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CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF *Stachys acerosa* AND ITS ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES

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Prevention of oxidants and microorganisms activity has been the scope of a lot of recent studies [1–4]. Although synthetic antioxidants such as butylated hydroxy toluene (BHT), and butylated hydroxy anisole (BHA) are frequently employed in food industries, there are reports on their side effects [5]. On the other hand, in recent years due to an upsurge in antibiotic-resistant infections, the search for new prototype drugs to combat infections is an absolute necessity [6]. The use of essential oils and plant extracts is considered an advantageous alternative of synthetic antioxidants and antibiotics and there are many reports in this regard [7–12].

The hydrodistillation of aerial parts of *S. acerosa* gave an oil in 0.46% (w/w) yield based on the dry weight of the plant. The essential oil composition along with retention indices and their percentages are listed in Table 1 in order of their elution on the DB-1 column. Forty components were identified, accounting for 98.8% of the total oil. The main constituents of the oil were *cis*-chrysanthenyl acetate (33.4%), 1,8-cineole (10.2%), α-pinene (10.1%), linalool (9.6%), and limonene (6.0%). The acetylated compounds comprised 36% of the oil. The classification of the oil compounds based on functional groups is presented at the end of Table 1. As shown, the oxygenated monoterpenes with 63.3% of the total oil were the principal compound group followed by monoterpene hydrocarbons (22.4%). Sesquiterpenoids comprised 13.1% of the oil, with viridiflorol (3.4%), *trans*-caryophyllene (2.9%), and caryophyllene oxide (2.2%) as the major compounds. In the present study, 22.4% of the oil belongs to monoterpene hydrocarbons, with α-pinene (10.1%) and limonene (6.0%) as the main components, while in previous investigation [13] monoterpene hydrocarbons constituted 1.4% of the total oil. 1,8-Cineole (10.2%), one of the major oil components of our study, was not found in the previous report.

The antibacterial activity of the essential oil of *S. acerosa* and its three main components are shown in Table 2. *B. subtilis* and *S. epidermidis* with 17 and 18 mm zones of growth inhibition and similar MIC values of 3.75 mg/mL were more sensitive to the oil than other examined bacteria (Table 2). The oil has no activity against *K. pneumoniae* and *P. aeruginosa*. The antibacterial activity of the essential oil may well be due to the presence of synergy between the tested major components and other constituents of the oil. Considering the fact that *S. acerosa* oil contained 1,8-cineole (10.2%), linalool (9.6%), and α-pinene (10.1%), the results obtained may be attributed to the presence of these compounds. The various extracts of *S. acerosa* showed moderate or no antibacterial activity against the tested bacteria.

The antioxidant activity of the *S. acerosa* extracts were measured by DPPH assay. The free radical scavenging activity of the *n*-butanol subfraction of methanol extract (IC_{50} = 22.7 μg/mL) was superior to all other extracts. This stronger antioxidant activity could be related to its higher phenolic content (182.1 mg/L) as measured by gallic acid test (Table 3).

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TABLE 1. Essential Oil Composition of *Stachys acerosa*

Compound	RI (DB-WAX)	RI (DB-1)	%	Identification method	Compound	RI (DB-WAX)	RI (DB-1)	%	Identification method
α-Thujene	-	924	0.8	RI, MS	Myrtenyl acetate	1676	1295	0.4	RI, MS
α-Pinene	1013	936	10.1	RI, MS, Co-I	α-Terpinyl acetate	-	1309	1.2	RI, MS
Camphene	-	947	Tr.	RI, MS	Neryl acetate	1708	1335	Tr.	RI, MS
Sabinene	1095	968	1.0	RI, MS	Geranyl acetate	1739	1340	1.0	RI, MS
β-Pinene	1110	974	0.2	RI, MS, Co-I	*trans*-Caryophyllene	1629	1359	2.9	RI, MS
Myrcene	1154	981	1.3	RI, MS	α-Humulene	-	1427	0.1	RI, MS
α-Phellandrene	1151	1000	0.3	RI, MS	*ar*-Curcumene	1751	1456	0.2	RI, MS
δ-3-Carene	1134	1009	1.5	RI, MS	Germacrene D	-	1471	0.5	RI, MS
α-Terpinene	-	1012	Tr.	RI, MS	α-Selinene	-	1481	Tr.	RI, MS
p-Cymene	-	1015	Tr.	RI, MS	Zingiberene	-	1485	Tr.	RI, MS
Limonene	1184	1026	6.0	RI, MS	Bicyclogermacrene	1704	1487	0.6	RI, MS
1,8-Cineole	1193	1028	10.2	RI, MS	α-Panasinsen	1731	1498	0.7	RI, MS
Ocimene	1225	1038	0.6	RI, MS, Co-I	Spathulenol	2046	1521	1.1	RI, MS
γ-Terpinene	1231	1051	0.6	RI, MS, Co-I	Caryophyllene oxide	1945	1575	2.2	RI, MS
Linalool	1565	1089	9.6	RI, MS	Viridiflorol	2025	1581	3.4	RI, MS
allo-Ocimene	1267	1118	Tr.	RI, MS	Ledol	1992	1595	0.2	RI, MS
trans-Pinocarveol	-	1129	Tr.	RI, MS	*t*-Muurolol	-	1603	1.2	RI, MS
Verbenol	1670	1133	Tr.	RI, MS	Monoterpene hydrocarbons			22.4	
4-Terpineol	1650	1151	0.6	RI, MS	Oxygenated monoterpenes			63.3	
α-Terpineol	1684	1168	4.8	RI, MS	Sesquiterpene hydrocarbons			5.0	
Nerol	1780	1179	Tr.	RI, MS	Oxygenated sesquiterpenes			8.1	
Chrysanthenyl acetate	1595	1220	33.4	RI, MS	Total			98.8	
trans-2-Caren-4-ol	-	1259	2.1	MS					

RI: retention indices relative to C6-C24 *n*-alkanes on the DB-Wax and DB-1 columns; MS: mass spectrum; Co-I: coinjection with an authentic sample; Tr.: trace (<0.1%).

TABLE 2. Antibacterial Activity of the Essential Oil and the Three Main Components of *S. acerosa*

Microorganism	Essential oil[a]		1,8-Cineole[a]		α-Pinene[a]		Linalool[a]		Antibiotic
	IZ[b]	MIC[c]	IZ	MIC	IZ	MIC	IZ	MIC	ampicillin[d]
Bacillus subtilis	17±0.5	3.75	25±0.2	0.93	10±0.6	7.5	28±0.6	0.2	14±0.4
Enterococcus faecalis	9±0.4	N.t.	10±0.2	7.5	N.a	N.t	10±0.6	2.5	11±0.3
Staphylococcus aureus	13±0.2	7.5	15±0.4	3.75	8±0.4	>15	17±0.9	0.6	13±0.3
Staphylococcus epidermidis	18±0.2	3.75	18±0.5	1.87	9±0.5	15	26±1.0	0.2	19±0.5
Escherichia coli	12±0.3	7.5	20±0.6	1.87	11±0.1	15	20±0.9	1.2	12±0.2
Klebsiella pneumoniae	N.a	N.t	8±0.2	7.5	N.a	N.t	13±0.9	0.6	N.a
Pseudomonas aeruginosa	N.a	N.t	N.a	N.t	N.a	N.t	N.a	N.t	9±0.2

[a]Essential oil and its main components tested at a volume of 10 µL/disc., [b]inhibition zone values in mm; [c]minimum inhibitory concentration values in mg/mL; [d]tested at 10 µg/disc.

N.a: not active; N.t: not tested.

Values for inhibition zones are given as mean ± SD.

TABLE 3. Total Phenolic Compounds and Radical Scavenging Capacity of Various Extracts of *S. acerosa* against DPPH (IC_{50})

Extracts	Gallic acid equivalents, mg/L	IC_{50}, µg/mL
Methanol extract	115.7	41.0
n-Butanol subfraction of methanol extract	182.1	22.7
Chloroform subfraction of methanol extract	81.8	145.0
Water soluble of methanol extract	132.4	42.4
Water extract	108.3	104.5
Control	-	26.5

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