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# Chemical Composition and Antimicrobial Activity of the Essential Oils of Three Closely Related *Hypericum* Species Growing Wild on the Island of Crete, Greece

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Received: 31 March 2020; Accepted: 15 April 2020; Published: 19 April 2020



**Abstract:** The volatile compositions of three closely related *Hypericum* species growing wild on the island of Crete were studied, all belonging to the section Coridium. Hydro-distillation in a modified Clevenger-type apparatus was performed according to the Hellenic Pharmacopoeia in order to obtain the essential oils, which were analyzed by GC-MS. Identification of the compounds was carried out by comparison of MS spectra and retention indices with literature data, as well as by co-chromatography with authentic samples. In total, 123 different compounds were identified and the main compounds were by order of their abundance as follows: *H. empetrifolium*:  $\alpha$ -pinene, germacrene D,  $\beta$ -pinene, E-caryophyllene; *H. amblycalyx*:  $\beta$ -elemene,  $\beta$ -selinene,  $\alpha$ -pinene, E-caryophyllene,  $\alpha$ -selinene; *H. jovis*: trans-calamenene,  $\alpha$ -selinene,  $\beta$ -elemene. The chemical results revealed the differences and similarities (qualitative and quantitative) between the studied oils, supporting the hypothesis that essential oils from *Hypericum* spp. do not serve as chemotaxonomic markers. Moreover, the essential oils were subjected to antimicrobial screening. According to the given results, the essential oils possessed better antifungal and anticandidal activities than antibacterial activities.

**Keywords:** *Hypericum*; section Coridium; essential oils; GC-MS; antifungal; anticandidal

## 1. Introduction

The genus *Hypericum* L. of the family Hypericaceae consists of more than 460 species divided in 36 sections [1] and has a long-term use in traditional medicine. It has been described by Hippocrates [2], Dioscorides [3], and later on in the Medieval era by Nikolaos Myrepsos [4,5]. *H. perforatum* (St. John’s Wort) has been used as an ancient folk cure for diseases including wounds, kidney and lung ailments, and depression, while in modern times, its monograph was included in 2009 in the European Pharmacopoeia [6]. *Hypericum* plants are also used in foodstuff, after the evaluation of the concentration of hypericin and xanthone derivatives (Council of Europe, 2000) [7]. Externally, *Hypericum* is a valuable healing and anti-inflammatory remedy for burns, wounds, sores, bruises, and other skin disorders [6].

The genus produces a plethora of bioactive secondary metabolites, mainly phenols, flavonoids, tannins, xanthenes, phloroglucinols, naphthodianthrones and essential oil (EO) [8]. Apart from the well-established antidepressant activity, many extracts and substances have become subject of intense studies concerning a variety of biological activities [8]. Although volatile constituents have been reported to contribute to these activities, only few *Hypericum* species have been investigated regarding their essential oil content [9–11]. *Hypericum* is considered to be an essential oil-poor genus [9], and so far studies have revealed that its volatile oil demonstrates antimicrobial, antioxidant, anti-angiogenic, gastroprotective, larvicidal, insecticidal activities [10–14].

In continuation to our work on *Hypericum* spp., we investigated the volatile constituents of three closely related taxa [15], *H. empetrifolium* Willd., *H. amblycalyx* Coustur. and Gand, and *H. jovis* Greuter, which were collected across a relatively restricted geographic range, in the island of Crete (Greece). All three belong to the section Coridium; *H. empetrifolium* is considered the ancestor of *H. amblycalyx* and *H. jovis*, the eastern and western derivatives, respectively [15]. The section Coridium comprises six species. It is worth mentioning that all three species under investigation grow on the island of Crete, while the rest are totally absent from Greece. Therefore, we have undertaken the present study, in order to compare the EOs of the Greek taxa belonging to the section Coridium, and based on the results revealed from those closely related *Hypericum* taxa, examine the hypothesis that essential oils from *Hypericum* taxa serve as chemotaxonomic markers. Regarding the chemotaxonomy, many studies describe volatile compounds as chemotaxonomic markers for the genus *Hypericum*, while Crockett [9] mentioned that statistical analysis of essential oils should not be used to infer phylogeny. The present results corroborate with the latter study, since significant differences (qualitative and quantitative) have been observed. In parallel, a second aim of the present study was the investigation of the antimicrobial activity from the obtained EOs, against a variety of fungi and bacteria. This is the first report of the chemical composition of the essential oils obtained from *H. amblycalyx* and *H. jovis*, as well as the first report of the antimicrobial activity of all three *Hypericum* taxa.

## 2. Materials and Methods

### 2.1. Plant Material

Aerial parts from the three *Hypericum* taxa under-investigation were collected from natural populations in the island of Crete (Greece), during the flowering stage. The plants were collected and identified by Dr. Z. Kypriotakis and voucher specimens of each population were deposited in the Personal Herbarium of Dr. Kypriotakis (Heraklion-Crete). In Table 1, collection sites on the island of Crete, dates of collection and essential oil yield are summarized, while the collection sites are also presented in Figure 1.

**Table 1.** Collection sites, dates and essential oil yield of the three under-investigation *Hypericum* spp.

<i>Hypericum</i> spp.	Abbreviations	Collection Sites	Date of Collection	EO Yield (% v/dry Weight)
<i>H. empetrifolium</i> Willd.	HE	Potamies Village, Heraklion, Crete; altitude: 215 m	13/05/2018	0.9
<i>H. amblycalyx</i> Coustur. and Gand	HA	Avdou Village, Heraklion, Crete; altitude: 520 m	13/05/2018	0.4
<i>H. jovis</i> Greuter	HJ	Kameron gorge, Crete; altitude: 680 m	22/06/2017	0.5



**Figure 1.** Collection sites of the three under-investigation *Hypericum* spp.; Map of Crete, Greece; ○ *H. jovis*, □ *H. empetrifolium*, △ *H. amblycalyx*.

## 2.2. Hydro-Distillation of Essential Oils

In order to obtain the EOs, the air-dried plant material from each sample was finely comminuted and separately subjected to hydro-distillation for 3 h. About 45 g from each plant in 500 mL of distilled water were used and the distillation was performed in a modified Clevenger-type apparatus with a water-cooled oil receiver to reduce artifacts produced during hydro-distillation by over-heating, according to the Hellenic Pharmacopoeia [16]. The EO yield (% *v/w*; Table 1) was calculated based on dry weight. GC (Gas Chromatography) grade n-pentane was used for the collection of the EOs, with the addition of anhydrous sodium sulphate to reduce any moisture. The EOs were subsequently analyzed by GC-MS (Gas chromatography-mass spectrometry analysis) and finally stored at  $-20\text{ }^{\circ}\text{C}$ .

## 2.3. Gas Chromatography-Mass Spectrometry Analysis

GC-MS analyses were carried out using a Hewlett-Packard 7820A-5977B MSD system operating in EI mode (70 eV), equipped with a HP-5MS fused silica capillary column (30 m  $\times$  0.25 mm; film thickness 0.25  $\mu\text{m}$ ), and a split-splitless injector. The temperature program was from 60  $^{\circ}\text{C}$  at the time of the injection, raised to 300  $^{\circ}\text{C}$  at a rate of 3  $^{\circ}\text{C}/\text{min}$  and subsequently held at 300  $^{\circ}\text{C}$  for 10 min. Helium was used as a carrier gas at a flow rate of 2.0 mL/min. The injected volume of the samples was 2  $\mu\text{L}$ . The analyses were carried out twice, resulting in reproducible results.

## 2.4. Identification of Compounds

Retention indices for all compounds were determined according to the Van der Dool approach [17], with reference to a homologous series of n-alkanes from  $\text{C}_9$  to  $\text{C}_{25}$ . The identification of the chemical components was based on comparison of both relative retention times and mass spectra with those reported in the NIST/NBS and Wiley libraries, as well as those described by Adams [18] and other literature data. In many cases, the essential oils were subjected to co-chromatography with authentic compounds (Fluka, Sigma). Component relative percentages were calculated based on GC peak areas without using correction factors. Optical rotation values of the Eos  $[\alpha]_{\text{D}}^{20}$  were determined at 20  $^{\circ}\text{C}$  at 589 nm in cyclohexane (cHex), Polarimeter: Perkin Elmer 341.

## 2.5. Microbial Cultures

The Gram-positive bacteria *Bacillus cereus* (human isolate), *Micrococcus flavus* (ATCC 10240), *Staphylococcus aureus* (ATCC 11632), and the Gram-negative bacteria *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Salmonella typhimurium* (ATCC 13311) were used in order to determine the potential antimicrobial activity of EOs of the three *Hypericum* species. For the determination of the antifungal activity, three yeast species *Candida albicans* (clinical isolate), *Candida tropicalis* (ATCC 750) and *Candida krusei* (clinical isolate) and four micromycetes *Aspergillus fumigatus* (ATCC 9197), *Aspergillus niger* (ATCC 6275), *Penicillium funiculosum* (ATCC 36839), *Penicillium verrucosum* var. *cyclopium* (food isolate) were tested for their susceptibility. The bacterial strains were cultured on solid Tryptic Soy agar (TSA), while micromycetes were cultured on solid malt agar (MA) and yeast were sustained on Sabouraud dextrose agar (SDA) medium. The cultures were sub-cultured once a month and stored at 4  $^{\circ}\text{C}$  for further utilization. All the tested microorganisms

are deposited at the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research “Siniša Stankovic”, University of Belgrade.

### 2.6. *In Vitro* Antibacterial and Antifungal Assays

The minimum inhibitory and bactericidal/fungicidal concentrations (MICs, MBCs and MFCs) were determined using 96-well microdilution plates with a flat bottom [19,20]. The oils were dissolved in 5% dimethylsulfoxide (DMSO) solution that contained 0.10% Tween 80 (*v/v*) and added appropriate medium with bacterial/fungal inoculum.

For antibacterial assay, bacterial suspensions were adjusted with sterile saline to a concentration of  $1.0 \times 10^5$  CFU mL<sup>-1</sup>. Afterward, the bacterial inoculum was added to this mixture in order to achieve the appropriate concentrations. The microplates were incubated for 24 h at 37 °C. The MIC/MBC values for bacteria were detected following the addition of 40 µL of p-iodonitrotetrazolium violet (INT) 0.2 mg/mL (Sigma I8377) and incubation at 37 °C for 30 min [21]. The lowest concentration with no visible growth was defined as the MBC, indicating 99.5% killing of the original inoculum. Streptomycin (Sigma-Aldrich S6501, St Louis, MO, USA) and ampicillin (Sigma-Aldrich A9393, Germany) were used as positive controls for bacteria (1 mg mL<sup>-1</sup> in sterile physiological saline). Sterilized distilled water containing 0.1% Tween 80 and 5% DMSO was used as negative control.

The assay for antifungal activity was performed in the following manner: fungal spores/yeast cells were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (*v/v*). The spore suspension was adjusted with sterile saline to a concentration of approximately  $1.0 \times 10^5$  in a final volume of 100 µL per well. MIC/MFC determination was performed by a serial dilution technique using 96-well plates. Microplates were incubated for 24 h at 37 °C for yeasts and for other fungi 72 h at 28 °C. Commercially available antifungal agent—ketoconazole (Zorkapharma, Šabac, Serbia) was used as a positive control. Sterilized distilled water containing 0.1% Tween 80 and 5% DMSO was used as negative control.

## 3. Results

### 3.1. Chemical Analysis

As presented in Table 2, the EOs of the under-investigation *Hypericum* spp., namely *H. empetrifolium* (HE), *H. amblycalyx* (HA) and *H. jovis* (HJ), were complex mixtures; in total 123 individual compounds were identified representing more than 94% of the total EOs, while even the main constituents never exceeded 19% ( $\alpha$ -pinene in HE). More specifically, 94.2%, 94.5% and 95.2% of the total EOs were identified regarding HE, HA and HJ, respectively. The main compounds have been revealed as follows: HE:  $\alpha$ -pinene (19.0%), germacrene D (12.5%),  $\beta$ -pinene (8.7%), E-caryophyllene (5.3%); HA:  $\beta$ -elemene (17.4%),  $\beta$ -selinene (10.5%),  $\alpha$ -pinene (10.2%), E-caryophyllene (8.8%),  $\alpha$ -selinene (8.7%); HJ: *trans*-calamenene (13.5%),  $\alpha$ -selinene (8.3%),  $\beta$ -elemene (7.6%). Chemical structures of the most abundant compounds are presented in Figure 2. The under investigation *Hypericum* spp. yielded EO 0.9%, 0.4% and 0.5 *v/w*% for HE, HA and HJ respectively, which was calculated in dry weight (Table 1).

**Table 2.** Qualitative and quantitative composition (% *v/v*) of EOs.

	Compounds	RI	HE	HA	HJ
1	(3E)-2,3-dimethylhepta-1,3-diene	902	0.2	-	-
2	$\alpha$ -thujene	913	1.0	-	-
3	$\alpha$ -pinene	928	19.0	10.2	-
4	$\alpha$ -fenchene	938	0.5	0.1	-
5	camphene	940	0.3	0.3	-
6	3-methyl-nonane	962	3.5	-	-
7	$\beta$ -pinene	972	8.7	1.5	-
8	6-methyl-5-hepten-2-one	979	-	0.1	0.1
9	myrcene	984	1.8	0.5	-
10	hexenyl acetate	985	-	-	0.1
11	n-decane	994	0.2	-	-
12	$\alpha$ -phellandrene	1001	tr	-	-
13	$\alpha$ -terpinene	1011	0.1	-	-
14	p-cymene	1018	0.8	0.2	-
15	limonene	1022	1.6	1.2	0.1
16	<i>cis</i> -ocimene	1030	0.7	0.2	-
17	<i>trans</i> -ocimene	1040	1.9	0.5	-
18	$\gamma$ -terpinene	1050	0.3	0.1	tr
19	2-methyl-decane	1056	1.8	-	-
20	terpinolene	1080	0.2	0.2	-
21	n-undecane	1094	1.0	2.0	1
22	n-nonanal	1097	-	0.2	0.2
23	endo-fenchol	1108	0.2	0.1	-
24	$\alpha$ -campholenal	1119	0.1	-	-
25	allo-ocimene	1128	0.1	-	-
26	<i>trans</i> -pinocarveol	1129	0.2	0.2	-
27	neo-allo-ocimene	1133	-	0.1	-
28	<i>trans</i> -verbenol	1135	0.2	-	-
29	camphor	1137	tr	-	-
30	camphene hydrate	1141	tr	-	-
31	isoborneol	1152	0.1	-	-
32	pinocarvone	1153	-	0.2	-
33	borneol	1158	0.3	0.3	-
34	3-methyl-undecane	1162	-	-	-
35	terpinen-4-ol	1167	0.1	-	-
36	$\alpha$ -terpineol	1181	0.2	0.5	-
37	myrtenol	1186	0.2	-	-
38	verbenone	1198	0.1	-	-
39	citronellol	1219	tr	-	-
40	linalool acetate	1246	0.4	-	-
41	2-undecanone	1285	0.1	-	-
42	tridecane	1290	0.1	-	-
43	carvacrol	1292	-	0.2	-
44	$\alpha$ -longipinene	1337	2.1	0.3	-
45	$\alpha$ -cubebene	1338	-	-	0.4
46	cyclosativene	1359	-	-	0.2
47	$\alpha$ -ylangene	1360	0.3	-	0.6
48	$\alpha$ -copaene	1363	0.4	0.2	0.6
49	$\beta$ -bourbonene	1370	0.4	-	-
50	geranyl acetate	1373	0.1	-	-
51	$\beta$ -cubebene	1376	0.1	-	-
52	$\beta$ -elemene	1380	0.2	17.4	7.6
53	$\beta$ -longipinene	1384	0.3	-	-
54	$\alpha$ -cedrene	1389	0.1	-	-
55	E-caryophyllene	1406	5.3	8.8	2.9
56	$\beta$ -cedrene	1409	0.9	0.2	0.5
57	$\beta$ -gurjunene	1424	0.5	0.3	0.2

Table 2. Cont.

	Compounds	RI	HE	HA	HJ
58	aromadendrene	1430	0.6	0.7	0.7
59	$\alpha$ -himachalene	1433	0.4	-	-
60	$\alpha$ -humulene	1437	0.6	1.2	2.1
61	E- $\beta$ -farnesene	1445	1.6	-	2.8
62	allo-aromadendrene	1450	0.4	-	-
63	<i>cis</i> -cadina-1(6).4-diene	1453	-	-	0.1
64	ishwarane	1453	2.0	2.3	-
65	4,5 di-epi-aristolochene	1453	-	0.3	-
66	$\gamma$ -muurolene	1461	0.7	3.5	6.2
67	germacrene D	1466	12.5	2.9	3.7
68	$\beta$ -selinene	1473	1.0	10.5	10
69	valencene	1476	-	0.6	-
70	$\alpha$ -selinene	1480	1.0	8.7	8.3
71	$\alpha$ -muurolene	1483	0.8	2.1	5.6
72	$\beta$ -himachalene	1485	-	2.0	-
73	epizonarene	1488	-	-	6.5
74	<i>trans</i> - $\beta$ -guaiene	1492	-	-	0.3
75	E.E- $\alpha$ -farnesene	1492	0.8	-	-
76	$\delta$ -amorphene	1494	-	0.1	1.6
77	$\gamma$ -cadinene	1496	1.5	0.5	4.5
78	7-epi- $\alpha$ -selinene	1499	-	0.4	0.2
79	$\delta$ -cadinene	1506	3.1	-	-
80	<i>trans</i> -calamenene	1508	-	4.4	13.5
81	$\gamma$ -dehydro-ar-himachelene	1514	-	-	1.2
82	cadina-1.4-diene	1514	0.2	-	0.3
83	$\alpha$ -cadinene	1519	0.4	0.1	0.3
84	$\alpha$ -calacorene	1524	0.1	0.2	1.5
85	$\beta$ -calacorene	1544	tr	-	0.3
86	E-nerolidol	1547	0.5	-	-
87	3Z-hexenyl-benzoate	1553	0.1	0.3	0.2
88	himachalene epoxide	1556	-	-	-
89	spathulenol	1558	1.5	-	-
90	caryophyllene oxide	1563	1.8	1.2	1.1
91	cubeban-11-ol	1573	0.1	-	-
92	salvial-4(14)-en-1-one	1574	-	-	0.3
93	viridiflorol	1578	0.2	-	0.4
94	rosifoliol	1581	0.3	-	-
95	humulene epoxide II	1589	tr	-	0.3
96	junenol	1597	0.3	0.2	0.2
97	cubenol 1.10-di-epi	1608	-	0.2	-
98	1-epi-cubenol	1608	0.1	-	-
99	epi- $\alpha$ -cadinol	1609	-	-	0.5
100	caryophylla-4(12).8(13)-dien-5- $\alpha$ -ol	1619	-	0.4	-
101	cubenol	1620	0.5	-	0.3
102	$\tau$ -muurolol	1626	2.3	0.5	1.3
103	Torreyol = $\alpha$ -muurolol	1632	0.5	0.2	0.4
104	$\beta$ -eudesmol	1635	-	-	0.2
105	epoxide-allo-aromadendrene	1637	-	-	-
106	$\alpha$ -cadinol	1640	0.8	-	1.9
107	selin-11-en-4- $\alpha$ -ol	1643	-	3.8	-
108	<i>cis</i> -calamenen-10-ol	1647	-	-	0.2
109	intermedeol	1652	-	0.4	-
110	calamenen-10-ol <i>trans</i>	1656	0.2	-	-
111	14-hydroxy-9-epi-E-caryophyllene	1661	-	-	0.3
112	cadalene	1664	tr	-	0.6
113	germacra-4(15).5.10(14)-trien-1- $\alpha$ -ol	1665	-	0.1	0.2
114	eudesma-4(15).7-dien-1 $\beta$ -ol	1676	-	-	0.9

Table 2. Cont.

	Compounds	RI	HE	HA	HJ
115	amorpha-4.9-dien-2-ol	1703	-	-	0.5
116	nootkatol	1710	-	-	0.2
117	$\gamma$ -costol	1714	-	0.6	0.5
118	cyclocolorone	1743	-	0.2	0.3
119	benzyl-benzoate	1757	0.1	-	-
120	6.10.14-trimethyl-pentadec-2-one	1843	-	-	0.2
121	n-hexadecanol	1877	0.3	-	-
122	nonadecane	1898	0.1	-	-
123	heneicosane	2097	0.1	-	-
Total identification			94.2	94.5	95.2
$[\alpha]_D^{20}$			-14.89	1.13	-1.66
			(c 0.10)	(c 1.06)	(c 0.48)
Grouped Components			HE	HA	HJ
Monoterpene hydrocarbons			36.9	15.1	0.1
Oxygenated monoterpenes			2.1	1.4	0
Sesquiterpene hydrocarbons			38.3	67.9	83.3
Oxygenated sesquiterpenes			9.2	7.6	10.0
Others			7.6	2.5	1.8

Components listed in order of elution from a HP 5MS column. RI: Retention indices calculated against C<sub>9</sub>–C<sub>25</sub> n-alkanes on the HP 5MS column; tr: traces; concentrations below 0.01% are marked as -.

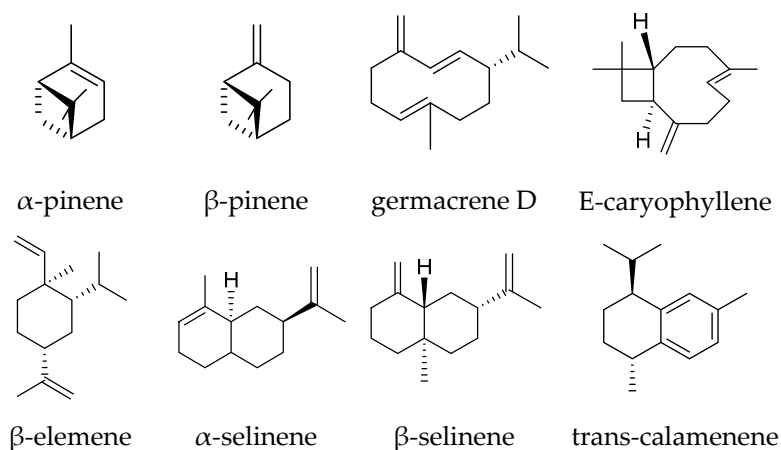


Figure 2. Chemical structures of the most abundant compounds.

### 3.2. Antimicrobial Activity

The antibacterial and antifungal activities of the three *Hypericum* EOs are presented as MIC and MBC/MFC in Tables 3 and 4.

**Table 3.** Antibacterial activity of *Hypericum* EOs mg/mL.

Bacteria	HJ MIC MBC	HE MIC MBC	HA MIC MBC	Streptomycin MIC MBC	Ampicilin MIC MBC
<i>Staphylococcus aureus</i>	0.015 0.030	0.030 0.060	0.015 0.030	0.05 0.10	0.25 0.40
<i>Bacillus cereus</i>	0.0075 0.015	0.015 0.030	0.0025 0.0050	0.10 0.20	0.25 0.40
<i>Listeria monocytogenes</i>	0.020 0.040	-	0.010 0.020	0.20 0.40	0.40 0.50
<i>Pseudomonas aeruginosa</i>	0.0015 0.0030	0.005 0.010	0.0025 0.0050	0.20 0.40	0.75 1.25
<i>Salmonella typhimurium</i>	-	-	-	0.20 0.40	0.40 0.50
<i>Escherichia coli</i>	0.0015 0.0030	0.010 0.015	0.0025 0.0050	0.20 0.40	0.25 0.50

-: the determination of the MIC and MBC values was not possible since expected MIC and MBC values were not in the tested range of concentrations (0.16 mg/mL).

**Table 4.** Antifungal activity of *Hypericum* EOs mg/mL.

Fungi	HJ MIC MFC	HE MIC MFC	HA MIC MFC	Ketoconazole MIC MFC
<i>Aspergillus fumigatus</i>	0.015 0.030	0.030 0.060	0.010 0.020	0.20 0.50
<i>Aspergillus niger</i>	-	-	-	0.20 0.50
<i>Penicillium funiculosum</i>	0.015 0.030	0.030 0.060	0.010 0.020	1.50 2.00
<i>Penicillium verucosum</i>	0.025 0.05	0.030 0.060	0.010 0.020	0.20 0.50
<i>Candida albicans</i>	-	0.005 0.010	-	0.50 1.00
<i>Candida tropicalis</i>	0.010 0.030	0.001 0.002	0.005 0.010	0.30 0.50
<i>Candida krusei</i>	0.010 0.030	0.001 0.002	0.005 0.010	0.50 1.00

-: the determination of the MIC and MFC values was not possible since expected MIC and MFC values were not in the tested range of concentrations (0.16 mg/mL).

#### 4. Discussion

The three under investigation species contained EOs in low amounts, consistent with previous studies reporting *Hypericum* as an EO-poor genus [9]. Despite this fact, many studies have been conducted on *Hypericum* spp. regarding the volatile constituents [9–11]; in many cases, the plant material has been collected in Greece [22–26]. Regarding chemotaxonomy, some controversial hypotheses exist; some researchers accept volatile constituents of the genus *Hypericum* as possible chemotaxonomic markers [27,28] and make use of statistical analysis to divide *Hypericum* spp. into taxonomic sections [24,29,30], while Crockett [9] mentioned the variability of the EOs from this genus concerning a broader distribution, taxonomic rank or phenological state. Our results are in accordance with the latter author, as both differences and similarities (qualitative and quantitative) were observed between the studied oils. The investigated *Hypericum* species are closely related taxa, as all belong



to the section Coridium; HA and HJ are very close to HE, referred as recent derivatives of the latter and they are considered as the eastern and western member of a species pair, respectively [15]. The *Hypericum* taxa of the section Coridium (six species in total) are distributed in disjunct geographic areas, except from the derivative taxa of HE in Crete, while the rest of them are totally absent from Greece [15]. HA and HJ are distributed in restricted areas of Crete only, thus being narrow endemic, and this is the first report of the chemical composition of their EOs, although studies have been conducted concerning their content in phloroglucinols derivatives [31,32]. Regarding HE, its populations occupy a wider geographical range: Crete and other Greek Islands, Central Greece, Albania, W. Turkey, Cyrenaica [15,33]. Intraspecific-variability has been observed, in comparison to some previous studies using plant material collected from Mount Parnitha, Attica [21] and Kos Island, Aegean Sea [26]. In those studies, the plant material has also been subjected to hydro-distillation using a Clevenger apparatus [24,26], allowing the comparison of the chemical composition of the EOs. The main compound of the EO from HE was  $\alpha$ -pinene (19.0%), a result that is consistent with previous studies for the dominant compound. However, both Petrakis et al. [24] and Fanouriou et al. [26] reported similarly higher percentages (approx. 35%), while the latter study also describes a high amount of iswarane (30.5%), a compound totally absent in the analysis of Petrakis et al. [21], which was revealed in small amounts (2.0%) in our study. The same compound was also identified in HA (2.3%). Generally, the most abundant group of compounds was found to be sesquiterpene hydrocarbons; however, some frequently reported constituents to other *Hypericum* spp., such as germacrene-D and  $\beta$ -caryophyllene [9,10], were either absent or revealed only in small amounts regarding HA and HJ. Such differences have also been found in the composition of *H. perforatum* growing wild in Greece [11]. It is noteworthy that HJ was found to be deficient in monoterpene hydrocarbons (0.1%), while this group of compounds was present in HE (36.9%) and HA (15.1%). HA and HJ were also found to be deficient in aliphatic hydrocarbons, like n-nonane, n-decane and n-undecane, which are common compounds in other *Hypericum* spp. [9,10]; e.g., *H. caprifolium* and *H. myrianthum* are characterized by the production of n-nonane and n-undecane, 55% and 20%, respectively [34]. Similar results have been reported for *H. coris*, the type species of the section Coridium [35]. Finally, *H. ericoides*, also belonging to the section Coridium, has been studied regarding its EO; however, these studies report quite diverse results, which could be explained by the different extraction methods that have been applied: isolation of the EO from the hexane extract of the plant [36], hydro-distillation [37], and headspace solid phase microextraction [38].

Regarding their anti-microbial potential, the obtained EOs were further subjected to the evaluation of antimicrobial activity in a variety of bacteria and fungi. The selected bacteria were some clinical and food isolates. More specifically, most food-borne illness is caused by infection of *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella typhimurium* and *Escherichia coli*, while *S. aureus*, *Pseudomonas aeruginosa*, *E. coli* give rise to some hospital-acquired infections. The tested fungi of *Aspergillus* and *Penicillium* genera are plant, animal and human pathogens, food contaminators and producers of potentially carcinogenic mycotoxins. *Penicillium* species can produce nephrotoxic, carcinogenic, teratogenic and immunotoxic metabolites, such as ochratoxin A, and contaminate cereals and cereal-based products [39]. Thus, in European countries, *P. verrucosum*, which produces this mycotoxin, is a major cause of cereal contamination [40]. Therefore, the presence of toxigenic fungi in cereals and food poses a potential risk to human and animal health. Moreover, *Aspergillus fumigatus* can cause invasive aspergillosis in immunocompromised patients [41]. Regarding the selected *Candida* species, they are responsible for the fungal infections called candidiasis. *C. albicans* is the cause for oral and genital infections, while *C. krusei* is the pathogen for fungemia (bloodstream infection).

According to given results of the antibacterial tests, EOs showed good activity against tested strains, but in variable degree. Minimal inhibitory concentrations ranged between 0.0025–0.030 mg/mL, and minimal bactericidal concentration was 0.005–0.060 mg/mL. All EOs showed higher antibacterial activity in comparison to commercial drugs Ampicillin and Streptomycin. The Gram-negative *E. coli* and *P. aeruginosa* were the most sensitive bacteria, since they were inhibited by all tested oils (MIC

values in the range of 0.015–0.010 mg/mL). In contrast, the tested oils did not exhibit antibacterial activity against *S. typhimurium*.

The examined oils were more effective compared to ketoconazole (MIC 0.200–1.500 mg/mL and MFC 0.500–2.000 mg/mL), a commercially available fungicide used as positive control. The most sensitive fungi were the yeasts *C. tropicalis* and *C. krusei* with MIC 0.001–0.010 mg/mL and MFC 0.002–0.030 mg/mL. No remarkable activity was detected against *A. niger* and *C. albicans*.

Generally, among all tested essential oils, antimicrobial potential could be presented as following: HA > HJ > HE. The *Hypericum* EOs possessed better antifungal and anticandidal activities than antibacterial activities, with MIC values ranging between 0.001 mg/mL to 0.030 mg/mL, whereas fungicidal activities were within the 0.002 mg/mL–0.060 mg/mL range. Their inhibition and bactericidal/fungicidal concentrations (<100 mg/mL) indicate a good potency level as antibiotics [42], and support their use in the traditional medicine against diseases caused by different species of microorganisms. This is in accordance with previous reports on the antimicrobial activities of EOs from the genus *Hypericum*, and could be explained due to the presence of  $\alpha$ -pinene,  $\beta$ -pinene and (E)-caryophyllene, which are known for their antimicrobial effects [11].

**Author Contributions:** Conceptualization, M.-E.G. and H.S.; Formal analysis, M.-E.G. and A.D.; Investigation, M.-E.G., A.D. and A.Ć.; Resources, E.A. and Z.K.; Supervision, H.S.; Writing—original draft, M.-E.G.; Writing—review and editing, A.Ć., M.S. and H.S. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research is in part co-financed by Greece and the European Union (European Social Fund-ESF) through the Operational Programme “Human Resources Development, Education and Lifelong Learning” in the context of the project “Strengthening Human Resources Research Potential via Doctorate Research” (MIS-5000432), implemented by the State Scholarships Foundation (IKY).

**Conflicts of Interest:** The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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