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Full Length Research Paper

Chemical composition and antibacterial activity of essential oils from different parts of some *Bupleurum* L. species

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The composition of the essential oils obtained by hydrodistillation and microdistillation from different parts of *Bupleurum croceum* Fenzl, *Bupleurum lancifolium* Hornem., *Bupleurum intermedium* Poiret, *Bupleurum cappadocicum* Boiss., *Bupleurum gerardii* All. and *Bupleurum falcatum* L. subsp. *cernuum* including flowers, fruits and roots were investigated by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) systems, simultaneously. The antibacterial activity of the oils which was obtained by hydrodistillation was assessed with micro-dilution assays. The main components of *B. croceum* were germacrene D (12.7%) in flowers, undecane (13.0%) in fruits and hexadecanoic acid (34.8%) in roots. The main components of *B. lancifolium* were spathulenol (15.4%) in flowers, hexacosane (13.0%) in fruits and hexadecanoic acid (13.9%) in roots. The main components of *B. intermedium* were methyl linoleat (21.2%) in flowers, germacrene D (25.9%) in fruits and undecane (62.8%) in roots. The main components of *B. cappadocicum* were heptanal (46.5%) in flowers, undecane (50.3%-23.1%) in fruits and roots. The main components of *B. gerardii* were undecane (36.9%-49.2%) in flowers and fruits, hexanal (21.7%) in roots. The main components of *B. falcatum* subsp. *cernuum* were α-pinene (41.2%-42.4%) in flowers and fruits, amyl furan (23.1%) in roots. The essential oils of *B. croceum*, *B. lancifolium* obtained from flowers and fruits, *B. intermedium* and *B. cappadocicum* from fruits used in the study did not have any effect against bacteria. The MIC values of essential oils of the roots for the bacterial strains tested, which were sensitive to the essential oils of roots of *B. croceum*, *B. lancifolium*, *B. cappadocicum* and *B. falcatum* subsp. *cernuum* were in the ratio of 2 mg/ml.

Key words: Antibacterial activity, essential oil composition, microdilution, *Bupleurum* species.

INTRODUCTION

Bupleurum L. is a genus of family Umbelliferae (Apiaceae), comprising about 200 species and primarily located in the Northern Hemisphere, Eurasia, and North Africa (Pan, 2006). The genus *Bupleurum* L. comprises 49 taxa in Turkey, of which 21 taxa are endemic (Davis, 1982; Güner et al., 2000).

Under the name of Chaihu (Saiko in Japanese and Shiho in Korean), the roots of several species from the genus have been frequently used in the prescriptions of oriental traditional medicine for the treatment of common cold with fever, influenza, inflammation, hepatitis, malaria, and also menopausal syndrome in China for 2000 years (Pan, 2006).

There are more than 150 species in the genus *Bupleurum*, nearly a quarter of which have been subjected to phytochemical investigation. The main constituents from the genus are triterpene glycosides of the oleanane series. Furthermore, the occurrence of essential oils, lignans, flavanoids, coumarins,

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Table 1. Tested *Buplerum* species.

Species	Location of the sample	Herbarium number	Fl.	Fr.	Ro.
B. croceum	Konya: Altinapa, nearby Degirmenkoy, 1200 m	HT1007 KNYA	HD	HD	HD
B. lancifolium	Karaman: Ermenek, Ermenek-Kazanci road intersection, 1125 m	HT1009 KNYA	HD	HD	HD
B. intermedium	Karaman: Ermenek, Ermenek-Kazanci road intersection, 1450 m	HT1010 KNYA	MD	HD	MD
B. cappadocicum	Karaman: nearby Sertavul Gateway, 1400 m	HT1008 KNYA	MD	HD	HD
B. gerardii	Konya: Altinapa, nearby Degirmenkoy, 1200 m	HT1013 KNYA	MD	MD	MD
B. falcatum subsp. *cernuum*	Konya: Between Altinapa and Basakavak, Han position, 1280 m	HT1011 KNYA	MD	MD	HD

FI: Flower, Fr: fruit,Ro:Root,HD: Hydrodistillation, MD: Microdistillation.

polysaccharides, polyacetylenes, phytosterols, and phenylpropanoids are also reported (Pan, 2006). Extracts and essential oils of *Bupleurum* genus plants have been largely used in traditional medicine for their anti-inflammatory and antiseptic activity (Nose et al., 1989).

Recent studies have shown that natural products and especially essential oils and components thereof display potential as antimicrobial agents for various uses in medical applications (Hammer et al., 1999).

This study concerns the analysis of the essential oils of different parts of *B. croceum*, *B. lancifolium*, *B. intermedium*, *B. cappadocicum*, *B.* gerardii. and *B. falcatum* subsp. *cernuum* including roots, flowers and fruits by gas chromatography (GC) and gas chromatography/ mass spectrometry (GC/MS) and the antibacterial evaluation against gram (+) and gram (-) human and animals pathogenic bacteria. To the best of our knowledge, there is no previous study on the essential oil and antibacterial activity of *Bupleurum* species used in this study.

MATERIALS AND METHODS

Plant material

Six species of *Bupleurum* samples were collected from Turkey (Table 1), between May and August in 2009 during their flowering and fruiting periods, and were identified by T. Uysal of Selcuk University, Turkey, through a systematic source (Davis, 1982).

Isolation of essential oil

The essential oils from air-dried plant materials were isolated by hydrodistillation and microdistillation.

Chromatographic analysis

Gas chromatography (*GC) conditions*

The GC analysis was carried out using an Agilent 6890N GC system. FID detector temperature was 300°C. To obtain the same elution order with GC-MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

Gas chromatography/mass spectrometry (GC/MS) conditions

The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 μm film thickness) was used with helium as carrier gas (0.8 ml/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 35 to 450. Library search was carried out using the Baser Library of Essential Oil Constituents, Wiley GC/MS Library, Adams Library, MassFinder Library. The individual compounds identified in the essential oils are given in Tables 2, 3 and 4.

Antimicrobial assay

In microbiological tests, standard 11 bacteria strain that exists in human beings, animals and food are used. These are *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 3166 09:K35:K99, *E. coli* ATCC 25922, *E. coli* ATCC 25923, *E. coli* ATCC 29988, *Bacillus cereus* ATCC 11778, *Streptococcus salivarius* RSHE 606, *Pseudomonas aeruginosa* ATCC 29853, *P. aeruginosa* ATCC 15442, *Proteus mirabilis* ATCC 43071. Bacterial cultures were activated in Mueller Hinton Broth (MHB, Merck) for 24 h at 37°C. At the end of the period of incubation, the cultures developed in the liquid medium were standardized at 10^8 cfu/ml (McFarland No: 0.5). The essential oils dissolved in 25% dimethylsulfoxide (DMSO) was first diluted to the highest concentration 4 mg/ml to be tested and then two-fold serial of dilutions were made in concentration range from 2 mg/ml to 3.906 μg/ml. Antibacterial activity was assayed using the microdilution technique (Koneman et al., 1997; Zgoda and Porter, 2001). For antibacterial tests, pre-sterilized micro titration petri dish (Brand) having 96 “U” type wells were used. Serial solutions of the essential oils were performed at microtitration petris. 100 μl of each

Table 2. Chemical compositions of the flower essential oils of *Bupleurum* species.

No	Compounds	RRI	A (%)	B (%)	C (%)	D (%)	E (%)	F (%)
1	Decane	1000				0.4	0.8	
2	α-Pinene	1032	9.0	10.8	14.1			41.2
3	Hexanal	1093				1.2	8.2	
4	Undecane	1100	5.7	1.5	2.1	36.6	36.9	
5	β-Pinene	1118	0.7					
6	Sabinene	1132	2.2					
7	Myrcene	1174	0.8		1.6			
8	Heptanal	1194				46.5		4.3
9	Limonene	1203	1.4	0.5	2.1		5.4	
10	β-Phellandrene	1218	4.5	0.5	7.1			
11	Amyl furan (*2-Pentyl furan*)	1244	0.3	0.6			3.4	
12	γ-Terpinene	1255	0.5					
13	Tridecane	1300	4.3					
14	1-Octen-3-ol	1452					2.4	
15	α-Cubebene	1466						4.3
16	(*Z*)-3-Hexenyl-2-methyl butyrate	1482	1.1		0.3			
17	(Z)-3-Hexenyl 3-methylbutyrate (=*(Z)-3-hexenyl isovalerate*)	1494	1.6					
18	Bicycloelemene	1495			0.1			
19	α-Copaene	1497	2.3	3.6	0.7			
20	Pentadecane	1500	2.4			0.2		
21	Dihydroedulan II	1505		0.6		0.1		
22	β-Bourbonene	1535	0.9	1.0	0.3			
23	Pinocamphone	1536						0.5
24	(E)-2 Nonenal	1548				0.5		
25	β-Cubebene	1549	0.8		0.3			
26	Pinocarvone	1586						1.2
27	β-Ylangene	1589			1.7			
28	β-Copaene	1597		0.4	1.1			
29	β-Elemene	1600			0.3			
30	Calerene	1610					2.0	
31	β-Caryophyllene	1612	3.8	0.6	5.8			
32	Aromadendren	1628				1.0		
33	Thyopsene	1644						0.3
34	Myrtenal	1648						0.7
35	(E)-2-Decanal	1655		0.4				
36	γ-Gurjunen	1659			0.7			
37	*cis*-Verbenol	1663						1.9
38	(*Z*)-β-Farnesene	1668	3.6					
39	*trans*-Pinocarveol	1670						2.8
40	(*Z*)-3-Hexenyl tiglat	1681			0.2			
41	*Trans*-Verbenol	1683		0.8				1.6
42	α-Humulene	1687		0.3	0.5			
43	Heptadecane	1700		0.5				
44	γ-Muurolene	1704			0.4	1.3		
45	Verbenone	1725						1.0
46	Germacrene D	1726	12.7	10.3	16.4			
47	Valensen	1740			0.4			
48	Phellandral	1744	1.0					
49	Bicyclogermacrene	1755	0.6	1.9	1.0			
50	δ-Cadinene	1773	1.1	1.0	0.9	0.6		0.5

Table 2. Contd.

51	γ-Cadinene	1776				0.6		
52	Myrtenol	1804						0.1
53	(*E,E*)-2,4-Decadienal	1827	0.7					
54	Cuparene	1849						2.2
55	Calamenene	1849						0.9
56	(*E*)-Geranyl acetone	1868	0.8	1.1				
57	α-Calacorene	1941	0.4	0.6				2.0
58	1,5-Epoxy-salvial 4(14)-ene	1945	2.5	2.3		0.8	1.7	
59	Heptanoic acid	1981				0.1		
60	γ-Calacorene	1984						0.6
61	Caryophyllene oxide	2008	8.0	4.3	1.1		5.8	
62	Salvial-4(14)-en-1-one	2037	1.7	2.5	0.4	0.4		
63	Humulene epoxide-II	2071		1.1			1.5	
64	Globulol	2098				0.6		
65	Viridiflorol	2104				0.5		
66	Salviadienol	2130	1.1	1.4	0.4			
67	Hexahydrofarnesyl acetone	2131	3.0	2.8	0.2	0.3	8.8	
68	Spathulenol	2144	5.6	15.4	1.9	3.8	2.5	10.4
69	Muurola-4,10(14)-dien-1-ol	2161						1.7
70	Methyl hexadecanat	2226				0.5		
71	Cadelene	2256				0.2		1.6
72	Torilenol	2278	1.3	2.7	3.4			
73	4-okso α-Ylangene	2289						2.1
74	Tricosane	2300						0.6
75	Eudesma-4(15),7-dien-1□-ol	2369	1.3		0.7			
76	Dihidroactinidiolide	2380					1.8	
77	Tetracosane	2400	1.4					tr
78	Methyl oleat	2456			6.2	0.2		
79	Pentacosane	2500	2.1	1.4				1.0
80	Dodecanoic acid	2503		2.6				
81	Methyl linoleat	2509			21.2	0.4		
82	Hexacosane	2600	2.4	2.3				0.8
83	Phytol	2622		1.0				
84	Tetradecanoic acid	2670		5.7				
85	Heptacosane	2700	2.7	0.5				0.9
86	Octacosane	2800	tr					
87	Hexadecanoic acid	2931		6.2				
	Monoterpene hydrocarbons		19.1	11.8	24.9		5.4	41.2
	Oxygenated monoterpenes		1.0	0.8				9.8
	Sesquiterpene hydrocarbons		26.2	20.3	30.6	3.7	2.0	12.4
	Oxygenated sesquiterpenes		21.5	29.7	7.9	6.1	11.5	14.2
	Fatty acid+esters			14.5		0.1		
	Diterpenes			1.0				
	Alkanes		21.0	6.2	2.1	37.2	37.7	3.3
	Others		7.5	5.5	28.1	49.7	24.6	4.3
	Total		96.3	89.8	93.6	96.8	81.2	85.2

RRI Relative retention indices calculated against n-alkanes % calculated from FID data tr Trace (< 0.1 %) A: *B. croceum*, B: *B. lancifolium*, C: *B. intermedium*, D: *B. Cappadocicum*, E: *B. gerardii*, F: *B. falcatum* subsp. *Cernuum.*

Table 3. Chemical compositions of the fruit essential oils of *Bupleurum* species.

No	Compounds	RRI	A (%)	B (%)	C (%)	D (%)	E (%)	F (%)
1	Decane	1000		0.9			0.8	
2	α-Pinene	1032	6.8		2.9			42.4
3	3-Hexanon	1058		0.8				
4	2- Hexanon	1087		1.2				
5	Hexanal	1093					6.1	2.9
6	Undecane	1100	13.0		1.4	50.3	49.2	
7	β-Pinene	1118	0.5		tr			1.7
8	Sabinene	1132	1.4		0.3		1.3	
9	Myrcene	1174			0.6			
10	Heptanal	1194				4.1		7.3
11	3- Hexanol	1202		1.8				
12	Limonene	1203	1.4		0.6		1.8	1.1
13	β-Phellandrene	1218	2.3		2.8			
14	2- Hexanol	1222		2.7				
15	Amyl furan (*2-Pentyl furan*)	1244	0.7				1.6	0.8
16	γ-Terpinene	1255	0.7					
17	*p*-Cymene	1280	0.3					0.2
18	Tridecane	1300	3.1					
19	1-Octen-3-ol	1452					1.1	
20	α-Cubebene	1466						0.3
21	(Z)-3-Hexenyl 3-methylbutyrate (=*(Z)-3-hexenyl isovalerate*)	1494	0.5					
22	α-Copaene	1497	1.3	1.6	1.9	0.1		6.8
23	Pentadecane	1500				0.2		
24	Dihydroedulan II	1505			0.4	0.4		
25	β-Bourbonene	1535	1.5		0.5			
26	(E)-2 Nonenal	1548				0.3		
27	β-Cubebene	1549			0.8			0.4
28	Pinocarvone	1586						0.2
29	β-Ylangene	1589		0.9				
30	Hexadecan	1600		0.8				
31	β-Caryophyllene	1612		0.1	19.1			
32	Aromadendren	1628				1.1		
33	Thyopsene	1644				0.5		1.0
34	*cis*-Verbenol	1663						0.6
35	*Trans*-Pinocarveol	1670						1.2
36	*Trans*-Verbenol	1683						1.9
37	α-Humulene	1687			1.6			
38	Heptadecane	1700		1.3			0.7	
39	γ-Muurolene	1704			0.9	1.7		
40	Leden	1708				0.3		
41	2-Undecanol	1722				0.4		
42	Verbenone	1725						0.8
43	Germacrene D	1726		1.2	25.9	0.3		
44	γ- Amorfen	1733				0.4		
45	α- Muurolene	1740				0.5		
46	Phellandral	1744	0.9					
47	Bicyclogermacrene	1755			2.0			
48	δ-Cadinene	1773		0.7	1.5	1.4		1.2
49	γ-Cadinene	1776				0.8		
50	Ar-Curcumen	1786					1.0	

Table 3. Contd

51	Octadecane	1800		0.8			
52	Myrtenol	1804					0.3
53	(E)-β Damassenone	1838				0.2	
54	Cuparene	1849				0.5	2.9
55	Calamenene	1849				0.5	0.4
56	(*E*)-Geranyl acetone	1868	1.3		0.5	0.8	
57	1-Undecanol	1871				0.2	
58	Nonadecane	1900		0.6			
59	α-Calacorene	1941		0.4	0.1	0.5	1.9
60	1,5-Epoxy-salvial 4(14)-ene	1945		1.2		1.4	0.8
61	Dodecanol	1973	0.5				
62	γ-Calacorene	1984					0.5
63	2-Phenylethyl-2-methylbutyrate	1988			0.5		
64	Eicosan	2000		0.6			
65	Caryophyllene oxide	2008	6.2	2.1	11.2		3.8
66	*Epi*-Globulol	2033				0.3	
67	Salvial-4(14)-en-1-one	2037		1.3	0.9	1.3	
68	Humulene epoxide-II	2071			0.4		1.2
69	1- *Epi*-Cubenol	2088				1.0	
70	Globulol	2098				0.9	
71	Heneicosane	2100		0.9			
72	Viridiflorol	2104				tr	
73	Salviadienol	2130		0.8	0.8	0.8	
74	Hexahydrofarnesyl acetone	2131	8.7	3.5	0.6	3.2	9.6
75	Spathulenol	2144	2.3	4.7	3.1	7.4	2.3	8.5
76	Muurola-4,10(14)-dien-1-ol	2161						1.5
77	Docosane	2200		1.2			
78	Phenylethyltiglate	2214			0.6		
79	*Trans*-α-Bergamotol	2247				0.2	
80	α-Cadinol	2255			0.7	1.0	
81	Cadelene	2256					1.1
82	Torilenol	2278			1.0	1.2	
83	4-okso α-Ylangene	2289				0.3		1.3
84	Tricosane	2300	0.6	2.8	0.7		
85	Caryophylladienol-II	2324			1.7		
86	Eudesma-4(15),7-dien-1□-ol	2369			1.5	0.5	
87	Dihidroactinidiolide	2380					tr
88	Pharnecyl acetone	2384				0.7	
89	Hexadecanol	2384	1.5				1.7
90	Caryophyllenol-II	2392			0.9		
91	Tetracosane	2400	1.0	6.5	0.8	0.8	
92	4-iso propil-6-methyl-1-tetra-1one	2411						0.3
93	Pentacosane	2500	2.4	12.0	0.9	1.2	
94	Dodecanoic acid	2503	1.4			1.1	
95	Hexacosane	2600	2.3	13.0	1.3	1.2	
96	Phytol	2622	1.3				
97	Tetradecanoic acid	2670	11.9			2.2	
98	Heptacosane	2700	1.5	10.0	1.5	tr	
99	Octacosane	2800		7.5	1.1		
100	Nonacosane	2900		5.1	tr	0.4	
101	Hexadecanoic acid	2931	6.8		tr	1.7	

Table 3. Contd.

102	Triacontane	3000		1.4			
	Monoterpene hydrocarbons		13.4		7.2	3.1	45.4
	Oxygenated monoterpenes		0.9				5.0
	Sesquiterpene hydrocarbons		2.8	4.9	54.3	8.6	1.0	16.5
	Oxygenated sesquiterpenes		8.5	10.1	22.2	16.3	8.1	11.3
	Fatty acid+esters		20.1			5.0		
	Diterpenes		1.3					
	Alkanes		23.9	65.4	7.7	54.1	50.7	
	Others		13.2	10.0	2.6	10.3	20.1	11.3
	Total		84.1	90.4	94.0	94.3	83.0	89.5

RRI Relative retention indices calculated against n-alkanes % calculated from FID data tr Trace (< 0.1 %) A: *B. croceum*, B: *B. lancifolium*, C: *B. intermedium*, D: *B. cappadocicum* E: *B. gerardii*, F: *B. falcatum* subsp. *Cernuum.*

Table 4. Chemical compositions of the roots essential oils of *Bupleurum* species.

No	Compounds	RRI	A (%)	B (%)	C (%)	D (%)	E (%)	F (%)
1	α-Pinene	1032			3.2		17.9	1.7
2	Hexanal	1093			8.1		21.7	7.1
3	Undecane	1100	14.3	4.9	62.8	23.1	16.6	
4	Heptanal	1194			2.8			2.1
5	Limonene	1203					1.0	
6	β-Phellandrene	1218			5.7			
7	Amyl furan (*2-Pentyl furan*)	1244		0.4	1.8		2.7	23.1
8	Octanal	1296						2.0
9	Tridecane	1300	3.1			2.4		
10	1-Hexanol	1360					0.8	
11	Nonanal	1400						4.9
12	3-Octen-2-one	1416					1.8	
13	(*E*)-2-Octenal	1441						3.5
14	1 Octen-3-ol	1452			0.6		2.3	
15	α-Copaene	1497						3.9
16	Pentadecane	1500				3.4		
17	Benzaldehyde	1541			0.7			
18	(E)-2 Nonenal	1548						2.6
19	Octanol	1562						2.3
20	Bornyl acetate	1591		11.9		0.9		
21	Calerene	1610					3.9	
22	(E)-2-Decanal	1655						2.2
23	*Trans*-Pinocarveol	1670					1.3	
24	*Trans*-Verbenol	1683					3.3	
25	Decyl acetate	1687		0.9				
26	Myrtenyl acetate	1704		3.4				
27	Dodecanal	1722		1.1				
28	Verbenone	1725					2.2	
29	α-Muurolene	1740						1.7
30	Decanol	1766		0.7				
31	δ-Cadinene	1773						2.3
32	(*E,Z*)-2,4-Decadienal	1779						1.2
33	(*E,E*)-2,4-Decadienal	1827						6.9
34	Cuparene	1849		1.9		2.7	1.2	1.4

Table 4. Contd.

35	(*E*)-Geranyl acetone	1868			0.2		0.7	
36	Nonadecene	1915			1.2			
37	α-Calacorene	1941						5.4
38	Dodecanol	1973		1.3				
39	Eicosane	2000		0.8				
40	Caryophyllene oxide	2008			1.0			
41	İsopropyl myristate	2045		2.2				
42	Heneicosane	2100		0.9				
43	Hexahydrofarnesyl acetone	2131	3.1	1.0		2.7		
44	Spathulenol	2144			1.0	4.5	2.9	7.2
45	Nonenoic acid	2192						1.2
46	Docosane	2200		1.3		0.9		
47	Ar-Turmerol	2214	2.6					
48	Tricosane	2300	4.6	1.9		2.0		
49	Hexadecanol	2384		1.9			0.6	
50	Tetracosane	2400	2.9	3.8		3.5		tr
51	Pentacosane	2500	3.5	5.9		5.8		1.8
52	Dodecanoic acid	2503	1.8	2.2		2.2		0.4
53	Hexacosane	2600	2.6	7.0		6.0		
54	1-Octadecanol	2607	0.8					
55	Tetradecanoic acid	2670		4.0		3.5	1.3	
56	Heptacosane	2700	19.8	12.0		10.5		tr
57	Octacosane	2800		2.4		3.9		
58	Pentadecanoic acid	2822		1.1		2.6		
59	Nonacosane	2900		2.4		1.8		
60	Hexadecanoic acid	2931	34.8	13.9		14.2	1.0	3.9
	Monoterpene hydrocarbons				8.9		18.9	1.7
	Oxygenated monoterpenes			15.3		0.9	6.8	
	Sesquiterpene hydrocarbons			1.9		2.7	5.1	14.7
	Oxygenated sesquiterpenes		2.6		2.0	4.5	2.9	7.2
	Fatty acid+esters		36.6	21.2		22.5	2.3	5.5
	Diterpenes							
	Alkanes		50.8	43.3	62.8	63.3	16.6	1.8
	Alkenes				1.2			
	Others		3.9	9.5	14.2	2.7	30.6	57.9
	Total		93.9	91.2	89.1	96.6	83.2	88.8

RRI Relative retention indices calculated against n-alkanes % calculated from FID data tr Trace (< 0.1 %) A: *B. croceum*, B: *B. lancifolium*, C: *B. intermedium*, D: *B. cappadocicum*, E: *B. gerardii*, F: *B. falcatum* subsp. *Cernuum.*

microbial suspension were added to the wells. The eleventh well containing only serial dilutions of antibacterial agents without microorganisms was used as negative control. The last well contained only microorganisms as positive control. Solvent DMSO as negative control and chloramphenicol (sigma) as positive control were used. The minimum inhibitory concentration (MIC) values were determined as the last well doesn't include turbidity at the end of the incubation for 24 h at 37°C.

RESULTS AND DISCUSSION

The essential oils were obtained by hydrodistillation and microdistillation from air-dried parts of *B. croceum*, *B. lancifolium*, *B. intermedium*, *B. cappadocicum*, *B. gerardii* and *B. falcatum* subsp. *cernuum* and subsequently analyzed by GC and GC/MS systems, simultaneously.

In total, 38 (flower), 29 (fruit) and 12 (root) constituents were identified and quantified in the various parts of *B. croceum*, respectively. In the flower oil of *B. croceum*, germacrene D (12.7%) and α-pinene (9.0%) were the main constituents. The flower essential oil comprised sesquiterpene hydrocarbons (26.2%), oxygenated sesquiterpenes (21.5%), alkanes (21.0%), monoterpene hydrocarbons (19.1%), 'others' (7.5%) and oxygenated

monoterpenes (1.0%), respectively. As for the fruit oil of *B*. *croceum*, the main constituents were undecane (13.0%) and tetraadecanoic acid (11.9%). Furthermore, fruit essential oil was composed of alkanes (23.9%), fatty acid+esters (20.1%), monoterpene hydrocarbons (13.4%), 'others' (13.2%), oxygenated sesquiterpenes (8.5%), sesquiterpene hydrocarbons (2.8%), diterpenes (1.3%) and oxygenated monoterpenes (0.9%), respectively. In the root oil of *B*. *croceum*, hexadecanoic acid (34.8%) and heptacosane (19.8%) were the main constituents. The root essential oil comprised alkanes (50.8%), fatty acid+esters (36.6%), 'others' (3.9%) and oxygenated sesquiterpenes (2.6%), respectively.

In total, 34 (flower), 32 (fruit) and 26 (root) constituents were identified and quantified in the various parts of *B*. *lancifolium*, respectively. In the flower oil of *B*. *lancifolium*, spathulenol (15.4%) and α-pinene (10.8%) were the main constituents. The flower essential oil comprised oxygenated sesquiterpenes (29.7%), sesquiterpene hydrocarbons (20.3%), fatty acid+esters (14.5%), monoterpene hydrocarbons (11.8%), alkanes (6.2%), 'others' (5.5%), diterpenes (1.0%) and oxygenated monoterpenes (0.8%), respectively. As for the fruit oil of *B*. *lancifolium*, the main constituents were hexacosane (13.0%) and pentacosane (12.0%). Furthermore, fruit essential oil was composed of alkanes (65.4%), oxygenated sesquiterpenes (10.1%), 'others' (10.0%) and sesquiterpene hydrocarbons (4.9%) respectively. In the root oil of *B*. *lancifolium*, hexadecanoic acid (13.9%) and heptacosane (12.0%) were the main constituents. The root essential oil comprised alkanes (43.3%), fatty acid+esters (21.2%), oxygenated monoterpenes (15.3%), 'others' (9.5%) and sesquiterpene hydrocarbons (1.9%), respectively.

In total, 31 (flower), 40 (fruit) and 12 (root) constituents were identified and quantified in the various parts of *B*. *intermedium*, respectively. In the flower oil of *B*. *intermedium*, methyl linoleat (21.2%) and germacrene D (16.4%) were the main constituents. The flower essential oil comprised sesquiterpene hydrocarbons (30.6%), 'others' (28.1%), monoterpene hydrocarbons (24.9%), oxygenated sesquiterpenes (7.9%) and alkanes (2.1%), respectively. In the fruit oil of *B*. *intermedium*, the main constituents were germacrene D (25.9%) and β-caryophyllene (19.1%). Furthermore, fruit essential oil was composed of sesquiterpene hydrocarbons (54.3%), oxygenated sesquiterpenes (22.2%), alkanes (7.7%), monoterpene hydrocarbons (7.2%), and 'others' (2.6%), respectively. As for the root oil of *B*. *intermedium*, undecane (62.8%) and hexanal (8.1%) were the main constituents. The root essential oil comprised alkanes (62.8%), 'others' (14.2%), monoterpene hydrocarbons (8.9%), oxygenated sesquiterpenes (2.0%) and alkenes (1.2%), respectively.

In total, 22 (flower), 45 (fruit) and 19 (root) constituents were identified and quantified in the various parts of *B*. *cappadocicum*, respectively. In the flower oil of *B*. *cappadocicum*, heptanal (46.5%) and undecane (36.6%) were the main constituents. The flower essential oil comprised 'others' (49.7%), alkanes (37.2%), oxygenated sesquiterpenes (6.1%), sesquiterpene hydrocarbons (3.7%) and fatty acid+esters (0.1%), respectively. As for the fruit oil of *B*. *cappadocicum*, the main constituents were undecane (50.3%) and spathulenol (7.4%). Furthermore, fruit essential oil was composed of alkanes (54.1%), oxygenated sesquiterpenes (16.3%), 'others' (10.3%), sesquiterpene hydrocarbons (8.6%) and fatty acid+esters (5.0%), respectively. In the root oil of *B*. *cappadocicum*, undecane (23.1%) and hexadecanoic acid (14.2%) were the main constituents. The root essential oil comprised alkanes (63.3%), fatty acid+esters (22.5%), oxygenated sesquiterpenes (4.5%), sesquiterpene hydrocarbons (2.7%) 'others' (2.7%) and oxygenated monoterpenes (0.9%), respectively.

In total, 13 (flower), 16 (fruit) and 18 (root) constituents were identified and quantified in the various parts of *B*. *gerardii*, respectively. In the flower oil of *B*. *gerardii*, undecane (36.9%) and hexahydrofarnesyl acetone (8.8%) were the main constituents. The flower essential oil comprised alkanes (37.7%), 'others' (24.6%), oxygenated sesquiterpenes (11.5%), monoterpene hydrocarbons (5.4%) and sesquiterpene hydrocarbons (2.0%), respectively. As for the fruit oil of *B*. *gerardii*, the main constituents were undecane (49.2%) and hexahydrofarnesyl acetone (9.6%). Furthermore, fruit essential oil was composed of alkanes (50.7%), 'others' (20.1%), oxygenated sesquiterpenes (8.1%), monoterpene hydrocarbons (3.1%), and sesquiterpene hydrocarbons (1.0%), respectively. In the root oil of *B*. *gerardii*, hexanal (21.7%) and α-pinene (17.9%) were the main constituents. The root essential oil comprised 'others' (30.6%), monoterpene hydrocarbons (18.9%), alkanes (16.6%), oxygenated monoterpenes (6.8%), sesquiterpene hydrocarbons (5.1%), oxygenated sesquiterpenes (2.9%) and fatty acid+esters (2.3%), respectively.

In total, 26 (flower), 27 (fruit) and 24 (root) constituents were identified and quantified in the various parts of *B*. *falcatum* subsp. *cernuum*, respectively. In the flower oil of *B*. *falcatum* subsp. *cernuum*, α-pinene (41.2%) and spathulenol (10.4%) were the main constituents. The flower essential oil comprised monoterpene hydrocarbons (41.2%), oxygenated sesquiterpenes (14.2%), sesquiterpene hydrocarbons (12.4%), oxygenated monoterpenes (9.8%), 'others' (4.3%) and alkanes (3.3%), respectively. In the fruit oil of *B*. *falcatum* subsp. *cernuum*, the main constituents were α-pinene (42.4%) and spathulenol (8.5%). Furthermore, fruit essential oil was composed of monoterpene hydrocarbons (45.4%), sesquiterpene hydrocarbons (16.5%), oxygenated sesquiterpenes (11.3%), 'others' (1.3%) and oxygenated monoterpenes (5.0%), respectively. As for the root oil of *B*. *falcatum* subsp. *cernuum*, amyl furan (23.1%) and spathulenol (7.2%)

were the main constituents. The root essential oil comprised 'others' (57.9%), sesquiterpene hydrocarbons (14.7%), oxygenated sesquiterpenes (7.2%), fatty acid+esters (5.5%), alkanes (1.8%) and monoterpene hydrocarbons (1.7%), respectively.

The essential oils of *B. croceum*, *B. lancifolium* obtained from flowers and fruits did not exhibit any activity against test microorganisms. The essential oils of *B. intermedium* and *B. cappadocicum* obtained from fruits did not exhibit any activity against test microorganisms. The essential oils of the roots of *B. croceum*, *B. lancifolium*, *B. cappadocicum* and *B. falcatum* subsp. *cernuum* showed activity against *E. coli* ATCC 25922, *Bacillus cereus* ATCC 11778, *Streptococcus salivarius* RSHE 606, *P. aeruginosa* ATCC 29853, *P. aeruginosa* ATCC 15442, without any difference compared to the Chloramphenicol. The essential oils of the roots of *B. croceum*, *B. cappadocicum* and *B. falcatum* subsp. *cernuum* had low activity against *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* ATCC 29213, *E. coli* ATCC 3166 09:K35:K99, *E. coli* ATCC 25923, *E. coli* ATCC 29988, *Proteus mirabilis* ATCC 43071 compared to the control antibiotic. The essential oil of the roots of *B. lancifolium* had low activity against *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* ATCC 29213, *E. coli* ATCC 25923, *E. coli* ATCC 29988, *Proteus mirabilis* ATCC 43071 compared to the control antibiotic. *E. coli* ATCC 3166 09:K35:K99 was not inhibited by the oil of the roots of *B. lancifolium*.

The antibacterial activity of the oil from flowers and roots of *B. intermedium*, from flowers of *B. cappadocicum*, from flowers, fruits and roots of *B. gerardii* and from flowers of *B. falcatum* subsp. *cernuum* which obtained by microdistillation not tested.

A wide variety of essential oils are known to possess antimicrobial properties and in many cases this activity is due to the presence of active constituents, mainly attributable to isoprenes such as monoterpenes, sesquiterpenes and related alcohols, other hydrocarbons and phenols (Dorman and Deans, 2000; Griffin et al., 1999). In fact, the synergistic effects of the diversity of major and minor constituents present in the essential oils should be taken into consideration to account for their biological activity (Akın et al., 2010).

This study also showed that the essential oil of the roots of *B. croceum*, *B. lancifolium*, *B. cappadocicum* and *B. falcatum* subsp. *cernuum* could be potential sources of new antimicrobial agents.

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