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Short communication

Essential oil composition of twenty-two *Stachys* species (mountain tea) and their biological activities

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ABSTRACT

The essential oils from twenty-two different *Stachys* species were obtained by hydrodistillation and analyzed by gas chromatography–mass spectrometry (GC–MS). Thirty-nine compounds, which accounted for 70.5–97.8% of the total composition of the oils, have been identified. Germacrene-D (2.9–45.3%), β-caryophyllene (2.3–62.3%), caryophyllene oxide (trace to 7.8%), spathulenol (trace to 7.8%) and α-cadinene (1.4–8.5%) have been identified as the main components of the essential oils. Antimicrobial assessments of the essential oils were evaluated against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Candida albicans* by disc diffusion method. Most of the essential oils showed moderate activity against the studied microorganisms.

1. Introduction

The Lamiaceae family is widely distributed in Anatolia and, one of the largest genera of the family is *Stachys* L. and contains about 300 taxa. In Turkey, the genus is represented by 83 species (109 taxa) belonging to 12 subsections, 15 sections and 2 subgenera. The section Eriostomum has three subsections, which are called subsect. *Germanicae* R. Bhattacharjee (13 taxa), subsect. *Creticae* R. Bhattacharjee (12 taxa) and subsect. *Spectabiles* R. Bhattacharjee (5 taxa) (Bhattacharjee, 1982; Davis et al., 1988; Duman, 2000; Ozhatay et al., 2009; Akcicek, 2010).

Stachys species are used as herbal remedies and consumed as wild tea in Anatolia as well as in Iran. Known as "mountain tea", decoctions or infusions of *Stachys* are applied as tonics to treat skin or taken internally for stomach disorders (Ozturk et al., 2009). Anti-inflammatory (Khanavi et al., 2005; Skaltsa et al., 2000), anti anxiety (Rabbani et al., 2003), antibacterial (Grujic-Jovanovic et al., 2004), anti-nephritic (Hayashi et al., 1994), anticancer (Amirghofran et al., 2006), anti-Helicobacter pylori (Stamatis et al., 2003), and antioxidant effects (Aydin et al., 2006) of genus desribed in the literature. Aerial parts of *S. inflata* Benth is used for infection, asthma, reheumatic and inflammatory disorders in Iranian folk medicine (Maleki et al., 2001; Khanavi et al., 2009). While *S. recta* used as wound healing agent, another species, *S. lavandulifolia* Vahl. is used for digestive disorders (Ozturk et al., 2009; Khanavi et al., 2009).

Although it is one of the largest genera of the Lamiaceae, there are limited reports in the literature on the phytochemistry of *Stachys* species. Several essential oil studies on the genus have been reported from Iran (Khanavi et al., 2004, 2005; Sajjadi and Somae, 2004; Norouzi-Arasi et al., 2004; Mehrabani et al., 2005; Sonboli et al., 2005; Rustaiyan et al., 2006; Rezazadeh et al., 2006), Greece (Skaltsa et al., 2003), Serbia and/or Montenegro (Radulović et al., 2007; Kukić et al., 2006), Italy (Giuliani et al., 2009; Piozzi and Bruno, 2009; Conforti et al., 2009), India (Bisht et al., 2008). β-Pinene, β-caryophyllene, germacrane D, linalool, linalyl acetate and caryophyllene oxide were found to be the main components of most of the reported species (Kaya et al., 2001; Skaltsa et al., 2001; Radulović et al., 2007; Harmandar et al., 1997). Moreover, recently, the presence of diterpenoids, such as labdane, abietane, kaurane and pimaranes were reported to be minor constituents of some *Stachys* essential oils (Piozzi and Bruno, 2009). On the contrary,

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aerial parts and roots of several *Stachys* species are very rich with many diterpenes, having neoclerodane, kaurane, labdane skeleta. Some diterpenes were found to have a novel, unprecedented tricyclic skeleton that does not conform to the isoprene rule (Piozzi and Bruno, 2011).

Essential oil compositions of four *Stachys* species, *S. recta* L., *S. balansae* L., *S. obliqua* L. and *S. athorecalyx* C. Koch from Turkey, were reported by the Harmandar group (Harmandar et al., 1997; Cakir et al., 1997; Duru et al., 1999). The main components of those species were determined to be oct-1-en-3-ol in *S. recta* (33.8%) and *S. athorecalyx* (18.7%), germacrene-D (25.4%) in *S. obliqua* and β-caryophyllene (24.3%) in *S. balansae*. Additionally, 71 compounds were characterized in the essential oil of *S. iberica* subsp. *stenotochya*, of which linalyl acetate (43.2%) was found to be the major component (Kaya et al., 2001). Nonacosane (23.1%) was the major component of the essential oil of *S. leativirens* Kotschy & Boiss ex Rech. fil., which is the endemic species growing in East Anatolia (Duman et al., 2005). The main components of the endemic species *S. aleurites*, which grows only in Antalya province, was determined as to be β-caryophyllene (33.7%) (Flamini et al., 2005). In the essential oil of *S. cretica*, *S. pinardii* α-curcumene (34.1%), cedrandiol (25.3%) and caryophyllene dioxide (22.2%) were reported (Ozturk et al., 2009). Recently, chemical composition and antimicrobial activity of essential oil of *S. cretica* L. subsp. *symrnaea*, which is endemic and distributed across north-west, west and south Anatolia, were reported. The major component of its oil was found to be β-caryophyllene (51.0%) (Ozturk et al., 2009).

In this study, we report the essential oil compositions and antimicrobial activities screening of 22 different *Stachys* species from Turkey and compare the chemical compositions with other *Stachys* essential oils. Moreover, this is the first report on the essential oil chemical compositions of *Stachys cretica* subsp. *cassia*, *S. cretica* subsp. *garana*, *S. cretica* subsp. *lesbiaca* (Çanakkale province, Turkey), *S. cretica* subsp. *kutahyensis*, *S. viticina*, *S. sericantha*, *S. pinetorum*, *S. bayburtensis*, *S. huber-morathii*, *S. huetii*, *S. tmolea*, *S. germanica* subsp. *bithynica*, *S. cretica* subsp. *bulgarica*, *S. spectabilis*, *S. thirkei*, *S. balansae* subsp. *carduchorum* and *S. longispicata*.

2. Results and discussion

Chemical compositions of the essential oils of twenty-two *Stachys* species were evaluated. Thirty-nine compounds, which accounted for 70.5–97.8% of the total composition of oils, are reported (Table 1). Germacrene-D (2.9–45.3%), β-caryophyllene (2.3–62.3%), caryophyllene oxide (trace to 12.8%), spathulenol (trace to 7.8%) and α-cadinene (1.4–8.5%) were identified as the major components of the essential oil of species. Additionally, α-cadinol, α-bisabolol, α-copaene and bicyclogermacrene were determined.

The essential oil of *Stachys cretica* L. subsp. *cassia* (Boiss.) Rech.f., of which 96.4% of the composition was determined, was characterized by its high proportion of sesquiterpene hydrocarbons (68.1%), followed by oxygenated sesquiterpenes (21.0%). Of the 24 components detected, the most abundant compounds were determined to be germacrene-D (27.8%), δ-elemene (14.9%) and β-caryophyllene (8.9%).

In *S. cretica* L. subsp. *garana* (Boiss.) Rech.f. oil, 24 components were identified representing 97.8% of the total oil. The oil consisted mainly of oxygenated sesquiterpenes (39.8%), sesquiterpene hydrocarbons (27.8%) and oxygenated monoterpenes (11.4%). α-Cadinol (16.4%), verbenol (11.4%) and dodecanoic acid (8.9%) were the major components.

In the oil of *S. cretica* L. subsp. *lesbiaca* (Boiss.) Rech.f., 27 components were determined representing 96.5% of the total oil, which consisted of sesquiterpene hydrocarbons (47.7%), oxygenated sesquiterpenes (34.8%), oxygenated monoterpenes (7.1%) and monoterpene hydrocarbons (2.6%). The major components of the oil were germacrene-D (13.9%), β-caryophyllene (12.5%) and α-cadinol (7.4%).

Twenty-three components of the essential oil of *S. cretica* L. subsp. *symrnaea* (Boiss.) Rech.f. were identified, accounting for 96.3% of the oil. The oil consisted mainly of sesquiterpene hydrocarbons (72.3%) and oxygenated sesquiterpenoids (24.0%). germacrene-D (38.9%), β-caryophyllene (14.8%) and caryophyllene oxide (8.7%) were the major components of the oil.

In the essential oil of *S. cretica* L. subsp. *kutahyensis* Akçiçek, 26 components were found and the oil contained sesquiterpene hydrocarbons (48.6%), oxygenated sesquiterpenoids (44.1%) and monoterpene hydrocarbons (0.6%). The major components were germacrene-D (28.1%), τ-muurolol (9.3%) and cubenol (8.8%).

In *S. viticina* Boiss. oil, 20 components were identified, representing 83.4% of the total oil. The oil consisted mainly of sesquiterpenes (65.4%), oxygenated sesquiterpenes (6.9%), monoterpene hydrocarbons (0.7%) 1 aliphatic acetate (8.9%) and 1 carboxylic acid (1.5%). β-Caryophyllene (62.3%), farnesyl acetate (8.9%) and α-bisabolol (4.4%) were the major components.

In the oil of *S. obliqua* Waldst. & Kit, 25 components were identified, representing 87.7% of oil, which was rich in sesquiterpene hydrocarbons (72.2%). Other components were classified as

Table 1
Essential oils composition of *Stachys* species[a].

RI	Compounds	1	2	3	4	5	6	7	8	9	10	11
930	Thujene	–	–	0.5	t	0.6	0.7	0.8	1.0	–	–	0.8
975	Sabinene	–	–	0.8	t	t	t	t	t	–	–	1.3
979	β-Pinene	–	1	1.3	t	t	t	1.6	0.9	–	–	1.9
1029	Limonene	t	t	t	t	t	t	8.3	t	t	–	1.3
1101	Nonanal	t	0.3	0.2	t	–	–	0.3	0.4	0.3	–	–
1143	*cis*-Sabinol	–	–	–	–	t	t	t	t	–	–	–
1145	Verbenol	t	11.4	6.2	t	t	t	t	t	t	–	1.5
1159	β-Pinene oxide	–	–	0.9	–	–	–	–	–	–	–	–
1194	2-Phenylethyl acetate	t	t	t	t	–	–	–	–	t	t	–
1265	Chrysanthenyl acetate	–	4.7	2.6	–	–	–	–	–	–	–	–
1276	*p*-Menth-10-en-7-al	t	1.3	1.5	–	–	–	–	–	t	t	3.9
1338	δ-Elemene	14.9	–	–	–	1.9	–	2.2	1.8	1.7	–	0.8
1377	α-Copaene	2.1	-	0.6	0.7	0.2	0.2	2.5	3.5	3.1	3.2	–
1385	β-Damascenone	-	3.6	–	–	–	–	–	–	–	t	–
1388	β-Bourbonene	–	–	5.3	5.8	t	t	t	t	–	–	6.0
1398	Unidentified	–	–	–	–	–	–	–	–	–	–	–
1419	**β-Caryophyllene**	**8.9**	**6.1**	**12.5**	**14.8**	**4.7**	**62.3**	**16.7**	**17.9**	**23.2**	**11.3**	**2.3**
1421	Cedrene	–	–	–	0.8	–	–	–	–	–	–	–
1439	α-Humulene	2.6	6.8	4.3	–	–	–	–	–	0.9	t	–
1455	Geranyl acetate	–	t	–	–	0.6	t	t	t		t	t
1457	*trans*-β-Farnesene	–	3.6	4.3	–	–	–	–	–	0.1	t	5.4

Table 1 (*Continued*)

RI	Compounds	1	2	3	4	5	6	7	8	9	10	11
1485	**Germacrene D**	**27.8**	**3.4**	**13.9**	**38.9**	**28.1**	**2.9**	**45.3**	**38.3**	**32.4**	**28.8**	**33.4**
1490	β-Selinene	0.3	t	1.4	1.7	2.5	t	t	t	0.1	t	1.3
1500	α-Muurolene	4.5	2.0	t	3.0	1.7	t	t	3.6	5.4	4.7	5.4
1502	Bicyclogermacrene	3.8	–	0.6	3.4	6.5	–	0.8	0.7	0.6	1.1	0.8
1523	**α-Cadinene**	**3.2**	**5.9**	**4.8**	**3.2**	**3.0**	**–**	**4.7**	**1.4**	**7.1**	**4.6**	**3.4**
1563	Nerolidol	2.1	–	2.4	–	–	–	–	–	0.4	–	–
1567	Dodecanoic acid	–	8.9	–	–	2.5	1.5	0.2	0.2	3.8	2.4	1.9
1569	Ledol	1.3	1.4	–	–	1.6	–	–	1.9	0.3	–	–
1578	**Spathulenol**	**2.9**	**6.8**	**6.3**	**3.9**	**6.5**	**–**	**–**	**7.8**	**0.2**	**6.8**	**5.2**
1583	**Caryophyllene oxide**	**2.0**	**t**	**3.8**	**8.7**	**6.8**	**1.1**	**2.3**	**5.4**	**0.3**	**8.1**	**6.1**
1585	Globulol	2.9	5.0	–	–	–	–	–	–	t	6.2	5.2
1608	Humulene epoxide II	–	–	1.4	1.2	1.6	–	–	–	–	–	–
1619	Cubenol	–	–	–	t	8.8	t	t	t	–	–	2.4
1642	τ-Muurolol	2.5	7.3	6.2	2.7	9.3	t	t	t	0.4	0.8	2.1
1654	α-Cadinol	4.8	16.4	7.4	5.4	5.7	1.4	1.6	1.4	0.4	t	1.4
1674	Valeranone	t	–	–	–	t	–	t	–	–	t	–
1686	α-Bisabolol	2.5	2.9	7.3	2.1	3.8	4.4	0.2	0.2	0.3	0.4	0.3
1726	Farnesyl acetate	7.3	–	–	–	–	8.9	0.2	0.2	0.7	–	–
Total		**96.4**	**97.8**	**96.5**	**96.3**	**96.4**	**83.4**	**87.7**	**86.6**	**81.7**	**78.4**	**94.1**

RI	Compounds	12	13	14	15	16	17	18	19	20	21	22
930	Thujene	0.8	0.9	0.9	t	t	–	0.4	0.3	t	t	1.4
975	Sabinene	1.3	3.4	–	t	1.5	9.1	3.1	1.2	1.1	t	t
979	β-Pinene	2.8	1.9	t	–	–	8.3	1.3	–	7.3	1.2	–
1029	Limonene	1.1	1.4	2.5	t	t	t	t	–	t	–	t
1101	Nonanal	1.5	–	–	1.1	2.1	–	0.9	1.3	–	0.5	0.2
1143	*cis*-Sabinol	–	–	t	t	t	1.7	1.2	t	1.3	–	t
1145	Verbenol	1.2	–	t	–	–	1.3	–	–	–	t	t
1159	β-Pinene oxide	–	–	t	0.8	0.9	0.9	t	–	2.4	–	–
1194	2-Phenylethyl acetate	–	–	–	–	–	–	–	1.3	–	–	1.1
1265	Chrysanthenyl acetate	–	–	–	–	–	–	–	–	t	–	–
1276	*p*-Menth-10-en-7-al	4.2		2.5	–	–	1.6	2.4	t	2.0	–	–
1338	δ-Elemene	0.5	0.2	–	–	–	–	t	–	–	3.1	1.4
1377	α-Copaene	1.6	0.2	1.7	3.2	7.7	2.4	3.7	4.7	0.9	1.9	t
1385	β-Damascenone	–	–	0.3	1.2	2.9	1.6	1.9	t	–	–	–
1388	β-Bourbonene	3.4	9.1	–	–	–	3.0	2.8	4.2	3.2	t	1.3
1398	Unidentified	–	–	3.0	1.0	1.6	–	1.0	–	t	–	–
1419	**β-Caryophyllene**	**14.5**	**12.4**	**19.7**	**15.7**	**14.8**	**2.5**	**11.8**	**9.9**	**3.8**	**29.9**	**9.7**
1421	Cedrene	–	0.2	–	–	–	–	–	0.1	–	1.3	–
1439	α-Humulene	–	0.5	–	1.6	0.6	0.5	0.2	–	2.6	–	1.6
1455	Geranyl acetate	t	–	0.4	3.1	t	t	t	–	t	–	–
1457	*trans*-β-Farnesene	9.6	6.1	1.4	t	6.8	t	4.8	t	6.8	–	–
1485	**Germacrene D**	**18.4**	**29.8**	**22.2**	**27.1**	**23.2**	**29.2**	**28.2**	**33.4**	**38.1**	**14.1**	**26.7**
1490	β-Selinene	1.2	2.4	1.5	t	t	t	–	t	t	–	t
1500	α-Muurolene	3.4	0.5	2.8	t	t	t	t	–	t	t	4.7
1502	Bicyclogermacrene	1.4	0.8	4.8	1.5	0.9	2.8	0.7	–	–	0.5	1.7
1523	**α-Cadinene**	**3.1**	**1.4**	**4.3**	**3.9**	**4.9**	**3.0**	**5.7**	**8.5**	**1.8**	**5.7**	**4.3**
1563	Nerolidol	–	0.4	0.6	0.5	t	t	t	–	t	–	1.1
1567	Dodecanoic acid	1.7	2.1	–	1.1	2.7	–	2.1	3.7	1.6	t	0.3
1569	Ledol	–	0.3	–	–	–	–	–	–	–	t	–
1578	**Spathulenol**	**2.5**	**2.9**	**6.3**	**3.7**	**5.8**	**4.5**	**7.4**	**t**	**3.8**	**4.2**	**5.8**
1583	**Caryophyllene oxide**	**5.9**	**5.2**	**1.6**	**12.8**	**6.9**	**4.7**	**6.6**	**3.2**	**7.9**	**3.8**	**5.1**
1585	Globulol	6.7	–	t	–	–	–	–	t	–	t	t
1608	Humulene epoxide II	–	–	–	4.0	3.4	1.4	1.4	2.1	0.8	–	–
1619	Cubenol	2,9	2.7	t	–	–	–	–	t	–	1.8	1.8
1642	τ-Muurolol	1.6	2.9	0.8	4.8	2.7	2.3	0.7	5.4	4.2	–	t
1654	α-Cadinol	1.8	5.2	1.7	1.9	2.0	2.1	1.9	1.7	1.3	1.0	5.3
1674	Valeranone	–	t	8.5	–	–	t	–	–	t	t	–
1686	α-Bisabolol	0.2	0.2	2.4	2.0	2.7	–	1.8	0.9	0.7	1.5	0.7
1726	Farnesyl acetate	–	–	3.3	3.1	1.7	0.8	2.6	1.6	t	–	t
Total		**93.3**	**93.1**	**93.2**	**94.1**	**95.8**	**83.7**	**94.6**	**83.5**	**91.6**	**70.5**	**74.2**

1: *Stachys cretica* subsp. *cassia* (ISTE 86160), **2**: *S. cretica* subsp. *garana* (ISTE 86161), **3**: *S. cretica* subsp. *lesbiaca* (ISTE 86162), **4**: S. *cretica* subsp. *smyrnaea* (ISTE 86129), **5**: *S. cretica* subsp. *kutahyensis* (ISTE 86123), **6:** *S. viticina* (ISTE 86138), **7**: *S. obliqua* (ISTE 86156), **8**: *S. balansae* subsp. *balansae* (ISTE 86150), **9**: *S. sericantha* (ISTE 86157), **10**: *S. pinetorum* (ISTE 86154), **11**: *S. bayburtensis* (ISTE 86142), **12**: *S. huber-morathii* (ISTE 86153), **13**: *S. huetii* (ISTE 86141), **14:** *S.tmolea* (ISTE 86159), **15**: *S. germanica* subsp. *heldreichii* (ISTE 86145), **16**: *S. germanica* subsp. *bithynica* (ISTE 86146), **17**: *S. cretica* subsp. *anatolica* (ISTE 86131), **18**: *S. cretica* subsp. *bulgarica* (ISTE 86127), **19**: *S. spectabilis* (ISTE 86136), **20**: *S. thirkei* (ISTE 86134). **21**: *S. balansae* subsp. *carduchorum* (ISTE 86151), **22**: *S. longispicata* (ISTE 86137).
[a]GC–MS analyses were replicated three times (mean RSD% value is 0.1); t: trace (<0.1%).

monoterpenes (10.7%) and oxygenated sesquiterpene hydrocarbons (4.1%). Germacrene-D (45.3%), β-caryophyllene (16.7%), and limonene (8.3%) were the major components of the oil.

S. balansae Boiss. & Kotschy subsp. *balansae* had 26 components which were accounted for 86.6% of the total composition of the oil. The oil consisted mainly of sesquiterpenes (67.2%), oxygenated sesquiterpenes (16.7%), monoterpene hydrocarbons (1.9%). Germacrene-D (38.3%), β-caryophyllene (17.9%) and spathulenol (7.8%) were the major components of the oil.

S. sericantha P.H. Davis was identified to have 25 components which were accounted for 81.7% of the total composition of the oil. It consisted of sesquiterpenes (74.6%), oxygenated sesquiterpenes

(2.3%), one aliphatic aldehyde (0.3%), one aliphatic acetate (0.7%) and one carboxylic acid (3.8%). Germacrene-D (32.4%), β-caryophyllene (23.2%) and α-cadinene (7.1%) were most abundant components of the oil.

In *S. pinetorum* Boiss. & Bal. oil, 21 components were identified, representing 78.4% of the total oil. It consisted of sesquiterpenes (53.7%), oxygenated sesquiterpenes (22.3%) and carboxylic acid (2.4%). Most abundant components of the oil were germacrene-D (28.8%), β-caryophyllene (11.3%) and caryophyllene oxide (8.3%).

In the oil of *S. bayburtensis* R. Bhattacharjee, 24 compounds, which were accounted for 94.1% of the total composition of the oil, were found. These compounds consisted of sesquiterpenes (58.8%), oxygenated sesquiterpenes (22.7%), monoterpenes (5.3%), oxygenated monoterpene (1.5%) one aliphatic aldehyde (3.9%) and one carboxylic acid (1.9%). Germacrene-D (33.4%), caryophyllene oxide (6.1%) and α-bourbonene (6.0%) were the major components of the oil.

In *S. huber-morathii* R. Bhattacharjee oil, 26 components were identified representing 93.3% of the total oil. It consisted mainly of sesquiterpenes (57.1%), oxygenated sesquiterpenes (21.6%), monoterpenes (6.0%) and oxygenated monoterpene (1.2%). Germacrene-D (18.4%), β-caryophyllene (14.5%) and *trans*-β-farnesene (9.6%) were the major components of the oil.

In the oil of *S. huetii* Boiss, 26 components were identified representing 93.1%, which was rich in sesquiterpene hydrocarbons (63.6%). Other components were classified as oxygenated sesquiterpenes (19.8%) and monoterpene hydrocarbons (7.6%). Germacrene-D (29.8%), β-caryophyllene (12.4%) and α-bourbonene (9.1%) were the major components of the oil.

The essential oil of *S. tmolea* Boiss, which 93.2% of the composition was identified, contained a high proportion of sesquiterpenes (58.4%) followed by oxygenated sesquiterpenoids (21.9%). Of the 28 components detected, the most abundant were germacrene-D (22.2%), β-caryophyllene (19.7%) and valeranone (8.5%).

Twenty-seven components were detected in the oil of *S. germanica* L. subsp. *heldreichii* (Boiss.) *hayek*, 20 of which represented 94.1% of the total oil. It consisted of mostly sesquiterpenes (53.0%), oxygenated sesquiterpenes (29.7%) and oxygenated monoterpenoids (0.8%). Germacrene-D (27.1%), β-caryophyllene (15.7%) and caryophyllene oxide (12.8%) were the major components of the essential oil.

In *S. germanica* L. subsp. *bithynica* (Boiss.) R. Bhattacharjee oil, 27 components were identified, 20 of which represented 95.8% of the total oil. The oil consisted mainly of sesquiterpenes (58.9%) and oxygenated sesquiterpenes (23.5%), followed by monoterpenes (1.5%) and oxygenated monoterpenes (0.9%). The major components of the oil were germacrene-D (23.2%), β-caryophyllene (14.8%) and α-copaene (7.7%).

In *S. cretica* L. subsp. *anatolica* Rech.f. oil, 27 components were identified, 20 of which represented 83.7% of the oil. The oil consisted of sesquiterpenes (43.4%), monoterpenes (17.4%), oxygenated sesquiterpenes (15.0%) and oxygenated monoterpenes (3.5%). The major components of the oil were germacrene-D (29.2%), sabinene (9.1%) and β-pinene (8.3%).

The essential oil of *S. cretica* L. subsp. *bulgarica* Rech.f. had 30 components, representing 94.6% of the oil. It consisted mainly of sesquiterpenes (57.9%), oxygenated sesquiterpenes (19.8%), monoterpenes (4.8%) and oxygenated monoterpenes (1.2%). Germacrene-D (28.2%), β-caryophyllene (11.8%) and spathulenol (7.4%) were the major components of the oil.

The essential oil of *S. spectabilis* Choisy ex DC had 25 components, which accounted for 83.5% of the oil. It consisted of sesquiterpene hydrocarbons (60.8%), followed by oxygenated sesquiterpenes (13.3%), monoterpene hydrocarbons (1.5%) two acetates (2.9%), one carboxylic acid (3.7%) and one aliphatic aldehyde (1.3%). Germacrene-D (33.4%), β-caryophyllene (9.9%) and α-cadinene (8.5%) were the major components of the oil.

In the oil of *S. thirkei* C. Koch, 29 compounds, 19 of which represented 91.6% of the total composition, were found. These compounds consisted of sesquiterpenes (57.2%), oxygenated sesquiterpenes (18.7%), monoterpenes (8.4%), oxygenated monoterpenes (3.7%), one aliphatic aldehyde (2.0%) and one carboxylic acid (1.6%). Germacrene-D (38.1%), caryophyllene oxide (7.9%) and β-pinene (7.3%) were the major components of the oil.

In the oil of *S. balansae* Boiss. & Kotschy subsp. *carduchorum* R. Bhattacharjee, 23 components were identified representing 70.5%, which was rich in sesquiterpene hydrocarbons (56.5%). Other components were classified as oxygenated sesquiterpene (12.3%) and monoterpene hydrocarbons (1.2%). β-caryophyllene (29.9%), germacrene-D (14.1%), and α-cadinene (5.7%) were major components of the oil.

The essential oil of *S. longispicata* Boiss. & Kotschy, of which 74.2% of the total composition of the oil was identified, contained a high proportion of sesquiterpenes (51.4%) followed by oxygenated sesquiterpenes (19.8%) and monoterpene hydrocarbons (1.4%). Of the 27 components detected, the most abundant were germacrene-D (26.7%), β-caryophyllene (9.7%) and spathulenol (5.8%).

The essential oils of *Stachys* species and some of ingredients such as α-pinene, β-caryophyllene, linalool oxide and caryophyllene oxide were tested against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Candida albicans*. Most of the essential oils showed moderate activity against the studied microorganism compared with sulphamethoxazole and nystatin. Terpenoid hydrocarbons such as pinene and caryophyllene tend to be relatively inactive compared to oxygenated terpenoids such as linalool, linalool oxide and caryophyllene oxide. This difference is likely due to the insolubility of the hydrocarbons and the hydrogen bonding capability of the oxygenated compounds with water during the MIC experiments (Grujic-Jovanovic et al., 2004). However, our results showed non-oxygenated terpenoids also to be active components of essential oils and their activities could easily be observed by the disc diffusion method. The antimicrobial and antifungal activities of α-pinene and β-caryophyllene demonstrate such activity. These non-oxygenated hydrocarbons showed better activity than oxygenated terpenoids linalool oxide and caryophyllene oxide (Table 2). Kubo et al. (1994) had found that monoterpenes such as α-pinene and camphene and, in particular, the sesquiterpene hydrocarbons β-caryophyllene and α-humulene to be notably more active against *Propionibacterium acnes* than oxygenated terpenoids. Schmidt et al. (2006) also found the sesquiterpenes β-caryophyllene, α-humulene, and germacrene-D to be moderately antimicrobial. We conclude, therefore, that the antimicrobial activities demonstrated by *Stachys* essential oils are attributable to the relatively high concentrations of sesquiterpenes.

In conclusion, *Stachys* species are used as herbal remedies and wild teas in all around of the world. Essential oil compositions of *Stachys* species and their antimicrobial potential are well defined in this study. Reported properties of seventeen of twenty-two *Stachys* species are reported herein for the first time. Finally, etnobotanical use of some of the *Stachys* species have been reported in Serbia, Montenegro, Iran and Turkey as antibacterial agents in the form of teas. We conclude that reported species of *Stachys* also can be used as an antibacterial agents.

3. Materials and methods

3.1. Plant material

Stachys species were collected during flowering period. Locality, altitude, collection time and Herbarium number of 22 species of

Table 2
Antibacterial and antifungal activity screening of essential oil of species of *Stachys* its pure compounds.[a,*]

Tested material	*E. coli*	*S. aureus*	*K. pneumoniae*	*P. aeruginosa*	*C. albicans*
S. cretica subsp. *cassia* (**1**)	NT	14	NT	15	10
S. cretica subsp. *garana* (**2**)	NT	NT	NT	NT	NT
S. cretica subsp. *lesbiaca* (**3**)	15	16	14	19	16
S. cretica subsp. *smyrnaea* (**4**)	15	18	13	19	17
S. cretica subsp. *kutahyensis* (**5**)	12	15	17	16	NT
S. viticina (**6**)	15	18	17	15	19
S. oblique (**7**)	NT	NT	NT	14	NT
S. balansae subsp. *balansae* (**8**)	15	14	17	15	13
S. sericantha (**9**)	16	15	12	17	18
S. pinetorum (**10**)	15	14	14	13	19
S. bayburtensis (**11**)	12	10	15	15	20
S. huber-morathii (**12**)	15	16	15	14	19
S. huetii (**13**)	15	14	NT	NT	18
S. tmolea (**14**)	NT	NT	NT	NT	19
S. germanica subsp. *heldreichii* (**15**)	13	12	NT	NT	NT
S. germanica subsp. *bithynica* (**16**)	12	14	15	14	14
S. cretica subsp. *anatolica* (**17**)	NT	NT	NT	NT	NT
S. cretica subsp. *bulgarica* (**18**)	NT	NT	NT	NT	14
S. spectabilis (**19**)	14	13	13	15	14
S. thirkei (**20**)	NT	NT	NT	NT	NT
S. balansae subsp. *carduchorum* (**21**)	16	17	17	19	19
S. longispicata (**22**)	NT	NT	NT	NT	18
α-Pinene	20	15	17	NA	13
β-Caryophyllene	20	24	23	22	21
Linalool oxide	NA	9	13	10	NA
Caryophyllene oxide	NA	NA	NT	NA	11
Sulphamethoxazole[*]	26	29	28	37	NT
Nystatin[*]	NT	NT	NT	NT	13

NA: non active NT: not tested.
[*] Sulphamethoxazole and nystatin were used as positive controls.
[a] The results are given in mm as zone diameter by disc diffusion and includes the 7 mm disc and the controls for the oil consisted of an equal volume of nutrient broth. Essential oil was used as 14 μL for each test and the pure compounds was used as 8 mg for each test. Three replicates have been done and mean value of them are reported for each assay.

Stachys are as follows; **1**: *Stachys cretica* L. subsp. *cassia* (Boiss.) Rech.f. (Osmaniye, Amanos Dağları, 850 m, July 2007, ISTE 86160), **2**: *S. cretica* L. subsp. *garana* (Boiss.) Rech.f. (Muş, Varto, 1770 m, August 2007, ISTE 86161), **3**: *S. cretica* L. subsp. *lesbiaca* (Boiss.) Rech.f. (Çanakkale, Ayvacık, 360 m, June 2009, ISTE 86162), **4**: *S. cretica* L. subsp. *symrnaea* (Boiss.) Rech.f. (Muğla, Marmaris, 120 m, June 2007, ISTE 86129), **5**: *S. cretica* L. subsp. *kutahyensis* Akçiçek (Kütahya, Tavşanlı, 850 m, June 2007, ISTE 86123), **6**: *S. viticina* Boiss. (Hatay, Yayladağı, 400 m, July 2007, ISTE 86138), **7**: *S. obliqua* Waldst. & Kit.(Balıkesir, Dursunbey, 900 m, July 2007, ISTE 86156), **8**: *S. balansae* Boiss. & Kotschy subsp. *balansae* (Bayburt, Kop Dağı, 2380 m, June 2008, ISTE 86150), **9**: *S. sericantha* P.H. Davis (Antalya, Kemer, 1200 m, June 2007, ISTE 86157), **10**: *S. pinetorum* Boiss. & Bal. (Osmaniye, Amanos Dağları, 850 m, July 2007, ISTE 86154), **11**: *S. bayburtensis* R. Bhattacharjee (Bayburt, Kop Dağı, 2020 m, June 2008, ISTE 86142), **12**: *S. huber-morathii* R. Bhattacharjee (Çorum, Kırkdilim Boğazı, 1050 m, June 2008, ISTE 86153), **13**: *S. huetii* Boiss. (Erzurum, Palandöken Dağı, 2500 m, June 2008, ISTE 86141), **14**: *S. tmolea* Boiss. (Balıkesir, Kaz Dağı, 1750 m, July 2007, ISTE 86159), **15**: *S. germanica* L. subsp. *heldreichii* (Boiss.) Hayek (Muğla, Ortaca, 5 m, June 2007, ISTE 86145), **16**: *S. germanica* L. subsp. *bithynica* (Boiss.) R.Bhattacharjee (Bursa, Uludağ, 2000 m, July 2008, ISTE 86146), **17**: *S. cretica* L. subsp. *anatolica* Rech.f. (Balıkesir, 150 m, May 2009, ISTE 86131), **18**: *S. cretica* L. subsp. *bulgarica* Rech.f. (Tekirdağ, 250 m, June 2009, ISTE 86127), **19**: *S. spectabilis* Choisy ex DC (Erzurum, Pasinler, 1680 m, August 2007, ISTE 86136), **20**: *S. thirkei* C. Koch (Yalova, 10 m, July 2008, ISTE 86134), **21**: *S. balansae* Boiss. & Kotschy subsp. *carduchorum* R.Bhattacharjee (Van, Bahçesaray, 2750 m, July 2009, ISTE 86151), **22**: *S. longispicata* Boiss. & Kotschy (Tunceli, Karakoçan, 1000 m, August 2008, ISTE 86137). The voucher specimens were deposited in the Herbarium of ISTE and Department of Biology Education, Necatibey Education Faculty, Balıkesir University.

3.2. Isolation of essential oils

250–650 g of dried aerial parts of the plants were cut into small pieces and subjected to hydro distillation with water for 3 h, using a Clevenger-type apparatus to produce the essential oils, which were stored under refrigeration until analysis. The yields of essential oils were determined to be between 0.1 and 0.25%. The essential oils of species were diluted by dichlorometane (1:3, v/v) before the GC run.

3.3. GC/MS conditions

GC/MS analyses were performed on Thermo Electron Trace 2000 GC model gas chromatography and Thermo Electron DSQ quadrupole mass spectrometry. A nonpolar Phenomenex DB5 fused silica column (30 m × 0.32 mm, ∅ with 0.25 μm film thickness) was used with helium at 1 mL/min (20 psi) as a carrier gas. The GC oven temperature was kept at 60 °C for 10 min and programmed to 220 °C at a rate of 4 °C/min and then kept constant at 220 °C for 15 min. The split ratio was adjusted to 1:20, the injection volume was 0.1 μL and EI/MS was recorded at 70 eV ionization energy. Mass range was *m/z* 35–500 amu. A homologous series of *n*-alkanes were used as reference in the calculation of Kovats Indices (KI). Identification of the compounds was based on the comparison of their retention times and mass spectra with those obtained from authentic samples and/or the NIST and Wiley spectra as well as the literature data.

3.4. Antibacterial and antifungal activity

The essential oil of twenty-two *species*, α-pinene, β-caryophyllene, linalool oxide and caryophyllene oxide were tested against standard bacterial strains which are *E. coli* ATCC 29995, *S.*

aureus ATCC 6538P, *K. pneumonia* CCM 2318, *P. aeruginosa* ATCC 27853, and the yeast *C. albicans* ATCC 10239. The agar diffusion method was used to determine the inhibition zones of the tested compounds and esssential oil against standard bacterial strains. Density of bacteria was determined by 0.5 McFarland standard which is used for visual comparison to adjust bacterial suspension to approximately 10^8 CFU/mL. *Mueller–Hinton Agar* (*BioLabs Cat. No: MHA2050*) *was used as a medium.* Agar was prepared as recommended by the manufacturer. All organisms were grown on Mueller–Hinton Agar and plates are incubated at 37 °C for 18 h. Inhibition effects of the essential oils and pure samples over bacteria were searched and the effects were given as diameter of inhibition zones (mm) which were determined as completely inhibiting visible growth by the naked eye (Goren et al., 2003; Fatimi et al., 2010).

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