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Chemical Composition and Antimicrobial Activity of Essential Oils from the Aerial Parts of *Salvia pinnata* L.

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Abstract: The composition of the essential oils obtained by hydrodistillation from the aerial parts of *Salvia pinnata* L. (Labiatae), collected during flowering and fruiting periods, were analyzed by GC and GC-MS. 37 compounds were identified representing 96.1 % of the essential oil obtained from the plant material collected during flowering period. 30 compounds were detected constituting 94.7 % of the essential oil of the plant material collected in fruiting period. The main components of the essential oils were characterized as bornyl acetate, camphor, camphene, bornyl formate, α-pinene and borneol. The oils were screened for antimicrobial activity by the micro-dilution assay against standard bacteria (*Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus*, *Enterococcus faecalis*) and yeast (*Candida albicans* and *Candida parapsilosis*). Both of the oils showed antimicrobial activity against the tested organisms.

Keywords: *Salvia pinnata*; Lamiaceae; essential oil composition; antimicrobial activity.

1. Plant Source

Salvia L., the largest genus of the family Lamiaceae, comprises of about 900 species, widespread throughout the world [1,2]. The genus *Salvia* is represented by about 95 taxa in Turkey [3]. The species of this genus are used traditionally all around the world to treat several ailments [2,4]. In Anatolia, members of *Salvia* have been commonly used against simple disorders such as colds and flu, tonsillitis, stomachache and flatulence especially in tea form [5-7]. Plants of this genus are shown to display many biological activities including antioxidant, anticholinesterase, anti-inflammatory, antinociceptive, antiprotozoal and antimicrobial activities [8-11].

The aerial parts of *Salvia pinnata* L. were collected during flowering and fruiting periods in 2011 from Sakran, Izmir, Turkey. The plant was identified by Umit Subası (Ege University, Faculty of

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Science) and voucher specimens (No: 1504 and 1521) are deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Ege University.

2. Previous Studies

The genus *Salvia* has been a subject of numerous chemical studies. It is a rich source of polyphenolic compounds. Terpenoids are also among the major secondary metabolites reported from this genus. *Salvia* species contain volatile components comprised mainly of monoterpenoids [12,13]. The chemical composition and biological activity of the essential oils from different *Salvia* species have been intensively reported in the literature [10,14,15,16]. A number of phytochemical studies reveal that *S. pinnata* is a source of triterpenoids and flavonoids [17,18]. There is only limited data on the essential oil composition of *S. pinnata* in the literature [19,20].

In the present study, a more detailed investigation was carried out on the essential oil of *S. pinnata*, collected during two different vegetation periods, from a different geographical region in Turkey. Antioxidant and anticholinesterase activities of this species have been reported elsewhere [8,20], however to the best our knowledge, there is no literature data available on the antimicrobial activity of *S. pinnata* essential oil.

3. Present Study

Isolation of the Essential Oils: Dried aerial parts of *S. pinnata*, collected during flowering and fruiting periods, were hydrodistilled for 3 h using a Clevenger apparatus to obtain essential oils in 0.11 and 0.06 % dry weight yields, respectively. The obtained oils were dried over anhydrous sodium sulphate and stored at +4 °C in the dark until analyzed.

Gas Chromatography-Mass Spectrometry Analysis (GC-MS): 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.

GC analysis: 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. The analysis results were given in Table 1.

Identification of components: Identification of the essential oil components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, Adams Library, MassFinder 3 Library) [21, 22], and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data [23, 24], was used for the identification.

Antimicrobial Activity: In vitro antimicrobial activity of the essential oils isolated from *S. pinnata* was investigated by the microdilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [25]. Minimum inhibitory concentrations (MICs) of the essential oils were determined against standard bacteria (*Escherichia coli* ATCC 25922, *Salmonella enterica* ATCC 13311, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212) and yeast (*Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019) strains. Antibacterial and antifungal activity assays were performed in Mueller-Hinton broth (MHB, Oxoid) and RPMI 1640 (Sigma, with glutamine, without bicarbonate) liquid mediums, respectively. Stock solutions of the essential oils were prepared in mixture of 25% DMSO + 75% MHB or RPMI-1640. The serial doubling dilutions of the essential oils were prepared in 96-well microplates, at concentrations ranging from 50 mg/mL to

0.024mg/mL. The standard bacteria and yeast strains were subcultured onto Mueller-Hinton agar (Oxoid) and Sabouraud Dextrose agar (Oxoid) plates, respectively, and incubated at 35 °C for 24-48 hours. Cell suspensions, which were prepared from recent cultures of the strains, were added to each well of the microplates to obtain final concentrations of approximately $5x10^5$ cells/mL for bacteria and $5x10^3$ cells/mL for yeasts. The plates were incubated at 35 °C for 24-48 hours. The MIC values were determined as the lowest concentrations of essential oils preventing visible growth of the microorganisms. All experiments were performed in triplicate. Ampicillin (Sigma) and fluconazole (Sigma) were used as reference standard antimicrobials. The possible inhibitory effect of the DMSO was also checked at the highest tested concentration.

Table 1. The chemical compositions of the essential oils of *S. pinnata*.

RRI	Compound	A %	B %
1014	Tricyclene	0.4	0.6
1032	α-Pinene	3.5	6.8
1035	α-Thujene	0.1	0.2
1076	Camphene	9.9	15.1
1118	β-Pinene	2.1	3.5
1203	Limonene	1.4	1.7
1213	1,8-Cineole	-	1.8
1218	β-Phellandrene	0.3	0.1
1255	γ-Terpinene	-	0.1
1280	*p*-Cymene	0.9	1.1
1393	3-Octanol	-	0.1
1400	Nonanal	-	0.2
1452	1-Octen-3-ol	0.8	1.4
1497	α-Copaene	2.0	1.9
1532	Camphor	12.0	18.4
1571	*trans*-*p*-Menth-2-en-1-ol	t	0.5
1588	Bornyl formate	6.5	5.3
1591	Bornyl acetate	43.3	25.6
1597	β-Copaene	0.4	0.6
1611	Terpinen-4-ol	0.6	0.9
1638	*cis*-*p*-Menth-2-en-1-ol	0.2	0.4
1664	Nonanol	-	0.2
1689	*trans*-Piperitol	0.3	0.3
1704	γ-Muurolene	0.5	0.8
1719	Borneol	4.1	3.9
1726	Germacrene D	t	0.4
1740	α-Muurolene	0.6	0.4
1758	*cis*-Piperitol	0.6	0.3
1766	Decanol	0.4	0.3
1773	δ-Cadinene	t	0.2
1776	γ-Cadinene	0.5	0.4
1800	Octadecane	-	0.3
1815	2-Tridecanone	1.1	0.3
1849	Calamenene	0.6	0.4
2088	1-*epi*-Cubenol	-	0.3
2256	Cadalene	0.7	0.6
2384	Hexadecanol	0.9	0.7
	Total	**94.7**	**96.1**

A: Fruiting stage, B: Flowering stage; *n*-Alkanes (C9-C20) were used as reference points in the calculation of retention indices (RRI); % calculated from FID data; t : Trace (< 0.1 %)

The GC analysis of the essential oils of *S. pinnata* collected in flowering and fruiting periods, resulted in the identification of different compounds, representing 96.1 and 94.7 % of total oil constituents, respectively (Table 1). The main components of the essential oils were found as bornyl acetate (flowering: 25.6 %, fruiting: 43.3 %) camphor (flowering: 18.4 %, fruiting: 12.0 %), camphene (flowering: 15.1 %, fruiting: 9.9 %), bornyl formate (flowering: 5.3 %, fruiting: 6.5 %), α-pinene (flowering: 6.8 %, fruiting: 3.5 %) and borneol (flowering: 3.9 %, fruiting: 4.1 %). Among these, bornyl acetate was detected as the major compound in both of the essential oils. According to the

classification of the main components in *Salvia* oils of Turkish origin, the oil of *S. pinnata* may be included in the group of *Salvia* oils which are rich in other monoterpene esters [16].

In a previous study, camphor (29.21 %), α-pinene (16.39 %), camphene (15.39 %) and 1,8-cineole (10.28 %) were identified as the major constituents of the essential oil of *S. pinnata* leaves, collected during flowering season from Kayseri province in Central Anatolia [19]. In another study, the GC-MS investigation of the essential oil of the flowering aerial parts of *S. pinnata* collected from Mugla province in Southwest of Turkey, yielded α-pinene (6.45 %), camphene (13.86 %), β-pinene (5.79 %), camphor (8.97 %), borneol (20.24 %), isobornyl formate (7.02 %) and isobornyl acetate (26.24 %) [20]. It is interesting to note that bornyl acetate, the major component of *S. pinnata* essential oils investigated in the present study, was previously found as 1.50 % in the essential oil of *S. pinnata*, collected from Kayseri. Also, bornyl acetate was not detected in the essential oil of *S. pinnata* growing in Mugla, but isobornyl acetate was reported as the major component in this plant sample. Moreover, in comparison with previous work, significant variations were observed in camphor [19,20], α-pinene [19], borneol [20], and 1,8-cineole [19] contents of the essentials oils of *S. pinnata*. In conclusion, the results of the present and previous studies indicate that there are some quantitative and qualitative differences in the oil compositions which may be due to several factors such as different growth stage and/or collection site of the plant material.

The results of antimicrobial activity of the essential oils obtained from *S. pinnata* are shown in Table 2. The essential oil of *S. pinnata* collected during flowering period was found to be more effective against Gram positive bacteria (*S. aureus* and *E. faecalis*) than the essential oil of *S. pinnata* collected during fruiting period. Essential oils collected during flowering and fruiting periods, were equally effective against Gram negative bacteria (*E. coli* and *S. enterica*). Hence, anti-Gram negative bacterial activity of these two essential oils were similar. Also, there was no difference observed between the antifungal activity of the tested essential oils. In general, MICs of the essential oils against yeast strains were lower than that of the bacterial strains, which indicated the presence of slightly more powerful antifungal activity.

Table 2. Antimicrobial activity of the essential oils obtained from *S. pinnata.*

Microorganism	MIC (mg/mL)	
	Flowering stage	Fruiting stage
E. coli ATCC 25922	50	50
S. enterica ATCC 13311	25	25
S. aureus ATCC 25923	25	50
E. faecalis ATCC 29212	12.5	50
C. albicans ATCC 10231	12.5	12.5
C. parapsilosis ATCC 22019	12.5	12.5

MIC: Minimal inhibitory concentration

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