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Chemical Composition and Antimicrobial Activity of the Essential Oil of *Chrysanthemum indicum* Against Oral Bacteria

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The chemical components of the essential oil obtained from *Chrysanthemum indicum* L. were analyzed by GC-MS. Seventy-three compounds accounting for 96.65% of the extracted essential oil were identified. The main compounds in the oil were α-pinene (4.4%), 1,8-cineole (10.4%), α-thujone (6.05%), camphor (10.12%), terpinen-4-ol (3.4%), bornyl acetate (6.1%), borneol (3.6%), *cis*-chrysanthenol (3.4%), β-caryophyllene (5.1%), germacrene D (10.6%), and α-cadinol (3.0%). The essential oil of *C. indicum* exhibited stronger antibacterial activity against all oral bacteria tested (MICs, 0.1 to 1.6 mg/ml; MBCs, 0.2 to 3.2 mg/ml) than their major compounds. Furthermore, the MICs/MBCs were reduced to one half ~ one sixteenth as a result of the combinations included the essential oil with ampicillin or gentamicin for all oral bacteria. A strong bactericidal effect was exerted in drug combinations. The *in vitro* data suggest that the essential oil of *C. indicum* with other antibiotics may be microbiologically beneficial and synergistic.

Key Words: *Chrysanthemum indicum*, Essential oil, Antibacterial activity, Oral bacteria

INTRODUCTION

Chrysanthemum indicum L. (Compositae), spreading widely in Korea is a well-known herb and medicinal plant with small yellow flowers (1, 2). *C. indicum* has been used in mixed spices, as a food additives for masking flavors, and used in teas and alcoholic beverages (combined with the flowers) in Korea from the ancient times (1~3). The *C. indicum* has a long history using as an Oriental traditional medicine for the treatment of several infectious diseases such as pneumonia, colitis, stomatitis, cancer, fever, and sore and used to treat vertigo, pertussis, and hypertensive symptoms (2, 4~7). Its flowers are also commonly used as tea to treat some eye diseases (8). Furthermore, recently its extracts has been reported to have central and peripheral analgesic properties, lowering blood pressure as well as anti-inflammatory activities, also exhibited inhibitory activity against bacteria and viruses (4, 5, 7, 9). Several chemical compounds isolated from *C. indicum* flowers were also found to exhibit inhibitory activity against nitric oxide (NO) in lipopolysaccharide-activated macrophages and rat lens aldose reductase (5, 10).

In the present study, the chemical compositions of essential oil from *C. indicum* were identified by GC-MS method, and their antibacterial properties against oral bacteria were evaluated using broth microdilution method and the checkerboard method to obtain a fractional inhibitory concentration (FIC) index.

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MATERIALS AND METHODS

Plant material and extraction of the essential oil

The aerial parts of *C. indicum* were collected in September 2003 from the area of Mt. Mireuk in Korea. The identity was confirmed by Dr. Bong-Seop Kil, College of Natural

Science, Wonkwang University. A voucher specimen (DJ-03-C25) was deposited at the herbarium of the College of Natural Science, Wonkwang University. The aerial parts of *C. indicum* (1 kg) were air dried and then distilled for 3 h, using a modified Clevenger type apparatus in order to obtain the essential oil. Anhydrous sodium sulphate was used to absorb the little water that the essential oil contained. The essential oil was stored in a deep freezer (-70℃) to minimize the loss of volatile compounds.

Analysis of the chemical composition of the essential oil

The oil was analyzed by GC and GC-MS. GC analysis was performed on a Hewlett Packard (HP) model 5890 series II gas chromatograph, with a flame ionization detector (FID), a split ratio of 1:35 using fused silica capillary column, Supelcowax 10 (60 m × 0.32 mm. i.d., 0.25 μm film thickness). The temperature of the column was programmed from 50℃ to 230℃ at 2℃/min, then kept constant at 230℃ for 30 min for the Supelcowax 10 column. The injector and detector temperatures for both analyses were 250℃, respectively. The carrier gas was nitrogen, at a flow rate of 1.86 ml/min for the Supelcowax 10 column. Peak areas were measured by electronic integration. The relative amounts of the individual components were based on the peak areas. The GC-MS was carried out on an HP model 5970 mass spectrometer operating in the EI mode at 70 eV, combined with the GC described above, fitted with an innowax column (60 m × 0.25 mm, i.d., 0.25 μm film thickness) and SPB-1 column (30 m × 0.32 mm, i,d., 0.25 μm film thickness). The temperature of the column was programmed from 40℃ to 230℃ at 2℃/min. The injector and ion source temperatures were the same as above. The carrier gas was helium at a flow rate of 1.25 ml/min for both analyses. The identification of the chemical constituents was based on comparisons of their relative retention times and mass spectra with those obtained from authentic sample and/or the NIST/NBS and Wiley libraries spectra.

Bacterial strains

Antimicrobial activities of the essential oil and some of its major compounds against some oral bacteria and a few reference strains were determined by the broth dilution method. The oral bacterial strains used in this study were: facultative anaerobic bacteria [*Streptococcus mutans* ATCC 25175, *Streptococcus sanguinis* ATCC 10556, *Streptococcus sobrinus* ATCC 27607, *Streptococcus ratti* KCTC (Korean collection for type cultures) 3294, *Streptococcus criceti* KCTC 3292, *Streptococcus anginosus* ATCC 31412, and *Streptococcus gordonii* ATCC 10558], microaerophilic bacteria (*Actinobacillus actinomycetemcomitans* ATCC 43717), and obligate anaerobic bacteria (*Fusobacterium nucleatum* ATCC 10953, *Prevotella intermedia* ATCC 25611, and *Porphylomonas gingivalis* ATCC 33277). The reference strains as facultative anaerobic bacteria used in this study were: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, and *Streptococcus pyogenes* ATCC 21059. Brain-heart infusion broth supplemented with 1% yeast extract (Difco Laboratories, Detroit, MI, USA) was used for all bacterial strains except *P. intermedia* and *P. gingivalis*. For *P. intermedia* and *P. gingivalis*, brain-heart infusion broth containing hemin 5 μg/ml (Sigma Chemical Co., St. Louis, MO, USA) and menadione 1 μg/ml (Sigma) was used.

Minimum inhibitory concentrations/minimum bactericidal concentrations assay

The minimum inhibitory concentrations (MICs) were determined for the essential oil and some of its major compounds by the broth dilution method (11, 12), and it was carried out in triplicate. The antibacterial activities were examined after incubation at 37℃ for 18 h (facultative anaerobic bacteria), for 24 h (microaerophilic bacteria), and for 1~2 days (obligate anaerobic bacteria) under anaerobic conditions. MICs were determined as the lowest concentrations of test samples that resulted in a complete inhibition of visible growth in the broth. Following anaerobic incubation of MICs plates, the minimum bactericidal concentrations (MBCs) were determined on the basis of the lowest concentration of the essential oil that kills 99.9% of the test bacteria by plating out onto each appropriate agar plate. Ampicillin and gentamicin were used as standard

Table 1. Constituents of the essential oil of *Chrysanthemum indicum*

Compounds	RI[a]	RI[b]	*C. indicum* Peak Area (%)[c]
Monoterpene hydrocarbons			**(14.88)**
Tricyclene	1006	919	0.16
α-Pinene	1012	930	4.40
α-Thujene	1029	924	0.08
Camphene	1071	943	2.14
β-Pinene	1111	966	0.94
Sabinene	1124	965	1.40
Myrcene	1167	984	0.91
α-Terpinene	1184	1007	0.43
Limonene	1196	1021	0.31
α-Phellandren	1212	994	1.40
cis-β-Ocimene	1240	1029	1.10
γ-Terpinene	1247	1050	0.74
trans-β-Ocimene	1257	1035	0.03
p-Cymene	1273	1011	0.83
Terpinolene	1283	1078	0.01
Oxygenated monoterpenes			**(52.14)**
1,8-Cineole	1215	1023	10.40
α-Terpinolene	1286	1078	0.23
cis-3-Hexen-1-ol	1386	836	0.05
α-Thujone	1423	1080	6.05
β-Thujone	1440	1091	0.32
trans-Sabinene hydrate	1465	1052	0.41
α-Terpinyl acetate	1470	1329	1.63
Chrysanthenone	1508	1096	2.30
Camphor	1517	1124	10.12
Linalool	1546	1106	0.37
Pinocarvone	1562	1134	0.87
cis-Chrysanthenyl acetate	1570	1243	0.40
Bornyl acetate	1578	1265	6.10
Terpinen-4-ol	1601	1158	3.40
Umbellulone	1639	1165	0.70
trans-Chrysanthenyl acetate	1675	1186	0.30
trans-Piperitol	1676	1205	t
α-Terpineol	1701	1169	0.70
Borneol	1703	1150	3.60
Piperitone	1721	1220	0.13
Carvone	1732	1210	0.14
cis-Chrysanthenol	1754	1150	3.40
Myrtenol	1793	1177	0.01
trans-Carveol	1832	1181	0.20
p-Cymen-8-ol	1835	1165	0.21
cis-Carveol	1862	1196	0.10
Sesquiterpene hydrocarbons			**(22.91)**
α-Copaene	1493	1370	0.30
α-Gurjunene	1525	1389	0.10
Berkheyaradulen	1531	1377	0.16
β-Elemene	1589	1381	1.20
β-Caryophyllene	1594	1418	5.10
α-Humulene	1665	1440	0.50
trans-β-Farnesene	1668	1454	0.85
α-Muurolene	1686	1464	0.20
γ-Cadinene	1690	1497	0.20
Germacrene D	1705	1465	10.6
α-Zingiberene	1715	1490	1.30
β-Selinene	1717	1471	0.21
cis,trans-α-Farnesene	1726	1447	0.30
δ-Cadinene	1762	1505	1.50
β-Sesquiphellandrene	1770	1515	0.18
ar-Curcumene	1773	1472	0.21
Oxygenated sesquiterpenes			**(5.97)**
Caryophyllene oxide	1979	1556	0.78
trans-Nerolidol	2043	1555	0.09
Globulol	2058	1568	0.10
Guaiol	2105	1576	0.20
Spathulenol	2125	1551	0.40
Eugenol	2146	1323	0.11
α-Cedrol	2150	1575	0.06
α-Cadinol	2173	1628	3.00
Torreyol	2179	1619	0.21
T-Muurolol	2234	1615	1.00
cis,trans-Farnesol	2298	1695	0.02

Table 1. Continued

Compounds	RI[a]	RI[b]	*C. indicum* Peak Area (%)[c]
Others			**(0.75)**
1,2,4-Trimethylbenzene	1236	979	0.07
n-Hexanol	1355	851	0.02
1-Octen-3-ol	1453	969	0.04
Tricosane	2300	2300	0.50
Tetracosane	2400	2400	0.12
Total identified			**(96.65%)**

[a] Retention index on a polar Supelcowax 10 column.
[b] Retention index on an apolar SPB-1 column.
[c] Peak area percentage is based on a polar Supelcowax 10 column, and values represent average of three determinations.
t; Trace (<0.05%)

antibiotics in order to compare the sensitivity with the essential oil and some of its major compounds against test bacteria.

Checker board dilution test

The antibacterial effects of a combination of the essential oil, which evidenced the most profound antimicrobial activity, and antibiotics were evaluated via the checkerboard test, as described previously (12). The antimicrobial combinations assayed herein included the essential oil with ampicillin or gentamicin. The FIC index (FICI) is the sum of the FICs of each of the drugs, which were defined as the MICs of each drug when used in combination divided by the MICs of each drug when used alone. The interaction was defined as synergistic in cases in which the (FICI) was less than or equal to 0.5, additive in cases in which the (FICI) was greater than 0.5 and less than or equal to 1.0, indifferent when the (FICI) was greater than 1.0 and less than or equal to 2.0, and antagonistic in cases in which the (FICI) was greater than 2.0 (12, 13).

RESULTS

Chemical composition of the essential oil

A light golden yellow essential oil of *C. indicum* was obtained with a yield of 0.5% dry weight. Results of the GC and GC-MS analyses for the essential oil are shown in Table 1, where the compounds were listed in order of their elution from the Supelcowax 10 column. Seventy-three compounds accounting for 96.65% of the extracted essential oil were identified. Fifteen monoterpene hydrocarbons (14.88%), twenty-six oxygenated monoterpenes (52.14%), sixteen sesquiterpene hydrocarbons (22.91%), eleven oxygenated sesquiterpenes (5.97%), and other compounds (0.75%) were identified in the essential oil. The main compounds in the oil were α-pinene (4.40%), 1,8-cineole (10.40%), α-thujone (6.05%), camphor (10.12%), terpinen-4-ol (3.40%), bornyl acetate (6.10%), borneol (3.60%), *cis*-chrysanthenol (3.40%), β-caryophyllene (5.10%), germacrene D (10.60%), and α-cadinol (3.00%).

Antibacterial activities of the essential oil

Antibacterial activities of the essential oil of *C. indicum* or some of its major compounds against some oral bacteria and reference strains were determined by the MICs through the broth dilution method carried out in triplicate (11). The results of the antibacterial activity (Table 2) showed that the essential oil exhibited moderate activities against most of tested streptococci species (MICs, 0.2 to 0.8 mg/ml; MBCs, 0.4 to 1.6 mg/ml), expect *S. ratti*, and also the essential oil showed the strong antimicrobial activity against obligate anaerobic bacteria: *F. nucleatum*, *P. intermedia*, and *P. gingivalis* (MICs, 0.1 to 0.2 mg/ml; MBCs, 0.2 to 0.8 mg/ml), while *E. coli* and *S. aureus* appeared to be less sensitive (MICs, 0.8 to 12.8 mg/ml; MBCs, 3.2 to >12.8 mg/ml). The monoterpene hydrocarbons α-pinene, oxygenated monoterpenes camphor, 1,8-cineole, terpinon-4-ol, and borneol and sesquiterpene hydrocarbon β-caryophyllene showed moderate antimicrobial activity against all bacteria tested (MICs, 0.4 to 12.8 < mg/ml; MBCs, 0.8 to 12.8 < mg/ml). The tested components of the essential oil showed less antimicrobial activity than the essential oil.

In combination with the essential oil, the MICs/MBCs for ampicillin were reduced by ≥8-fold in *S. mutans*, *S. sanguinis*, *S. ratti*, *S. anginosus*, *S. gordonii*, *F. nucleatum*, *P. intermedia*, and *P. gingivalis*, suggesting a synergistic effect as defined by a FICI of ≤ 0.5, except *S. sobrinus*, *S.*

Table 2. The essential oil and its major components of *C. indicum* for some oral bacteria with a few reference strains

Strains	Essentail oil	α-pinene	Camphor	1,8-Cineole	Terpinen-4-ol	Borneol	β-Caryophyllene	Ampicillin	Gentamicin
Escherichia coli ATCC 25922	12.8/12.8<	12.8/12.8<	12.8/12.8<	6.4/6.4	1.6/3.2	0.8/0.8	12.8/12.8<	128/256 × 10^{-3}	4/16 × 10^{-3}
Staphylococcus aureus ATCC 29213	0.8/3.2	6.4/12.8	12.8/12.8<	12.8/12.8	1.6/1.6	1.6/3.2	12.8/12.8<	8/16 × 10^{-3}	1/4 × 10^{-3}
Staphylococcus epidermidis ATCC 12228	0.4/1.6	12.8/12.8<	12.8/12.8<	1.6/3.2	1.6/3.2	1.6/1.6	12.8/12.8<	32/64 × 10^{-3}	2/2 × 10^{-3}
Streptococcus pyogenes ATCC 21059	0.4/1.6	12.8/12.8	12.8/12.8<	12.8/12.8	0.8/1.6	0.4/0.8	12.8/12.8	4/8 × 10^{-3}	8/16 × 10^{-3}
Streptococcus mutans ATCC 25175	0.4/0.8	6.4/12.8	12.8/12.8	12.8/12.8	1.6/3.2	0.8/1.6	3.2/3.2	2/4 × 10^{-3}	4/8 × 10^{-3}
Streptococcus sanguinis ATCC 10556	0.4/0.8	6.4/12.8	6.4/12.8	12.8/12.8	1.6/3.2	1.6/3.2	1.6/3.2	8/32 × 10^{-3}	8/16 × 10^{-3}
Streptococcus sobrinus ATCC 27607	0.4/0.8	12.8/12.8	6.4/12.8	12.8/12.8	1.6/3.2	0.2/0.8	6.4/12.8	2/4 × 10^{-3}	4/8 × 10^{-3}
Streptococcus ratti [a]KCTC 3294	1.6/3.2	12.8/12.8<	12.8/12.8<	12.8/12.8	3.2/3.2	1.6/6.4	12.8/12.8<	4/8 × 10^{-3}	8/8 × 10^{-3}
Streptococcus criceti KCTC 3292	0.8/1.6	12.8/12.8<	12.8/12.8	12.8/12.8	3.2/3.2	3.2/6.4	12.8/12.8<	2/4 × 10^{-3}	4/8 × 10^{-3}
Streptococcus anginosus ATCC 31412	0.2/0.8	6.4/12.8	6.4/12.8	6.4/6.4	1.6/3.2	0.4/0.8	12.8/12.8<	4/8 × 10^{-3}	8/16 × 10^{-3}
Streptococcus gordonii ATCC 10558	0.2/0.4	3.2/6.4	6.4/12.8	3.2/6.4	0.1/0.2	0.4/0.8	1.6/3.2	1/2 × 10^{-3}	2/4 × 10^{-3}
Actinobacillus actinomycetemcomitans ATCC 43717	0.2/0.4	6.4/12.8	6.4/12.8	6.4/12.8	1.6/3.2	0.8/1.6	0.8/1.6	64/128 × 10^{-3}	1/2 × 10^{-3}
Fusobacterium nucleatum ATCC 10953	0.2/0.4	3.2/3.2	6.4/12.8	3.2/6.4	0.1/0.4	0.1/0.2	6.4/12.8	0.125/0.25 × 10^{-3}	32/32 × 10^{-3}
Prevotella intermedia ATCC 25611	0.2/0.8	0.8/0.8	3.2/3.2	3.2/6.4	0.2/0.4	0.2/0.4	0.8/1.6	8/32 × 10^{-3}	1/1 × 10^{-3}
Porphylomonas gingivalis ATCC 33277	0.1/0.2	0.8/1.6	3.2/6.4	6.4/6.4	0.1/0.4	0.4/0.8	0.4/0.8	1/1 × 10^{-3}	256/512 × 10^{-3}

Data represent as MIC/MBC (mg/ml)

Table 3. Checkerboard assay of the essential oil of *C. indicum* and ampicillin against some oral bacteria with a few reference strains

Strains	Agent	MIC/MBC[a]		FIC[c]	FICI[c]	Outcome
		Alone	Combination[b]			
Escherichia coli ATCC 25922	Essential oil	12.8/12.8<	6.4/6.4	0.5	0.75	Additive
	Ampicillin	128/256	32/128	0.25		
Staphylococcus aureus ATCC 29213	Essential oil	0.8/3.2	0.4/0.4	0.5	0.75	Additive
	Ampicillin	8/16	2/4	0.25		
Staphylococcus epidermidis ATCC 12228	Essential oil	0.4/1.6	0.2/0.4	0.5	1	Additive
	Ampicillin	32/64	16/32	0.5		
Streptococcus pyogenes ATCC 21059	Essential oil	0.4/1.6	0.2/0.4	0.5	1	Additive
	Ampicillin	4/8	2/2	0.5		
S. mutans ATCC 25175	Essential oil	0.4/0.8	0.1 /02	0.25	0.5	Synergistic
	Ampicillin	2/4	0.5/1	0.25		
S. sanguinis ATCC 10556	Essential oil	0.4/1.6	0.1/0.2	0.25	0.5	Synergistic
	Ampicillin	8/32	2/4	0.25		
S. sobrinus ATCC 27607	Essential oil	0.4/0.8	0.2/0.2	0.5	0.75	Additive
	Ampicillin	2/4	0.5/1	0.25		
S. ratti KCTC 3294	Essential oil	1.6/3.2	0.4/0.8	0.25	0.5	Synergistic
	Ampicillin	4/8	1/2	0.25		
S. criceti KCTC 3292	Essential oil	0.8/1.6	0.4/0.4	0.5	0.75	Additive
	Ampicillin	2/4	0.5/1	0.25		
S. anginosus ATCC 31412	Essential oil	0.2/0.8	0.05/0.1	0.25	0.375	Synergistic
	Ampicillin	4/8	0.5/2	0.125		
S. gordonii ATCC 10558	Essential oil	0.2/0.4	0.05/0.1	0.25	0.5	Synergistic
	Ampicillin	1/2	0.025/0.5	0.25		
A. actinomycetemcomitans ATCC 43717	Essential oil	0.2/0.4	0.1/0.2	0.5	0.625	Additive
	Ampicillin	64/128	8/16	0.125		
F. nucleatum ATCC 10953	Essential oil	0.2/0.4	0.05/0.1	0.25	0.375	Synergistic
	Ampicillin	0.125/0.25	0.015/0.031	0.125		
P. intermedia ATCC 25611	Essential oil	0.2/0.8	0.05/1	0.25	0.5	Synergistic
	Ampicillin	8/32	2/4	0.25		
P. gingivalis ATCC 33277	Essential oil	0.1/0.2	0.025/0.1	0.25	0.5	Synergistic
	Ampicillin	1/1	0.25/0.5	0.25		

[a] Essential oil MIC/MBC: mg/ml; ampicillin MIC/MBC: μg/ml.
[b] The checkerboard test as previously described (13). The MICs and MBCs of the essential oil of *C. indicum* with ampicillin against some oral bacteria with a few reference strains are indicated.
[c] The interaction was defined as synergistic if the FICI was less than or equal to 0.5, additive if the FICI was greater than 0.5 and less than or equal 1.0, indifferent if the FICI was greater than 1.0 and less than or equal to 2.0, and antagonistic if the FICI was greater than 2.0 (12, 13)

criceti, and *A. actinomycetemcomitans* and tested some reference strains (FICI of ≥ 0.625) (Table 3). The combination of gentamicin with the essential oil resulted in a reduction of the MICs/MBCs by ≥8-fold for most of tested

Table 4. Checkerboard assay of the essential oil of *C. indicum* and gentamicin against some oral bacteria with a few reference strains

Strains	Agent	MIC/MBC[a]		FIC[c]	FICI[c]	Outcome
		Alone	Combination[b]			
Escherichia coli ATCC 25922	Essential oil	12.8/12.8<	6.4/6.4	0.5	0.75	Additive
	Gentamicin	4/16	1/2	0.25		
Staphylococcus aureus ATCC 29213	Essential oil	0.8/3.2	0.2/0.4	0.25	0.5	Synergistic
	Gentamicin	1/4	0.25/1	0.25		
Staphylococcus epidermidis ATCC 12228	Essential oil	0.4/1.6	0.2/0.4	0.5	0.75	Additive
	Gentamicin	2/2	0.5/0.5	0.25		
Streptococcus pyogenes ATCC 21059	Essential oil	0.4/1.6	0.2/0.2	0.5	1	Additive
	Gentamicin	8/16	4/8	0.5		
S. mutans ATCC 25175	Essential oil	0.4/0.8	0.1/0.2	0.25	0.5	Synergistic
	Gentamicin	4/8	1/2	0.25		
S. sanguinis ATCC 10556	Essential oil	0.4/0.8	0.1/0.2	0.25	0.5	Synergistic
	Gentamicin	8/16	2/4	0.25		
S. sobrinus ATCC 27607	Essential oil	0.4/0.8	0.1/0.2	0.25	0.5	Synergistic
	Gentamicin	4/8	1/2	0.25		
S. ratti KCTC 3294	Essential oil	1.6/3.2	0.4/0.8	0.25	0.75	Additive
	Gentamicin	8/8	4/4	0.5		
S. criceti KCTC 3292	Essential oil	0.8/1.6	0.2/0.4	0.25	0.5	Synergistic
	Gentamicin	4/8	1/2	0.25		
S. anginosus ATCC 31412	Essential oil	0.2/0.8	0.05/0.1	0.25	0.375	Synergistic
	Gentamicin	8/16	1/4	0.125		
S. gordonii ATCC 10558	Essential oil	0.2/0.4	0.05/0.1	0.25	0.5	Synergistic
	Gentamicin	2/4	0.5/1	0.25		
A. actinomycetemcomitans ATCC 43717	Essential oil	0.2/0.4	0.05/0.1	0.25	0.375	Synergistic
	Gentamicin	1/2	0.125/0.5	0.125		
F. nucleatum ATCC 10953	Essential oil	0.2/0.4	0.05/0.1	0.25	0.5	Synergistic
	Gentamicin	32/32	8/8	0.25		
P. intermedia ATCC 25611	Essential oil	0.2/0.8	0.05/0.1	0.25	0.5	Synergistic
	Gentamicin	1/1	0.25/0.5	0.25		
P. gingivalis ATCC 33277	Essential oil	0.1/0.2	0.025/0.05	0.25	0.375	Synergistic
	Gentamicin	256/512	32/32	0.125		

[a] Essential oil MIC/MBC: mg/ml; gentamicin MIC/MBC: μg/ml.
[b] The checkerboard test as previously described (13). The MICs and MBCs of the essential oil of *C. indicum* with gentamicin against some oral bacteria with a few reference strains are indicated.
[c] The interaction was defined as synergistic if the FICI was less than or equal to 0.5, additive if the FICI was greater than 0.5 and less than or equal 1.0, indifferent if the FICI was greater than 1.0 and less than or equal to 2.0, and antagonistic if the FICI was greater than 2.0 (12, 13)

bacteria except *E. coli*, *S. epidermidis*, *S. pyogenes*, and *S. ratti* with the MICs/MBCs of 1/1 to 256/512 μg/ml for gentamicin becoming 0.125/0.3 to 32/32 μg/ml (Table 4).

DISCUSSION

Seventy-three compounds were identified in the oil extracted from *C. indicum*, representing 96.65% of the total oil. The main compounds with concentrations higher than 3% as percentage peak area of GC analysis were α-pinene (4.4%), 1,8-cineole (10.4%), α-thujone (6.05%), camphor (10.12%), terpinen-4-ol (3.4%), bornyl acetate (6.1%), borneol (3.6%), *cis*-chrysanthenol (3.4%), β-caryophyllene (5.1%), germacrene D (10.6%), and α-cadinol (3.0%). It was noteworthy that the compositions of the *C. indicum* essential oil were in partial agreement with those reported by other authors previously by high content of camphor on the essential oils of other *Chrysanthemum* species (7, 14, 15). Phytochemical profile of this plant has shown the presence of flavonoids, terpenoids and phenolic compounds (1, 4, 16, 17). The essential oil is very rich in terpenoids which exert inhibitory action against microorganisms such as *S. aureus*, *E. coli* and *Streptococcus pneumoniae* by disrupting their membranes (18).

The results of the antibacterial activity showed that the essential oil of *C. indicum* exhibited moderate activities against most of tested streptococci species (MICs, 0.2 to 0.8 mg/ml; MBCs, 0.4 to 1.6 mg/ml), and also the essential oil showed the strong antimicrobial activity against obligate anaerobic bacteria: *F. nucleatum*, *P. intermedia*, and *P. gingivalis* (MICs, 0.1 to 0.2 mg/ml; MBCs, 0.2 to 0.8 mg/ml). The major components of the essential oil from *C. indicum*, terpinon-4-ol, borneol, and β-caryophyllene were indicated as stronger antibacterial activity than α-pinene, camphor, and 1,8-cineole. In combination with the essential oil, the MICs/MBCs for ampicillin and gentamicin were reduced by ≥8-fold in tested some of oral bacteria and reference bacteria, suggesting a synergistic effect as defined by a FICI of ≤ 0.5.

Some oils have been shown to have applications in food preservation, aromatherapy, and pharmacological properties as antibacterial, antifungal, antioxidant, spasmolytic, antiplasmodial, anti-inflammatory, and anticancer activities (11, 15, 18, 19). The essential oils of *Chrysanthemum* have been found to have antibacterial, antifungal, and antioxidant activities (7, 10, 20). In general, there is a correlation between the antifungal activity and the percentage of some major components (20). Camphor and 1,8-cineole as well as borneol were well-known chemical with their pronounced antimicrobial properties (7, 11, 15). In this study, the essential oil of *C. indicum* exhibited stronger antibacterial activity than some of its major compounds. In addition, our results are consistent with previous findings that the components in lower amount such as α-terpineol, terpinen-4-ol, ρ-cymene, and linalool could also contribute to the antimicrobial activity of the oils (7, 15, 19). In fact, it was also possible that the components in lower percentage might be involved in some type of synergism with the other active compounds, synergistic activity of 1,8-cineole and camphor against some bacteria had already been reported (11, 15, 19).

These results also indicate the possibility of exploitation of the essential oil of *C. indicum* as an effective inhibitor of oral bacteria, for example, a component of toothpaste and/or gargling solution. However, for medicinal purposes, the safety and toxicity of this essential oil need to be addressed.

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