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Chemical compositon, antibacterial and antioxidant activities of *Cnidium silaifolium* ssp. *orientale* (Boiss.) Tutin essential oils

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SUMMARY: The chemical compositions of the essential oils (EOs) obtained by hydrodistillation from different parts of *Cnidium silaifolium* ssp. *orientale* (Boiss.) Tutin were analyzed both by GC-FID and GC/MS, simultaneously. One hundred nine compounds representing 90.1% of the total volatiles in the EOs were identified with the main characteristic compounds α-pinene (50.3%) in the root, germacrene D (20.3%) in the fruit, and β-caryophyllene (18.7%) in the aerial parts of *C. silaifolium* ssp. *orientale.* The antimicrobial activity against human pathogenic Gram-negative and Gram-positive bacteria was evaluated by the *in vitro* microdilution method. Antibacterial succeptibility was observed from the root and aerial part EOs against *Staphylococcus aureus* (0.039 and 0.156 mg/mL, respectively); while the fruit EO was most effective against *Bacillus cereus* at 0.07 mg/mL. The antioxidant capacities of the EOs were also evaluated by *in vitro* DPPH• and ABTS•+ scavenging assays, where no significant activity was observed compared to ascorbic acid and Trolox.

KEYWORDS: *Antimicrobial; Antioxidant; Apiaceae; Cnidium silaifolium ssp. orientale*

RESUMEN: ***Composición química, actividades antibacterianas y antioxidantes de* Cnidium silaifolia *ssp.* orientale *(Boiss.) de aceites esenciales de tutin.*** Se analizó por GC-FID y GC-MS la composición química de los aceites esenciales (AE) obtenidos por hidrodestilación de diferentes partes de *Cnidium silaifolium* ssp. *orientale* (Boiss.) tutin. Ciento nueve compuestos, que representan el 90.1% del total de volátiles de los AE, se identificaron. Los compuestos característicos principales fueron α-pineno (50.3%) en la raíz, germacreno D (20.3%) en la fruta y β-cariofileno (18.7%) en las partes aéreas de *C. silaifolium* ssp. *orientale.* La actividad antimicrobiana contra bacterias Gram negativas y Gram positivas patógenas humanas se evaluó mediante el método de microdilución in vitro. La susceptibilidad antibacteriana contra *Staphylococcus aureus*se observó desde la raíz a la parte aérea AEs (0.039 y 0.156 mg/mL, respectivamente), mientras que la fruta EO fue más efectiva contra *Bacillus cereus* a 0.07 mg/mL. La capacidad antioxidante de los AE también se evaluó mediante ensayos de eliminación in vitro de DPPH• y ABTS•+, donde no se observó actividad significativa en comparación con el ácido ascórbico y Trolox.

PALABRAS CLAVE: *Antimicrobiano; Antioxidante; Apiacea; Cnidium silaifolium ssp. orientale*

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1. INTRODUCTION

Cnidium silaifolium ssp. *orientale* (Boiss.) Tutin of Apiaceae is the only representative of the *Cnidium* species in Turkey, and is known as "galyabişotu" (Yüzbaşıoğlu *et al*., 2018). Previous *Cnidium* studies reported acaricidal, antioxidant, antipruritic, anticancer, hepatoprotective, and anti-inflammatory activities (Oh *et al.,* 2002; Jeong *et al.,* 2009; Li *et al.,* 2015; Hong *et al.,* 2017; Lim *et al.,* 2018; Tran *et al.,* 2018; Kim *et al.,* 2018). However, there are only a few previous reports on the essential oil (EO) compositions,where aerial parts were investigated in two different studies (Kapetanos *et al.,* 2008; Polat *et al.,* 2011).

There are very few studies on *C. silaifolium* ssp. *orientale* and the EO compositions of its aerial parts were investigated previously in two different studies (Kapetanos *et al.,* 2008; Polat *et al.,* 2011). So far, the EO composition of *C. silaifolium* ssp. *orientale* fruit and root parts have not been characterized. Here, comparative EO compositions of the aerial parts, fruits, and roots of *C. silaifolium* ssp. *orientale* were reported using gas chromatography with flame ionization detector (GC-FID) and mass spectrometry (GC-MS) systems. Natural products are an important resource for antimicrobial agents, and the essential oils are useful for many applications due to their antimicrobial properties. Antimicrobial essential oils are used as aromas, cosmetics and pharmaceuticals (Arici *et al.,* 2005; Selim, 2011; Başer and Buchbauer 2016). Because of this, the antibacterial and antioxidant activities of the aforementioned EOs were determined by broth microdilution and DPPH - ABTS radical scavenging methods, respectively.

The aim of this present study was to evaluate the *in vitro* antimicrobial and antioxidant activities of the different parts of *C. silaifolium* ssp. *orientale* EOs. To the best of our knowledge, this is the first comparative study on the chemistry of the volatiles and biological activities of the EOs from different parts of *C. silaifolium* ssp. *orientale* from its natural habitat in Turkey. The EOs were extracted by hydrodistillation followed by chromatographic analyses, and *in vitro* biological evaluation using selected human pathogenic strains and DPPH and ABTS radicals as scavenger targets.

2. MATERIALS AND METHODS

2.1. Plant material

The aerial parts, fruits, and roots of *C. silaifolium* ssp. *orientale* were collected in 16 July 2018 in Ermenek, Balkusan Village. The plant was identified by Ömer Çeçen and the voucher specimen (Herbarium No: 28000) was deposited at the Herbarium of the Selcuk University (KNYA), Konya, Turkey.

2.2. Hydrodistillation

Air-dried aerial parts, fruits, and roots (100 g) were crushed and hydrodistilled by distilled water (200 mL) using a Clevenger apparatus (ILDAM LTD., Ankara, Turkey) for eight hours, individually. The obtained EOs were dried by anhydrous sodium sulfate (Sigma, Germany) and kept in suitable conditions at 4 °C until GC and GC/MS analyses as well as biological assays were performed.

2.3. Chromatographic analyses

GC/MS analyses of the essential oils were performed using an Agilent 5975 GC-MSD system, (SEM Ltd., Istanbul, Turkey) where an HP-Innowax FSC column (60 m × 0.25 mm, 0.25 μm film thickness, Agilent, Walt & Jennings Scientific, Delaware, USA) was used with Helium as carrier gas with a 0.8 mL/min flow rate. The GC oven temperature was maintained at 60 °C for 10 min. The oven was set to 220 °C (4°C/min), and kept for 10 min. and then heated to 240 °C (1°C/min). The split ratio was set to 40:1. The injection temperature was 250 °C. The Mass Spectra (MS) were recorded at 70 eV, and the mass ranges were from *m/z* 35 to 450.

FID temperature was set to 300 °C for GC analyses using an Agilent 6890N system (SEM Ltd., Istanbul, Turkey). Simultaneous auto-injection was applied using the same conditions as described in the GC/MS part. Relative percentages (%) of the detected volatile compounds were determined. Identification of these compounds was carried out by comparing their linear retention indexes (LRI) to a series of C_9–C_{20} *n*-alkane standard solutions (Fluka, Buchs, Switzerland). Computer matching was carried out using commercial (Wiley GC/MS Library, MassFinder Software 4.0), and the in-house 'Başer Library of Essential Oil Constituents' library as well as the literature was performed (Demirci *et al.*, 2018).

Table 1. The Chemical composition of *Cnidium silaifolium* ssp. *orientale* essential oils

*RRI	Compounds	**CsH %	**CsF %	**CsR %	***IM
1032	α-Pinene	0.2	0.6	50.3	t_R, MS
1035	α-Thujene	-	tr	-	MS
1076	Camphene	-	-	0.6	t_R, MS
1093	Hexanal	-	-	0.2	t_R, MS
1118	β-Pinene	0.1	0.1	1.8	t_R, MS
1132	Sabinene	0.1	0.2	1.2	t_R, MS
1174	Myrcene	0.1	0.8	4.0	t_R, MS
1176	α-Phellandrene	-	-	0.5	t_R, MS
1183	*p*-Mentha-1,7(8)-diene (=*Pseudolimonene*)	-	-	0.1	MS
1197	Methyl hexanoate	-	-	0.1	t_R, MS
1203	Limonene	0.1	0.5	5.4	t_R, MS
1218	β-Phellandrene	-	0.1	3.6	t_R, MS
1246	(*Z*)-β-Ocimene	0.1	0.2	4.5	MS
1255	γ-Terpinene	-	0.1	1.0	t_R, MS
1266	(*E*)-β-Ocimene	0.1	0.2	0.8	MS
1280	*p*-Cymene	tr	0.1	-	t_R, MS
1290	Terpinolene	-	tr	-	t_R, MS
1294	1,2,4-Trimethyl benzene	-	-	0.2	MS
1296	Octanal	-	-	0.1	t_R, MS
1355	1,2,3-Trimethyl benzene	-	-	0.1	MS
1400	Nonanal	-	0.1	-	t_R, MS
1429	Perillene	-	-	0.1	t_R, MS
1452	α,*p*-Dimethylstyrene	-	-	0.2	MS
1452	1-Octen-3-ol	-	-	0.1	MS
1466	α-Cubebene	0.3	0.1	-	MS
1477	4,8-Epoxyterpinolene	-	-	0.4	MS
1479	δ-Elemene	-	0.1	-	MS
1492	Cyclosativene	0.6	-	-	MS
1497	α-Copaene	7.5	5.3	0.3	MS
1499	α-Campholene aldehyde	-	-	0.6	MS
1519	1,7-Diepi-α-Cedrene (=α-Funebrene)	0.4	0.3	-	MS
1520	3,5-Octadien-2-one	-	-	0.1	MS
1535	β-Bourbonene	0.4	0.2	-	MS
1549	β-Cubebene	1.0	2.0	-	MS
1553	Linalool	tr	tr	0.1	t_R, MS
1571	*trans*-*p*-Menth-2-en-1-ol	-	-	0.1	MS
1577	α-Cedrene	1.1	0.7	-	t_R, MS
1586	Pinocarvone	-	-	0.2	t_R, MS
1597	β-Copaene	0.4	-	-	MS
1600	β-Elemene	2.1	13.7	-	MS
1604	Thymol methyl ether (=*Methyl thymol*)	-	-	0.4	t_R, MS
1611	Terpinen-4-ol	2.0	-	-	t_R, MS
1612	β-Caryophyllene	18.7	11.3	0.1	t_R, MS

*RRI	Compounds	**CsH %	**CsF %	**CsR %	***IM
1614	Carvacrol methyl ether (=*Methyl carvacrol*)	-	-	0.3	t_R, MS
1614	Acora-2,4-diene	-	0.3	-	MS
1648	Myrtenal		-	0.2	MS
1650	γ-Elemene	tr	0.1	-	MS
1670	*trans*-Pinocarveol	-	-	0.7	t_R, MS
1668	(*Z*)-β-Farnesene	1.2	0.4	-	MS
1683	*trans*-Verbenol	-	-	1.2	t_R, MS
1687	α-Humulene	2.5	6.0		t_R, MS
1690	Cryptone	-	-	0.4	MS
1693	β-Acoradiene	1.0	0.6	-	MS
1700	*p*-Mentha-1,8-dien-4-ol (=*Limonen-4-ol*)	-	-	0.1	t_R, MS
1704	γ-Muurolene	-	1.2	-	MS
1704	γ-Curcumene	-	1.6	-	MS
1706	α-Terpineol	2.2	-	-	t_R, MS
1725	Verbenone	-	-	0.3	t_R, MS
1726	Germacrene D	9.2	20.3	-	t_R, MS
1742	β-Selinene	2.2	3.4	-	MS
1744	α-Selinene	1.3	1.3	-	MS
1751	Carvone	-	-	0.1	t_R, MS
1755	Bicyclogermacrene	1.4	0.6	-	MS
1773	δ-Cadinene	5.7	-	-	MS
1783	β-Sesquiphellandrene	-	4.4	-	MS
1786	*ar*-Curcumene	2.9	5.1	0.2	MS
1796	Selina-3,7(11)-diene	-	1.5	-	MS
1797	*p*-Methyl acetophenone	-	-	0.2	MS
1804	Myrtenol	-	-	0.3	MS
1827	(*E,E*)-2,4-Decadienal	-	-	0.1	MS
1845	*trans*-Carveol	0.3	-	0.5	t_R, MS
1849	Cuparene		0.2	-	MS
1854	Germacrene-B	0.5	2.0	-	MS
1864	*p*-Cymen-8-ol	0.2	-	1.4	MS
1868	(*E*)-Geranyl acetone	-	0.1	-	MS
1870	Hexanoic acid	-	-	0.2	t_R, MS
1878	2,5-Dimethoxy-*p*-cymene	-	-	0.1	MS
1900	*epi*-Cubebol	-	0.1	-	MS
1925	2,3,4-Trimethyl benzaldehyde	-	-	0.2	MS
1941	α-Calacorene	0.7	tr	-	MS
1945	1,5-Epoxy-salvial(4)14-ene	0.2	0.1	-	MS
1957	Cubebol	-	0.1	-	MS
1984	γ-Calacorene	-	0.1	-	MS
2001	Isocaryophyllene oxide	-	0.1	-	MS
2008	Caryophyllene oxide	3.4	1.3	-	t_R, MS
2019	2,3,6-Trimethylbenzaldehyde	3.9	-	1.6	t_R, MS
2037	Salvial-4(14)-en-1-one	1.3	0.1	-	MS

*RRI	Compounds	**CsH %	**CsF %	**CsR %	***IM
2050	(*E*)-Nerolidol	0.4	-	-	t_R, MS
2071	Humulene epoxide-II	0.6	0.1	-	MS
2084	Octanoic acid	-	-	tr	t_R, MS
2100	Heneicosane	-	0.1	-	t_R, MS
2109	*cis*-Methyl isoeugenol	-	-	0.2	MS
2131	Hexahydrofarnesyl acetone	0.1	-	-	MS
2144	Spathulenol	5.9	0.8	-	MS
2161	Muurola-4,10(14)-dien-1-ol	tr	0.1	-	MS
2192	Nonanoic acid	tr	0.1	-	t_R, MS
2200	3,4-Dimetil-5-pentyl-5H-furan-2-one	tr	tr		MS
2239	Carvacrol	-	-	0.1	t_R, MS
2242	Methyl palmitate	-	-	0.2	MS
2255	α-Cadinol	-	0.1	-	MS
2262	Ethyl palmitate	-	-	tr	MS
2273	Selin-11-en-4α-ol	-	0.4	-	MS
2278	Torilenol	0.1	0.1	-	MS
2324	Caryophylla-2(12),6(13)-dien-5α-ol (=*Caryophylladienol II*)	0.7	-	-	MS
2369	Eudesma-4(15),7-dien-4β-ol	-	0.2	-	MS
2392	Caryophylla-2(12),6-dien-5β-ol (=*Caryophyllenol II*)	1.0	-	-	MS
2456	Methyl oleate	-	-	0.1	t_R, MS
2509	Methyl linoleate	-	-	0.3	t_R, MS
2655	Benzyl benzoate	5.9	0.4	0.7	t_R, MS
	Monoterpene Hydrocarbons	0.8	2.9	73.7	
	Oxygenated Monoterpenes	4.7	tr	7.3	
	Sesquiterpene Hydrocarbons	61.1	82.8	0.6	
	Oxygenated Sesquiterpenes	13.6	3.6		
	Fatty acid+esters	tr	0.1	0.9	
	Others	9.9	0.7	4.4	
	Total	90.1	90.1	86.9	

*RRI Relative retention indices calculated against *n*-alkanes , % calculated from FID data; tr Trace (< 0.1 %)

**CsH: aerial parts, CsF: fruits. CsR: *C.* roots

***Method of Identification by *t*R: retention times of standards on the HP Innowax column [a]; MS: Mass spectra identified on the basis of computer matching with those of the Wiley and MassFinder libraries and comparison with literature data

2.4. Antimicrobial activity

The antimicrobial activity of the Eos was determined using the broth microdilution assay as described before (Karadag *et al.,* 2019). *Acinetobacter baumanii* ATCC 19606, *Salmonella typhi* ATCC 6539, *Bacillus cereus* ATCC 14579, *Staphylococcus aureus* ATCC 6538, and *Listeria monocytogenes* ATCC 19115 strains were grown in Mueller Hinton Broth (MHB, Merck, Germany). All microorganisms were standardized to 1 × 10^8 CFU/mL using McFarland No: 0.5 in sterile saline (0.85%) using a tubidometer (Biolab, Turkey). Intially, stock solutions of each essential oil and standard antimicrobial agent were prepared in diluted DMSO, serial dilutions were prepared and each strain along with the diluted samples were added to the wells and then allowed to incubate at 37 °C for 24 hours (Karadağ *et al*., 2019).

Helicobacter pylori ATCC 43504 was inoculated for 24 hours in Brucella broth containing 5% (v/v)

horse blood Colombia agar (Oxoid, Germany) and containing 10% (v/v) fetal bovine serum (FBS, Sigma Aldrich, Germany) at 37 °C in an anaerobic incubation system (5% CO_2). After the incubation, 100 μL of 1:10 diluted and density adjusted pathogenic strain were put onto each microplate (Karadağ *et al.,* 2019).

Mycobacterium avium was inoculated in Middlebrook 7H11 agar (Sigma Aldrich) and incubated at 37 °C under aerobic conditions for 4-5 days. Subsequently cultures were vortexed, and after 30 min. diluted bacterial suspensions (10^6 CFU/mL) were added to each well and then allowed to incubate at 37 °C for 5 days. The minimum inhibitory concentrations (MIC) were determined by XTT staining and the results were calculated as a mean of three repetitions. The standard antimicrobial compounds were Chloramphenicol, as shown in Table 3. (Chung *et al.,* 1995; Sun *et al.,* 2007).

2.5. Antioxidant activity

2.5.1. DPPH radical scavenging assay

The antioxidant capacity was determined in terms of hydrogen donating or radical scavenging ability using 2,2-diphenyl-1-picrylhydrazyl (DPPH•) (Sigma, Germany) for its capability to bleach the stable radical (Blois 1958). The reaction mix contained 100 μM DPPH• in methanol and EOs at 1 mg/mL concentration. After 30 min, absorbance was read at 517 nm by using a UV–Vis spectrophotometer (UV-1800, Shimadzu, Japan) at 25±2 °C.

Ascorbic acid (Merck, USA) was used as the reference, methanol was used for negative control. IC_{50} values were determined from a calibration curve, where each experiment was performed in triplicate (Blois 1958; Okur *et al.,* 2018).

2.5.2. ABTS radical scavenging assay

The total antioxidant activity of the EOs was measured using the ABTS· assay (Re *et al.,* 1999). ABTS• was produced by reacting ABTS• (Sigma, Germany) with 2.45 mM potassium persulfate. The mixture was left at room temperature overnight. Then, the colored ABTS radical cation was diluted with ethanol. The absorbances were measured at 734 nm at room temperature. In the assay Trolox (Supelco, Italy) was used as a positive control, as well as the water-soluble α-tocopherol (Sigma-Aldrich, Germany) analogue and blank ethanol was used for negative control. The assays were performed in triplicate.

3. RESULTS AND DISCUSSION

Comparative EO compositions of the aerial parts, fruits, and roots of *C. silaifolium* ssp. *orientale* were were reported using gas chromatography with flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS) systems. The air-dried root, fruit, and aerial part materials were hydro distilled in a Clevenger-type apparatus for 8 hours to yield a light-yellow oil. The *C. silaifolium* ssp. *orientale* aerial part, fruit, and root oil yields were 0.9% (v/w), 1.2% (v/w), 0.7% (v/w), respectively which were consequently analyzed both by GC-FID and GC-MS simultaneously. One hundred-nine compound were identified in *C. silaifolium* ssp. *orientale* EOs obtained from different parts constituting approximately 90% of the total oil. The aerial part and fruit EOs were dominated by sesquiterpene hydrocarbons. Otherwise, the EO of the root consisted of monoterpene hydrocarbons, mainly. These compounds are listed in Table 1 with their relative percentages. The main components were found to be β-caryophyllene (18.7%), germacrene D (9.2%), α-copaene (7.5%), spathulenol (5.9%), benzyl benzoate (5.9%) for aerial part; α-pinene (50%), limonene (5.4%), (*Z*)-β-ocimene (4.5%) and myrcene (4%)

Table 2. Antioxidant activity of *C. silaifolium* ssp. *orientale* essential oils (1 mg/mL concentration)

	IC_{50} ±SD (mg/mL)			
	CsH	**CsF**	**CsR**	**References**
DPPH·	1.32 ± 0.05	1.28 ±0.17	1.45 ± 0.03	0.004± 0.001 (Ascorbic acid)
ABTS·	1.14± 0.06	0.91± 0.07	1.29± 0.07	0.015± 0.008(Trolox)

**CsH: aerial parts, CsF: fruits. CsR: *C.* roots

Table 3. Antimicrobial activity of *C. silaifolium* ssp. *silaifolium* essential oils (MICs in mg/mL)

Bacteria Sample	St	Sa	Lm	Ab	Hp	Bc	Ma
CsH	>10	**0.156**	0.625	>10	>10	0.625	>10
CsF	>10	>10	>10	>10	>10	**0.078**	>10
CsR	>10	**0.039**	0.625	>10	>10	1.25	>10
Chloramphenicol	0.062	0.007	0.001	0.125	0.007	0.031	-

(- control) DMSO. **CsH: aerial parts, CsF: fruits. CsR: *C.* roots *St: Salmonella typhii Sa: Staphyllococcus aureus Lm: Listeria monocytogenes Ab: Acinetobacter baumanii Hp: Helicobacter pylori Bc: Bacillus cereus Ma: Mycobacterium avium*

β-phellandrene (3.6%) for root; germacrene D (20.3%), β-elemene (13.7%), β-caryophyllene (11.3%) and α-humulene (6%) for fruit EO, respectively. In previous studies, the EOs of the aerial parts of *C. silaifolium* ssp. *orientale* from two different localities were analyzed (Kapetanos *et al.,*2008; Polat *et al.,*2011). One of the studies was *C. silaifolium* ssp. *orientale* from Central Balcan, which was investigated for its EO composition and α-pinene was found to be the main component in this study (Kapetanos *et al.,* 2008). In other respects, the EO composition of *C. silaifolium* ssp. *orientale* aerial parts from Turkey was analyzed and kessane was found to be the main component of the EO composition (Polat *et al.,* 2011). The first five main components were found to be completely different compared to these two previous studies. Also, in this present study, compared to previous studies, it was seen that the content in EO was investigated more as a percentage and that more compounds were detected than in other studies (Kapetanos *et al.,*2008; Polat *et al*., 2011). These differences can be considered to be due to the collection of plant materials from different locations and different seasons. It is possible to see from the results that location differences in plants can change the phytochemistry of plants and hence biological activities.

The results for DPPH and ABTS radical scavenging activities are shown in Table 2. According to the DPPH· testing system, free radical scavenging activity IC_{50} value of *C. silaifolium* ssp. *orientale* aerial part, fruit, and root EOs were determined as 1.32, 1.28, and 1.45 mg/mL, respectively. For the ascorbic acid results (0.004 mg/mL) the oils were less effective than those of the ascorbic acid standard. In addition, the ABTS radical scavenging activity was also found at moderate levels (1.14, 0.91, and 1.29 mg/mL) and the results were compared to the Trolox standard (0.015 mg/mL).

Selected Gram (-) and (+) bacteria are given in Table S3 and were subjected to *C. silaifolium* ssp. *orientale* EOs. Among the tested bacteria in this study, *S. aureus* was the most sensitive to the aerial part and root and *B. cereus* was the most sensitive to the fruit EOs. The growth of *S. aureus* was remarkably inhibited by the EO of *C. silaifolium* ssp. *orientale* aerial and root parts. These results show that aerial and root EOs of *C. silaifolium* ssp. *orientale* can be used as a natural antibacterial agent for the prevention of *S. aureus* infections. The results indicated that these volatile oils can be natural and potential antimicrobial agents for wound healing and throat infections.

The biological activities of EOs are often explained by synergistic effects caused by combinations of major components. In previous studies, it was found that a *Zantoxylum* species and *Phlomis cretia* EOs, the major components of EO similar to the root EO used in this study, had a moderate antimicrobial activity. as in this study (Tatsadjieu *et al.,*2003; Aligiannis *et al*., 2004). The essential oils of *C. officinale* leaves and rhizomes, another *Cnidium* species, were studied against some human pathogens and moderate activity was detected (Sim and Shin 2014). In particular, the high antimicrobial activity of *C. officinale* leaf essential oil against *B. cereus* is similar to the *C. silaifolium* ssp. *orientale* leaf essential oil used in this study.

In conclusion, the EOs of the different parts of *C. silaifolium* ssp. *orientale* have moderate antioxidant activity. In addition, aerial part and root EOs showed

significant inhibition against *S. aureus*, while *B. cereus* was susceptible to fruit EO. To the best of our knowledge, this is the first comparabe report on the volatiles and *in vitro* biological activities of *C. silaifolium* ssp. *orientale* aerial part, root, and fruit EOs.

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