.7	3.0	O
	6-methylhept-5-en-2-one	966	965	-	0.3	-	-	-	0.5	AO
	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	t	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

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

No.	Constituent	RI _{exp}	RI _{lit}	1	3	4	5	6	10	Class of compound
Content [%]										
	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 MTH			-	0.5	-	-	-	0.7	
	Oxygenated monoterpenes MTO			1.1	9.8	3.2	7.4	3.3	15.0	
	Diterpenes DT			0.7	4.2	5.5	1.2	0.6	0.7	
	Aliphatic hydrocarbons AH			3.7	19.4	35.4	48.3	4.7	5.7	
	Oxygenated aliphatic compounds AO			23.5	0.3	0.3	-	47.3	14.9	
	Other O			8.4	28.2	7.5	11.0	3.7	5.8	
	Oil yield			0.033	0.032	0.029	0.028	0.033	0.028	

LIL2017_1_01 #1364 RT: 28.19 AV: 1 SB: 15 61.55-61.80 NL: 1.59E8
T: + c Full ms [33.00-550.00]

Figure 2: GC-MS chromatogram of unidentified constituent (RI 1336).

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.

Figure 3: Zones of bacterial growth inhibition of EOs of *Heimerocallis* cultivars tested against Gram-negative and Gram-positive bacterial strains.

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 *Heimerocallis* 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 *Heimerocallis* cultivars using two protocols with 2,2-diphenyl-1-picrylhydrazyl (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 monoterpene hydrocarbons [2 (*H. citrina*), 7 (*H. 'Pink Solace'*), 8 (*H. 'Bożena'*), 9 (*H. 'Jaskółka'*), 10 (*H. 'Aten'*)] showed higher scavenging ability and they had IC_{50} values from 4.49 ± 0.28 $\mu\text{g/mL}$ to 19.62 ± 0.11 $\mu\text{g/mL}$, whereas those of the synthetic antioxidant (BHT) activity was 18.32 ± 0.92 $\mu\text{g/mL}$. Our results are in agreement with those obtained by Ruberto and Baratta [41], who showed that some monoterpene 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 IC_{50} values of 60.72 ± 1.10 and 51.59 ± 1.13 $\mu\text{g/mL}$, 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 IC_{50} 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 *Heimerocallis* 'Bożena' (8) and *H. citrina* (2) had the best antioxidant activity in both performed assays.

Figure 4: Range of antibacterial spectrum of EOs of *Hemerocallis* cultivars tested against Gram-negative and Gram-positive bacterial strains.

Table 4: The antioxidant activity of essential oils of *Hemerocallis* cultivars measured in DPPH[•] and β -carotene/linoleic acid tests. Values are expressed as mean $\pm$ SD (n=3). The different letters (^{a-g}) in the same column indicate a significant difference between oils (p<0.05). IC₅₀ (μ g/mL) is the concentration of the oil at which 50% is inhibited.

Essential oil	IC ₅₀ (μ g/mL) of DPPH	IC ₅₀ (μ g/mL) of β -carotene/linoleic acid
1	51.59 $\pm$ 1.13 ^b	26.54 $\pm$ 0.27 ^c
2	9.78 $\pm$ 0.22 ^f	3.70 $\pm$ 0.07 ^e
3	38.79 $\pm$ 0.73 ^c	34.04 $\pm$ 0.68 ^c
4	60.72 $\pm$ 1.10 ^a	2.87 $\pm$ 0.14 ^e
5	25.86 $\pm$ 0.64 ^d	8.58 $\pm$ 0.21 ^d
6	21.07 $\pm$ 0.23 ^d	53.12 $\pm$ 0.16 ^b
7	19.62 $\pm$ 0.11 ^e	70.44 $\pm$ 0.80 ^a
8	4.49 $\pm$ 0.28 ^g	1.81 $\pm$ 0.22 ^e
9	17.66 $\pm$ 0.43 ^e	12.68 $\pm$ 0.39 ^d
10	19.59 $\pm$ 0.37 ^e	67.42 $\pm$ 0.32 ^a
BHT	18.32 $\pm$ 0.92	38.24 $\pm$ 0.25

4 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.

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