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Antibacterial activity and essential oil composition of *Calendula arvensis* L.

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Abstract: Essential oil composition and antibacterial activity of *Calendula arvensis* L. were investigated. The essential oil of aerial part was obtained through hydro-distillation using a Clevenger type apparatus with 0.38 (v/w) yield. The essential oil components were determined by GC-MS analyses. Thirty-six components were identified in the essential oil that represented 91.8 % of the oil. The major components of the essential oil were δ-cadinene (14.8 %), *epi*-cubebol (10.7 %), α-cadinol (8.5 %), cubenol (7.7 %), cubebol (7.2 %), 1-*epi*-cubenol (5.4 %) and ledene (5.1 %). Antibacterial activity of essential oil was observed against *Staphylococcus aureus* ATCC 29213, *Bacillus cereus* ATCC 14579, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 by using a broth microdilution. The essential oil showed weak inhibitory activity against *E. coli* and *B. cereus* at 8 mg/mL. The oil didn't show any antibacterial activity against *S. aureus* and *P. aeruginosa*. These results revealed that the oil was rich in oxygenated sesquiterpenes as well as had a weak antimicrobial activity.

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1. INTRODUCTION

Calendula L. is a member of Calenduleae tribe of Asteraceae family. *Calendula* L. genus is illustrated in Turkey by three species which are *C. arvensis* M.Bieb., *C. officinalis* L., and *C. suffruticosa* Vahl [1]. *Calendula* species are used as an antipyretic and anti-inflammatory remedy in Italian folk medicine [2]. In European folk medicine, *Calendula* species are considered as an immune tonic that aids in prevention of sickness in winter [3]. *C. arvensis* is known by its local name as "Portakal nergisi" in Turkey and is used externally to treat varicose veins, eczema, fungus, warts and wounds [4]. *C. arvensis* has sedative, antibacterial, analgesic, lymphagogue, demulcent, choleretic, vulnerary anti-tumor, mild anodyne anti-inflammatory, antioxidants, anti-parasitic, antiviral, and antiseptic activities [5]. *C. officinalis* has immunostimulant, hepatoprotective, anti-HIV, antitumor, anti-inflammatory, spasmogenic and spasmolytic effects [6]. *C. suffruticosa* has mostly antioxidant and antifungal activity, especially its ethanolic extract [7]. Previously, the essential oils and hydrosol extract from aerial parts of *C. arvensis* L. were examined by using GC-FID and GC/MS. The main compounds were zingiberenol 1 (8.7-29.8%), eremoligenol (4.2-12.5%), β-curcumene (2.1-12.5%),

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zingiberenol 2 (4.6-19.8%) and (*E*, *Z*)-farnesol (3.5-23.4%). The antioxidant activity of essential oil and extract was checked by using DPPH, FRAP and β-carotene methods. The hydrosol extract showed high antioxidant activity. The antifungal activity of the essential oil and hydrosol extract was studied against *Penicillium expansum* and *Aspergillus niger*. The extract showed the highest inhibitory activity against *P. expansum* and *A. niger* [8]. The essential oil of aerial parts of *C. arvensis* L. was studied by GC-FID and GC-MS methods. The essential oil was obtained by hydrodistillation (HD) and microwave distillation (MD). Sesquiterpenes (HD: 30.5 % and MD: 23.4 %) and monoterpene compounds (HD: 26.3 % and MD: 24.3 %) were reported as the major groups. The main compounds were α-selinene (HD: 16.0 % and MD: 0.0 %), α-pinene (HD: 11.9 % and MD: 12.3 %), (*Z*)-α-santalol (HD: 8.2 % and MD: 7.4 %), λ-amorphene (HD: 0.0 % and MD: 8.0 %), (*Z*)-sesquilavandulol (HD: 4.8 % and MD: 0.0 %), 7-*epi*-silphiperfol-5-ene (HD: 2.6 % and MD: 3.7 %), viridiflorene (HD: 2.5 % and MD: 1.7 %) and β-pinene (HD: 1.8 % and MD: 2.4 %). The antimicrobial activities of the essential oils, hexane, ether and methanolic extracts of the *C. arvensis* L. were studied. The essential oil (HD) and methanolic extract had tolerable antibacterial activity against *Staphylococcus aureus* and *Bacillus cereus*. All the extracts were reported to have good antituberculosis activity against *Mycobacterium smegmatis* [9].

The first purpose of this research was to obtain essential oil from aerial parts of *C. arvensis* by using the hydrodistillation method as well as to determine antibacterial activity. And the second purpose was to determine the variation in the volatile oil composition of *C. arvensis* and to show that essential oil differences are related to geographical regions.

2. MATERIAL and METHODS

2.1. Plant Material

Plant material (natural) was collected in flowering periods from Istanbul (İkitelli-Başakşehir) province of Turkey in April 2017. Voucher specimen was deposited in the Herbarium of Marmara University (Voucher no: MARE20229), Turkey.

2.2. Isolation of the Essential Oil

The volatile oil of *C. arvensis* aerial parts (436 g) was obtained by Clevenger apparatus (3 h) with the hydrodistillation method. *C. arvensis* aerial parts produced 0.38% (v/w) essential oil yields. The oil was hold in amber vials under -20°C until analyzed.

2.3. Gas Chromatography/Mass Spectrometry Analysis

The GC-MS analysis was employed with an Agilent 5975C Inert XL EI/CI MSD system in EI mode. Essential oil of aerial part was kept in *n*-hexane was injected (1 µL) in split mode. The temperatures of the injector and MS transfer line were adjusted at 250˚C. Innowax FSC column (60 m x 0.25 mm, 0.25 µm film thickness) and helium as carrier gas (1 mL/min) were utilized in GC/MS analyses. The temperature of oven was adjusted to 60˚C for 10 min. and increased to 220˚C at a rate of 4˚C/min. The temperature kept stable at 220˚C for 10 min. and then increased to 240˚C at a rate of 1˚C/min. Mass spectra were saved at 70 eV with the mass range *m*/*z* 35 to 425. The relative percentage quantities of the separated compounds were calculated from integration of the peaks in MS chromatograms (given in Figure 1). The analysis was realized in triplicate.

2.4. Identification of Essential Oil Components

The determination of volatile oil compounds was realized by comparison with their relative retention indices got by a series of *n*-alkanes (C5 to C30) to the literature [10-23] (given in Table 1 and Figure 1) and with mass spectra comparison to the in-house libraries (Wiley W9N11, NIST11).

2.5. Antibacterial Assay

Antibacterial activity of the essential oil was studied against *Staphylococcus aureus* ATCC 29213, *Bacillus cereus* ATCC 14579, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922. The minimum inhibitory concentration (MIC) values were determined for the oil, on each organism by using microplate dilution method [24]. Stock solution of the oil (16 mg/mL) was prepared with %10 dimethyl sulfoxide (DMSO). Serial dilution of essential oil was done on 96-well microplates with using Mueller Hinton Broth (MHB). Bacteria were standardized in MHB according to McFarland No:0.5. Bacterial cultures were mixed with different concentrations of essential oils on microplates and were incubated 24 h at 37ºC. Minimum inhibitory concentrations (MIC: mg/mL) were detected at the minimum concentration where bacterial growth was not detected. All the experiments were performed in duplicate.

3. RESULTS and DISCUSSION

The essential composition from aerial parts of *C. arvensis* were analyzed by GC-MS. Thirty six compounds were identified comprising 91.8 ± 0.1% of the oil. The main compounds of the oil were δ-cadinene (14.8 ± 0.1%), *epi*-cubebol (10.7 ± 0.0%), α-cadinol (8.5 ± 0.1%), cubenol (7.7 ± 0.0%), cubebol (7.2 ± 0.0%), 1-*epi*-cubenol (5.4 ± 0.0%) and ledene (5.1 ± 0.0%). The essential oil composition is given in Table 1. The antibacterial activity of oil was studied against two Gram-positive (*Staphylococcus aureus*, *Bacillus cereus*) and two Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) by using a broth microdilution assay. The essential oil showed weak inhibitory activity against *E. coli*, and *B. cereus* at 8 mg/mL. The essential oil did not show any antibacterial activity against *S. aureus* and *P. aeruginosa* (given in Table 2). Also, it has been suggested in previous studies that δ-cadinene, *epi*-cubebol, α-cadinol, cubenol, cubebol, 1-*epi*-cubenol and ledene have antibacterial activity [25-32]. Along with these compounds in the essential oils of *C. arvensis* may have contributed to the activity of the oil.

Zingiberenol 1 (8.7-29.8%), eremoligenol (4.2-12.5%), β-curcumene (2.1-12.5%), zingiberenol 2 (4.6-19.8%) and (*E*,*Z*)-farnesol (3.5-23.4%) were detected in higher quantity in the essential oil of *Calendula arvensis* from Algeria [8] while zingiberenol contained at a low amount in the current study. And other main compounds were not detected in the essential oil of current study.

According to a study from Trabzon, *C. arvensis* was reported to contain α-selinene, α-pinene, (*Z*)-sesquilavandulol, 7-*epi*-silphiperfol-5-ene, viridiflorene, and β-pinene as main compounds [9]. But these compounds did not detect in the essential oil of this study except for α-pinene (0.2%). The oil from Trabzon had monoterpene and sesquiterpene as major groups. In the present study, the oil from Istanbul has oxygenated sesquiterpenes as a dominant group and showed a dissimilar chemical profile from the previous study. The difference in the composition of both oils may be correlated with the geographical region, collection time and specific climate conditions. The oil from Trabzon showed moderate antibacterial activity against *S. aureus* and *B. cereus* but in the current study, the oil did not show activity against *S. aureus*. Both oil samples had activity against *B. cereus* at different MIC values. The antibacterial activity differences in previous research and present data could be related to different main compounds of oils.

Another study from Corsica-France had common main compounds with the current study. The main compounds of the oil from France were δ-cadinene (15.1%) and α-cadinol (12.4%) [33]. The similarities were observed between essential oil constituents of Corsica-France and Istanbul-Turkey plants. But there are quantitative dissimilarities in main compounds of both essential oils.

Table 1. Essential oil composition from aerial parts of *Calendula arvensis* L.

No	R.T. min.[1]	RRI[2]	RRI Lit.[3]	Compound	I [4] (%)	II (%)	III (%)	Average[5] (%)	SD[6]	Id.Met.[7]
1	8.67	1020	1032	α-Pinene	0.1	0.2	0.2	0.2	0.1	RI,MS
2	27.332	1461	1466	α-Cubebene	0.3	0.3	0.3	0.3	0.0	RI,MS
3	28.304	1488	1482	α-Longifolene	0.2	0.2	0.2	0.2	0.0	RI,MS
4	28.58	1496	1497	α-Copaene	0.5	0.5	0.5	0.5	0.0	RI,MS
5	29.906	1536	1544	α-Gurjenene	0.1	0.1	0.1	0.1	0.0	RI,MS
6	30.165	1544	1547	β-Cubenene	0.4	0.4	0.4	0.4	0.0	RI,MS
7	32.169	1605	1612	β-Caryophyllene	0.3	0.3	0.3	0.3	0.0	RI,MS
8	34.09	1669	1677	*epi*-Zonarene	1.3	1.2	1.3	1.3	0.1	RI,MS
9	34.448	1680	1687	α-Caryophyllene	0.5	0.5	0.5	0.5	0.0	RI,MS
10	35.195	1705	1707	Ledene	5.1	5.1	5.1	5.1	0.0	RI,MS
11	35.623	1720	1726	Bicyclosesquiphallendrene	0.2	0.2	0.2	0.2	0.0	RI,MS
12	35.793	1726	1726	Germacrene D	1.4	1.4	1.4	1.4	0.0	RI,MS
13	35.989	1733	1740	α-Muurolene	1.2	1.2	1.2	1.2	0.0	RI,MS
14	36.976	1768	1772	δ-Cadinene	14.9	14.8	14.8	14.8	0.1	RI,MS
15	37.261	1778	1783	β-Sesquiphallendrene	0.1	0.1	0.1	0.1	0.0	RI,MS
16	37.707	1793	1799	Cadina-1,4-diene	1.0	1.0	1.0	1.0	0.0	RI,MS
17	39.13	1846	1849	Calamenene	0.4	0.4	0.4	0.4	0.0	RI,MS
18	40.486	1896	1900	*epi*-Cubebol	10.7	10.7	10.7	10.7	0.0	RI,MS
19	41.275	1927	1955	Neophytodiene isomer	3.2	3.2	3.2	3.2	0.0	RI,MS
20	41.703	1943	1953	Palustrol	0.3	0.3	0.3	0.3	0.0	RI,MS
21	41.837	1949	1957	Cubebol	7.2	7.2	7.2	7.2	0.0	RI,MS
22	44.261	2045	2057	Ledol	3.0	3.0	3.0	3.0	0.0	RI,MS
23	44.821	2068	2080	Cubenol	7.7	7.7	7.7	7.7	0.0	RI,MS
24	44.962	2074	2080	1,10-di-*epi*-cubenol	3.1	3.1	3.1	3.1	0.0	RI,MS
25	45.114	2080	2088	1-*epi*-cubenol	5.4	5.4	5.4	5.4	0.0	RI,MS
26	45.333	2089	2092	β-Oplopenene	0.2	0.2	0.2	0.2	0.0	RI,MS
27	45.56	2098	2100	Viridiflorol	3.1	3.2	3.1	3.1	0.0	RI,MS
28	46.074	2120	2096	Sesquisabinene hydrate	2.8	2.9	2.8	2.8	0.1	RI,MS
29	46.305	2130	2131	Hexahydrofarnesyl acetone	0.4	0.4	0.4	0.4	0.0	RI,MS
30	46.462	2137	2135	Spathulenol	0.3	0.3	0.3	0.3	0.0	RI,MS
31	47.629	2187	2191	Zingiberenol	1.8	1.8	1.8	1.8	0.0	RI,MS
32	47.921	2199	2192	τ-Cadinol	2.6	2.6	2.6	2.6	0.0	RI,MS
33	48.169	2210	2219	δ-Cadinol	1.0	1.0	1.0	1.0	0.0	RI,MS
34	48.495	2225	2232	α-Bisabolol	0.4	0.4	0.4	0.4	0.0	RI,MS
35	48.975	2246	2255	α-Cadinol	8.4	8.5	8.5	8.5	0.1	RI,MS
36	59.374	2613	2622	Phytol	1.9	1.9	1.9	1.9	0.0	RI,MS
				Total	91.8	91.9	91.8	91.8	0.1	

[1]R.T: Retention time; [2]RRI: Relative retention time; [3]RRI Lit.: Relative retention time in the literature; [4]The analysis results; [5,6]The average % area of analysis with ± standard deviation (SD) (*n*=3); [7]Identification method.

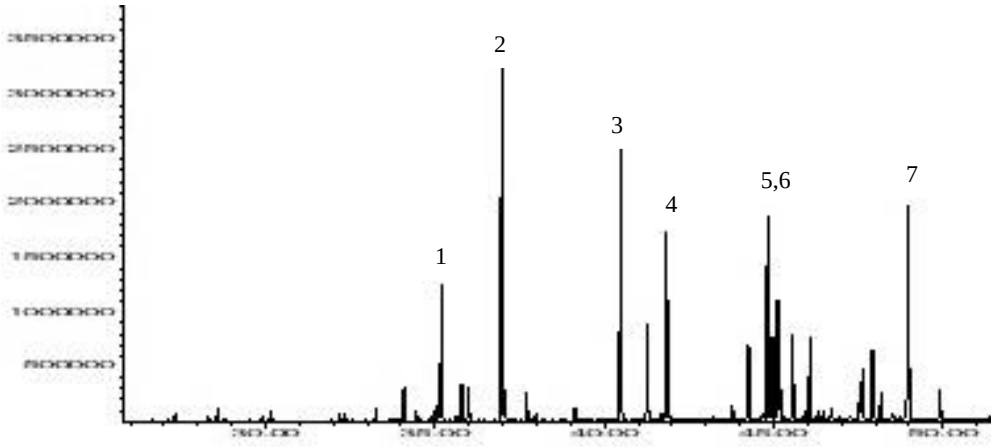

Figure 1. GC-MS Chromatogram of *Calendula arvensis* aerial parts essential oil. (**1:**Ledene; **2:**δ-Cadinene; **3:***epi*-Cubebol; **4:**Cubebol; **5:**Cubenol; **6:**1-*epi*-cubenol; **7:**α-Cadinol)

Table 2. Essential oil composition from aerial parts of *Calendula arvensis* L.

Bacteria	Essential oil (mg/mL)
Staphylococcus aureus	Not sensitive
Bacillus cereus	8 mg/mL
Escherichia coli	8 mg/mL
Pseudomonas aeruginosa	Not sensitive

4. CONCLUSION

The present study indicated that *C. arvensis* oil was rich in oxygenated sesquiterpenes and had antibacterial activity against *E. coli* and *B. cereus*. The oil did not show activity against *S. aureus* and *P. aeruginosa*. This result indicates a higher concentration of the oil is required to inhibit the growth of these bacteria. The essential oil of the current work is different from previous researches as quantitative and qualitative composition. The variations of essential oil ingredients and composition may be correlated with environmental factors such as temperature, humidity, and photoperiod. The quantitative composition of the essential oils can be related to plant age and harvesting time. Further studies on essential oil of *C. arvensis* are required to isolate main compounds of oil that are responsible for its antibacterial activities.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the author(s).

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