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

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Summary: The essential oils of *Bupleurum heldreichii* Boiss. & Bal., *Bupleurum sulphureum* Boiss. & Bal., *Bupleurum turcicum* Snogerup, and *Bupleurum lycaonicum* Snogerup flowers, fruits and roots were obtained using hydrodistillation and microdistillation techniques and their chemical compositions were analyzed by GC and GC/MS systems, simultaneously. The antibacterial activity of the oils which obtained by hydrodistillation was assessed with micro-dilution assays. The main components of *B. heldreichii* were germacrene D (% 47.5-48.4) in flowers and fruits, and hexadecanoic acid (% 46.2) in roots. The main components of *B. sulphureum* found undecane (% 14.0-20.2) in flowers and fruits, and calarene (% 26.9) in roots. The main components of *B. turcicum* were heptanal (% 33.2-23.5) in flowers and fruits, and pentacosane (% 9.0) in roots. The main components of *B. lycaonicum* were tridecane (% 14.9-37.3) in flowers and roots, spathulenol (% 14.4) in fruits. The essential oils of *B. heldreichii*, *B. sulphureum, B. turcicum* obtained from flowers and fruits, *B. lycaonicum* 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. heldreichii*, *B. sulphureum* and *B. turcicum* were in the ratio of 2 mg/ml. This investigation showed that the antibacterial activity of *B. heldreichii*, *B. sulphureum* and *B. turcicum* was attributed to the essential oil of roots, thus they can be a potential medicinal resource.

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

Endemik *Bupleurum* L. türlerinin farklı kısımlarının uçucu yağlarının kimyasal kompozisyonu ve antibakteriyel aktivitesi

Özet: *Bupleurum heldreichii* Boiss. & Bal., *Bupleurum sulphureum* Boiss. & Bal., *Bupleurum turcicum* Snogerup ve *Bupleurum lycaonicum* Snogerup'un çiçek, meyve ve köklerinin uçucu yağları hidrodistilasyon ve mikrodistilasyon yöntemleriyle elde edildi ve kimyasal bileşimleri GC ve GC/MS sistemleri ile eşzamanlı olarak incelendi. Hidrodistilasyon ile elde edilen uçucu yağların antibakteriyel aktiviteleri mikrodilüsyon yöntemiyle belirlendi. *B. heldreichii*'nin çiçek ve meyvelerinde germakren D (% 47.5-48.4), köklerinde hekzadekanoik asit (% 46.2) ana bileşenler olarak belirlendi. Ana bileşenler *B. sulphureum*'un çiçeklerinde ve meyvelerinde undekan (% 14.0-20.2), köklerinde kalaren (% 26.9) olarak bulundu. *B. turcicum*'un çiçeklerinde ve meyvelerinde heptanal (% 33.2-23.5), köklerinde pentakosan (% 9.0) ana bileşenler olarak belirlendi. *B. lycaonicum*'un çiçeklerinde ve köklerinde tridekan (% 14.9-37.3), meyvelerinde spatulenol (% 14.4) ana bileşenler olarak bulundu. *B. heldreichii*, *B. sulphureum, B. turcicum*'un çiçek ve meyvelerinden, *B. lycaonicum*'un meyvelerinden elde edilen uçucu yağların çalışmada kullanılan bakterilere karşı etkisinin olmadığı görüldü. *B. heldreichii*, *B. sulphureum* ve *B. turcicum*'un kök uçucu yağlarının test edilen bakteri suşları için MİK değerleri 2 mg/ml'dir. Bu araştırma *B. heldreichii*, *B. sulphureum* ve *B. turcicum*'un kök uçucu yağlarının antibakteriyel aktivitesinin olduğunu ve potansiyel tıbbi kaynak olabileceğini gösterdi.

Anahtar sözcükler: Antibakteriyel aktivite, endemik *Bupleurum* türleri, mikrodilüsyon, uçucu yağ bileşimi.

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 (11). The genus *Bupleurum* L. comprises 49 taxa in Turkey, of which 21 taxa are endemic (3, 6).

The roots of several *Bupleurum* species have been included either alone or in combination with other ingredients in many pharmaceutical preparations, based upon traditional Chinese medicine for the treatment of common cold (12), inflammation (2), hepatitis, cancer (9), and fever associated with malaria (13). Extracts and essential oils of *Bupleurum* genus plants have been largely used in traditional medicine for their anti-inflammatory and antiseptic activity (10).

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, polysaccharides, polyacetylenes, phytosterols, and phenylpropanoids are also reported (11).

To the best of our knowledge, there is no previous study on the essential oils of *B. heldreichii*, *B. sulphureum*, *B. turcicum*, and *B. lycaonicum*. 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 (7).

This study concerns the analysis of the essential oils of different parts of *B. heldreichii*, *B. sulphureum*, *B. turcicum*, and *B. lycaonicum* including roots, flowers and fruits by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) (Table 2,3,4) and the antibacterial evaluation against Gram positive and Gram negative pathogenic bacteria of human and animals. Antimicrobial broth dilution assay was used to determine the minimum inhibitory concentrations (MIC) of the essential oils to eleven different microorganisms.

Materials and Methods

Plant Material: The plant materials was collected between June and July in 2009 and was identified through a systematic source (3) (Table 1).

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

Chromatographic analysis

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.

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.

Identification of components: The components of essential oils were identified by comparison of their mass spectra with those in the Baser Library of Essential Oil Constituents, Wiley GC/MS Library, Adams Library, MassFinder Library and confirmed by comparison of their retention indices. Alkanes were used as reference points in the calculation of relative retention indices (RRI). Relative percentage amounts of the separated compounds were calculated from FID chromatograms. The individual compounds identified in the essential oils are given Table 2, 3, 4.

Detection of antibacterial activity: In microbiological tests, 11 strains of reference bacteria strain that originated from human beings, animals or food are used. These were *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 3166 09:K35:K99, *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 25923, *Escherichia coli* ATCC 29988, *Bacillus cereus* ATCC 11778, *Streptococcus salivarius* RSKK 606, *Pseudomonas aeruginosa* ATCC 29853, *Pseudomonas aeruginosa* ATCC 15442, *Proteus mirabilis* ATCC 43071. Bacterial strains were obtained from the Biotechnology Laboratory in Selcuk University. Bacterial cultures were activated in Mueller Hinton Broth (MHB, Merck) for 24h at 37^0C. At the end of the period of incubation, the cultures developed in the liquid medium were standardized to Mc Farland No: 5 standard. 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 (8, 14). For antibacterial tests, pre-sterilized microplates (Brand) having 96 "U" type wells were used. Serial dilutions of the essential oils were performed at microtitration plates. 100 μl of each bacterial suspension were added to the wells. The eleventh well containing only serial dilutions of antibacterial agents without bacteria was used as negative

Table 1. Tested Endemic *Buplerum* species.
Tablo 1. Test edilen endemik *Bupleurum* türleri.

Species	Location of the samples	Herbarium number	Fl	Fr	Ro
B. heldreichii	Konya: Karapinar, Erosion area, 1150 m	HT1006 KNYA	HD	HD	HD
B. sulphureum	Konya: Beysehir road, Stone quarry area, 1350 m	HT1003 KNYA	HD	HD	HD
B. turcicum	Konya: Cihanbeyli, Salt lake area, 910 m	HT1005 KNYA	HD	HD	HD
B. lycaonicum	Konya: Beyşehir road, Stone quarry area,1350 m	HT1004 KNYA	MD	HD	MD

Fl: Flower, Fr: Fruit, Ro: Root, HD: Hydrodistillation, MD: Microdistillation

control. The last well contained only bacteria as positive control. Solvent DMSO as negative control and Chloramphenicol (Sigma) as positive control were used. The minimum inhibitory concentration (MIC) values were determined by the determination of absence of turbidity in the last well which were incubated for 24 h at 37°C.

Table 2. Chemical compositions of the flower essential oils of *Bupleurum* species.
Tablo 2. *Bupleurum* türlerinin çiçek uçucu yağlarının bileşimi.

		%			
RRI	Compounds	A	B	C	D
1032	α-Pinene	1.5	9.3	0.8	
1035	α-Thujene		0.2		
1076	Camphene		0.4		
1093	*Hexanal*			0.9	6.3
1100	Undecane	1.1	14.0	6.6	
1118	β-Pinene		5.8		
1194	Heptanal			33.2	1.1
1203	Limonene		0.3	1.5	
1218	β-Phellandrene	0.9			
1225	(*Z*)-3-Hexanal				4.6
1244	2-Pentyl furan		0.7	1.3	2.4
1255	γ-Terpinene		0.3		
1280	*p*-Cymene		2.9		
1290	2-Octanone			0.3	
1296	Octanal			0.5	
1300	Tridecane			1.8	14.9
1345	2-Hexyl furan			0.3	
1400	Tetradecane			0.3	
1400	Nonanal		0.2	0.3	
1417	4,8-Dimethyl-1,3,7-nonatriene			0.2	
1441	(*E*)-2-Octenal			0.5	
1452	1-Octen-3-ol			0.2	
1466	α-Cubebene				2.1
1495	Bicycloelemene	2.8			
1497	α-Copaene	4.4	4.8		1.8
1500	Pentadecane			19.6	
1505	Dihydroedulan II				2.0
1535	Dihydroedulan I				0.3
1535	β-Bourbonene	0.8	0.5		
1541	Benzaldehyde				0.3
1548	(*E*)-2-Nonenal		0.2	1.5	
1549	β-Cubebene	1.0	0.6		2.2
1589	β-Ylangene	5.4			1.0
1597	β-Copaene	4.1			0.8
1600	Hexadecane			0.2	
1600	β-Elemene	0.5		0.2	2.6
1604	2-Undecanone			0.3	
1612	β-Caryophyllene		1.0	0.5	0.7
1648	Myrtenal		0.3		
1655	(*E*)-2-Decanal		0.4	0.4	
1659	γ-Gurjunene	2.8			
1668	(*Z*)-β-Farnesene	0.2			
1687	α-Humulene	0.3			
1700	Heptadecane			0.8	
1704	γ-Muurolene	1.6			0.5
1719	1-Heptadecene			0.5	
1726	Germacrene D	47.5	5.1		6.2
1740	α-Muurolene	1.8			0.7
1755	Bicyclogermacrene	5.5	1.0		
1773	δ-Cadinene	3.5	1.0		3.0
1776	γ-Cadinene	0.7			
1779	(*E,Z*)-2,4-Decadienal			0.2	
1800	Octadecane			0.2	
1827	(*E,E*)-2,4-Decadienal		0.9	0.8	
1834	(*Z*)-Geranyl acetone			1.4	
1849	Calamenene				0.6
1852	1-Octadecene		0.6	0.3	
1868	(*E*)-Geranyl acetone		0.7	3.1	
1900	*epi*-Cubebol				0.9
1933	Tetradecanal	0.2			
1941	α-Calacorene	0.4	1.1		1.0
1945	1,5-Epoxy-salvial-4(14)-ene	0.9	8.7		1.6
1957	Cubebol				1.5
1958	(*E*)-β-Ionone			0.8	
1981	Heptanoic acid			1.0	
1984	γ-Calacorene				0.4
2008	Caryophyllene oxide	1.8	4.5	3.5	3.9
2037	Salvial-4(14)-en-1-one	0.6	3.0		3.6
2071	Humulene epoxide-II				1.1
2080	Cubenol				0.5
2088	1-*epi*-Cubenol				1.2
2130	Salviadienol	0.4	0.8		1.9
2131	Hexahydrofarnesyl acetone	0.6	1.2	1.4	1.4
2144	Spathulenol	5.2	9.9	3.5	8.0
2161	Muurola-4,10(14)-dien-1-ol		1.7		
2200	3,4-Dimethyl-5-Pentyl-5H-Furan-2-one			0.4	
2209	T-Muurolol		1.3		
2256	Cadalene		1.0		1.5
2278	Torilenol		0.8		2.7
2289	4-oxo-α-Ylangene		2.5		
2300	Tricosane	0.7			
2369	Eudesma-4(15),7-dien-1β-ol				2.4
2384	Farnesyl acetone			0.1	
2384	1-Hexadecanol			0.3	
2400	Tetracosane			0.2	
2500	Pentacosane		0.6	0.2	0.7
2503	Dodecanoic acid		0.5	0.4	
2600	Hexacosane			0.2	
2670	Tetradecanoic acid		1.0	0.6	
2700	Heptacosane			0.3	
2800	Octacosane			0.1	
2900	Nonacosane			0.2	
2931	Hexadecanoic acid	0.3	1.2	1.9	
	Monoterpene Hydrocarbons	2.4	19.2	2.3	
	Oxygenated Monoterpenes		0.3		
	Sesquiterpene Hydrocarbons	83.3	16.1	0.7	25.1
	Oxygenated Sesquiterpenes	8.9	33.2	7.0	29.3
	Fatty acid+esters	0.3	2.7	3.9	
	Alkanes	1.8	14.6	30.7	15.6
	Others	0.8	4.9	49.2	18.4
	Total	97.5	91.0	93.8	88.4

RRI Relative retention indices calculated against n-alkanes % calculated from FID data

tr Trace (< 0.1 %) A: *B. heldreichii*, B: *B. sulphureum*, C: *B. turcicum*, D: *B. lycaonicum*

Table 3. Chemical compositions of the fruit essential oils of *Bupleurum* species.
Tablo 3. *Bupleurum* türlerinin meyve uçucu yağlarının bileşimi.

		%			
RRI	Compounds	A	B	C	D
1032	α-Pinene		5.8	2.0	0.7
1035	α-Thujene		tr		
1076	Camphene		0.3		
1093	*Hexanal*			0.5	
1100	Undecane	0.3	20.2	8.9	1.0
1118	β-Pinene		3.7		0.9
1194	Heptanal			23.5	
1203	Limonene			1.8	1.2
1244	2-Pentyl furan		0.4	1.0	
1259	Butyl isovalerate		0.3		
1280	*p*-Cymene		2.0		0.5
1296	Octanal			0.5	
1300	Tridecane			1.9	4.8
1345	2-Hexyl furan			0.3	
1348	6-Methyl-5-hepten-2-one			0.3	
1400	Tetradecane			0.2	
1400	Nonanal		0.3	0.4	
1441	(*E*)-2-Octenal			0.2	
1495	Bicycloelemene	3.4			
1497	α-Copaene	6.7	3.3		1.3
1500	Pentadecane			13.4	
1505	Dihydroedulan II			0.2	3.5
1535	β-Bourbonene		1.0		
1548	(*E*)-2-Nonenal			2.3	
1549	β-Cubebene				1.3
1589	β-Ylangene	6.3			
1589	Aristolene			0.2	
1597	β-Copaene	4.7	0.4		
1600	Hexadecane			0.2	
1600	β-Elemene			0.8	
1604	2-Undecanone		0.7	0.7	
1648	Myrtenal		0.4		
1655	(*E*)-2-Decanal			0.5	
1659	γ-Gurjunene	1.7			
1700	Heptadecane			0.8	
1704	γ-Muurolene	2.9			
1719	1-Heptadecene			0.5	
1726	Germacrene D	48.4	0.8		1.7
1755	Bicyclogermacrene	1.5			
1773	δ-Cadinene	4.5	0.5		2.5
1776	γ-Cadinene	1.7			
1827	(*E,E*)-2,4-Decadienal			0.7	
1834	(*Z*)-Geranyl acetone			2.5	
1838	(*E*)-β-Damascenone		0.7		
1852	1-Octadecene		1.1	0.7	
1868	(*E*)-Geranyl acetone		1.7	7.7	1.0
1900	Nonadecane		0.4		1.4
1941	α-Calacorene	0.8	0.8		0.8
1945	1,5-Epoxy-salvial-4(14)-ene	1.9	3.8		7.3
1958	(*E*)-β-Ionone		0.4	0.8	
1981	Heptanoic acid			0.7	
2008	Caryophyllene oxide	1.1	2.4	2.4	4.8
2037	Salvial-4(14)-en-1-one	0.6	2.9		3.4
2050	(*E*)-Neralidol			0.5	
2071	Humulene epoxide-II				1.1
2130	Salviadienol				1.1
2131	Hexahydrofarnesyl acetone	1.6	4.5	3.6	6.2
2144	Spathulenol	8.0	6.0	5.9	14.4
2161	Muurola-4,10(14)-dien-1-ol		1.1		
2179	*nor*-Copaanone				2.1
2209	T-Muurolol	tr	1.2		
2255	α-Cadinol	tr			
2256	Cadalene		1.3		2.2
2278	Torilenol		0.6		1.4
2289	4-oxo-α-Ylangene		2.2		3.1
2300	Tricosane		0.8		2.6
2369	Eudesma-4(15),7-dien-1β-ol				1.8
2384	Farnesyl acetone			0.4	
2384	1-Hexadecanol			0.7	
2400	Tetracosane				1.0
2500	Pentacosane		1.3		1.6
2503	Dodecanoic acid		0.7	0.8	2.2
2600	Hexacosane				2.3
2622	Phytol			1.0	
2670	Tetradecanoic acid		1.9	2.6	
2700	Heptacosane				4.3
2900	Nonacosane				0.1
2931	Hexadecanoic acid		1.4	2.8	2.6
	Monoterpene Hydrocarbons		11.8	3.8	3.3
	Oxygenated Monoterpenes		0.4		
	Sesquiterpene Hydrocarbons	82.6	8.1	1.0	9.8
	Oxygenated Sesquiterpenes	11.6	20.2	8.8	40.5
	Fatty acid+esters		4.0	6.9	4.8
	Diterpenes			1.0	
	Alkanes	0.3	22.7	25.4	19.1
	Others	1.6	10.1	48.0	10.7
	Total	96.1	77.3	94.9	88.2

RRI Relative retention indices calculated against n-alkanes % calculated from FID data

tr Trace (< 0.1 %) A: *B. heldreichii*, B: *B. sulphureum*, C: *B. turcicum*, D: *B. lycaonicum*

Table 4. Chemical compositions of the roots essential oils of *Bupleurum* species.
Tablo 4. *Bupleurum* türlerinin kök uçucu yağlarının bileşimi.

		%			
RRI	Compounds	A	B	C	D
1000	*Decane*			0.1	2.5
1032	α-Pinene	0.3			
1093	*Hexanal*				23.8
1100	Undecane	9.0	23.8	5.5	5.1
1244	2-Pentyl furan	0.3			1.6
1280	*p*-Cymene		0.5		
1296	Octanal	0.3			
1300	Tridecane	0.2		1.8	37.3
1400	Tetradecane			0.5	
1400	Nonanal	0.2		0.2	
1492	Cyclosativene	0.1			
1497	α-Copaene	0.2			
1500	Pentadecane			3.0	2.7
1506	Decanal	0.2		0.4	
1541	Benzaldehyde				1.2
1548	(*E*)-2-Nonenal	0.2			
1589	Aristolene		3.7		
1591	Bornyl acetate	0.8	8.2	0.2	
1600	Hexadecane			0.4	
1604	2-Undecanone			0.5	
1610	Calarene	0.5	26.9		
1617	Undecanal			0.5	
1655	(*E*)-2-Decanal	0.2		0.9	
1668	2-Decyl acetate			4.5	
1687	Decyl acetate	1.5			
1700	Heptadecane			0.6	
1704	Myrtenyl acetate	0.4			
1722	Dodecanal	0.2	1.2	0.4	
1744	α-Selinene		0.5		
1764	(*E*)-2-Undecanal			0.8	
1766	1-Decanol	0.2		0.8	
1800	Octadecane	0.2		0.5	
1827	(*E,E*)-2,4-Decadienal			0.8	
1849	Cuparene	0.5	0.9	0.5	2.5
1868	(*E*)-Geranyl acetone			0.8	
1871	1-Undecanol			8.8	
1878	2,5-Dimethoxy-*p*-cymene		1.2		
1893	Dodecyl acetate	2.4			
1900	Nonadecane			0.5	
1932	2,6-Dimethoxy-*p*-cymene		0.7		
1973	1-Dodecanol	1.0	1.6	6.3	
2045	Isopropyl myristate				0.9
2100	Heneicosane	0.5		1.1	
2131	Hexahydrofarnesyl acetone	2.5	1.0	3.7	0.9
2144	Spathulenol		1.7	6.3	1.7
2179	1-Tetradecanol	0.6		2.2	
2200	Docosane			1.4	
2298	Decanoic acid	0.2			
2300	Tricosane	1.1		3.3	
2308	Methyl dihydrojasmonate				1.0
2384	Farnesyl acetone			1.4	
2384	1-Hexadecanol	1.2			
2400	Tetracosane		1.9	5.4	
2500	Pentacosane	3.2	0.8	9.0	
2503	Dodecanoic acid	3.5	1.0		
2600	Hexacosane		2.0	8.0	
2607	1-Octadecanol	0.9			
2670	Tetradecanoic acid	14.7			
2700	Heptacosane		2.1	3.6	
2800	Octacosane			3.9	
2804	Benzyl salicylate			1.8	
2822	Pentadecanoic acid	3.8			
2857	γ-Palmitolactone	0.9			
2900	Nonacosane			2.4	
2931	Hexadecanoic acid	46.2	3.0	0.2	
	Monoterpene Hydrocarbons	0.3	0.5		
	Oxygenated Monoterpenes	1.2	10.1	0.2	
	Sesquiterpene Hydrocarbons	1.3	32.0	0.5	2.5
	Oxygenated Sesquiterpenes		1.7	6.3	1.7
	Fatty acid+esters	68.4	4.0	0.2	
	Alkanes	14.2	30.6	51.0	47.6
	Others	12.8	3.8	34.8	29.4
	Total	98.2	82.7	93.0	81.2

RRI Relative retention indices calculated against n-alkanes % calculated from FID data
tr Trace (< 0.1 %) A: *B. heldreichii*, B: *B. sulphureum*, C: *B. turcicum*, D: *B. lycaonicum*

Results

The essential oils were obtained by hydrodistillation and microdistillation from air-dried parts of *B. heldreichii*, *B. sulphureum*, *B. turcicum*, and *B. lycaonicum* subsequently analyzed by GC and GC/MS systems, simultaneously.

In total, 29 (flower), 19 (fruit) and 34 (root) constituents were identified and quantified in the various parts of *B. heldreichii*, respectively. The main components of essential oils own to *B. heldreichii* were germacrene D (% 47.5), bicyclogermacrene (% 5.5) and β-yılangen (% 5.4) in flower, germacrene D (% 48.4), spathulenol (% 8.0) and α-copaene (% 6.7) in fruit, hexadecanoic acid (% 46.2), tetradecanoic acid (% 14.7) and undecane (% 9.0) in root.

For the *B. sulphureum*, a total of 39 (flower), 37 (fruit) and 19 (root) constituents were identified and quantified, respectively. In the flower oil of *B. sulphureum*, undecane (% 14.0), spathulenol (% 9.9) and α-pinene (% 9.3) were the main constituents. The main constituents of fruit oil of *B. sulphureum* were undecane (% 20.2), spathulenol (% 6.0) and α-pinene (% 5.8). In the root oil of *B. sulphureum*, calarene (% 26.9), undecane (% 23.8) and bornyl acetate (% 8.2) were the major substances.

A total of 39 (flower), 39 (fruit), 39 (root) compounds were identified and quantified respectively from the *B. turcicum*. The major constituents of the flower oil were heptanal (% 33.2), pentadecane (% 19.6) and undecane (% 6.6). The main components of the fruit oil were heptanal (% 23.5), pentadecane (% 13.4) and undecane (% 8.9). The essential oil of root, was characterised by the presence of pentacosane (% 9.0), 1-undecanol (% 8.8) and hexacosane (% 8.0).

A total of 37, 34 and 12 constituents were identified and quantified in flower, fruit and root oils of *B. lycaonicum*, respectively. Tridecane (% 14.9), spathulenol (% 8.0) and hexanal (% 6.3) were the major compounds in flower essential oil. Spathulenol (% 14.4) was the most abundant constituent in fruit oil, followed by 1,5-Epoxy-salvial-4(14)-ene (% 7.3) and hexahydrofarnesyl acetone (% 6.2). In the root oil of *B. lycaonicum*, tridecane (% 37.3), hexanal (% 23.8), undecane (% 5.1) were the major components.

The essential oils of *B. heldreichii*, *B. sulphureum, B. turcicum* obtained from flowers and fruits did not exhibit any activity against tested bacteria. The essential oils of *B. lycaonicum* obtained from fruits did not exhibit any activity against tested bacteria. The essential oils of the roots of *B. heldreichii*, *B. sulphureum, B. turcicum* and *B. lycaonicum* showed activity against *Escherichia coli* ATCC 25922, *Bacillus cereus* ATCC 11778, *Streptococcus salivarius* RSKK 606, *Pseudomonas aeruginosa* ATCC 29853, *Pseudomonas aeruginosa* ATCC 15442, without any difference compared to the Chloramphenicol. The essential oil of the roots of *B. sulphureum* had low activity against *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 3166 09:K35:K99, *Escherichia coli* ATCC 25923, *Escherichia coli* ATCC 29988, *Proteus mirabilis* ATCC 43071 compared to the control antibiotic. The essential oils of the roots of *B. heldreichii* and *B. turcicum* had low activity against *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25923, *Escherichia coli* ATCC 29988, *Proteus mirabilis* ATCC 43071 compared to the control antibiotic. *Escherichia coli* ATCC 3166 09:K35:K99 was not inhibited by the oil of the roots of *B. heldreichii* and *B. turcicum*.

The antibacterial activity of the oil from flowers and roots of *B. lycaonicum*, which obtained by microdistillation was not tested.

Discussion and Conclusion

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 (4, 5). 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 (1).

This study also showed that the essential oil of the roots of *B. heldreichii*, *B. sulphureum* and *B. turcicum* could be used as potential sources for new antimicrobial agents.

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