Journal of Oleo Science
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J. Oleo Sci. **58**, (8) 443-446 (2009)

NOTE

In vitro Biological Activity of *Salvia leriifolia* Benth Essential Oil Relevant to the Treatment of Alzheimer's Disease

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Abstract: In this study the chemical composition, cholinesterase inhibitory property and anti-inflammatory activity of *S. leriifolia* Benth. essential oil was evaluated for the first time. GC and GC-MS analysis revealed the presence of camphor (10.5%), 1,8-cineole (8.6%), camphene (6.2%) and α-pinene (4.7%) as main constituents. *S. leriifolia* oil exhibited a promising antioxidant activity by DPPH assay with an IC_{50} 2.26 μL/mL. Interesting cholinesterase inhibitory activity was also found with IC_{50} values of 0.32 and 0.29 μL/mL for acetylcholinesterase (AChE) and butyrrylcholinesterase (BChE), respectively. Moreover, this oil inhibited LPS-induced NO production with an IC_{50} value of 165 μg/mL. The absence of cytotoxicity at 1000 μg/mL was evaluated by MTT assay in 142BR cells.

Key words: *Salvia leriifolia*, Essential oil, GC-MS, Cholinesterase inhibition, NO production inhibition

1 INTRODUCTION

Salvia has a long history of use in the treatment of a variety of disorders. Imanshahidi *et al.*[1] reviewed evidence for sage as a remedy which may be used for the treatment of disorders of the nervous system and in particular for the treatment of dementia. Volatile constituents of the essential oils of *Salvia* species are likely to readily cross the blood-brain barrier due to their small molecular size and lipophilicity. Alzheimer's disease (AD) is the most common form of neurodegenerative disorders. In spite of the multifactorial nature of AD, actually only the anti-cholinergic therapeutic approach is followed. Moreover, a recent study demonstrated as cholinergic up-regulation obtained with the use of acetylcholinesterase inhibitors was associated to an anti-inflammatory effect which is involved in AD. This identified new role of acetylcholinesterase inhibitors emphasizes the importance of cholinergic balance in this neurological disorder[2].

As a part of our research on essential oils biological property, in the present work we report for the first time the chemical composition of the oil from the aerial parts of *S. Salvia leriifolia* Benth. The antioxidant, cholinesterase inhibitory activity and the anti-inflammatory properties were also evaluated.

2 EXPERIMENTALS

2.1 Plant material

Aerial parts of *S. leriifolia* Benth. were collected at the full flowering stage from plants growing wild in Khorassan (Iran) and authenticated by Dr. F. Nadjafi. A voucher specimen was deposited in Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran,

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Accepted April 4, 2009 (received for review March 27, 2009)

Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online
http://www.jstage.jst.go.jp/browse/jos/

Iran.

2.2 Essential oil isolation and analysis

The oil from air-dried ground aerial parts of *S. leriifolia* was obtained by hydrodistillation for 3 h, using a Clevenger-type apparatus[3]. The essential oil analysis was carried out as previously described[4]. The identification of the compounds was achieved through retention indices (*I*), with those of the literature or with those of authentic compounds available in our laboratory[4].

2.3 Biological assay

Antioxidant activity was evaluated by DPPH assay[5]. Acetylcholinesterase- and butyrylcholinesterase-inhibiting activities were measured by slightly modifying the spectrophotometric method developed by Ellman *et al.*[5]. The presence of nitrite, a stable oxidized product of NO, was determined in cell culture media by Griess reagent as previously described. Cytotoxicity was determined using the MTT assay as previously described using murine monocytic macrophage cell line RAW 264.7 (ECCC, UK)[6].

3 RESULTS AND DISCUSSION

Hydrodistillation of *Salvia leriifolia* aerial parts yielded 1.5 w/w % essential oil. Forty-nine constituents, representing 97.3% of the total component of *S. leriifolia* oil, have been identified. Retention index, percentage composition and identification method are given in Table 1. Camphor (10.5%), 1,8-cineole (8.6%), camphene (6.2%) and α-pinene (4.7%) were the main components. Also β-pinene (2.7%), isoborneol (1.5%) and α-terpinene (1.2%) were present in considerable amounts. Several Iranian *Salvia* species were investigated. According to Mohammadhosseini *et al.*[7] camphor (19.0%) represents the main abundant compound in *S. multicaulis* oil. Differences in α-pinene content could be appreciated in several *Salvia* species collected in Iran[8].

S. leriifolia essential oil exhibited promising antioxidant activity with an IC_{50} 2.26 μL/mL. Interesting was also oil cholinesterase inhibitory properties with IC_{50} values of 0.32 and 0.29 μL/mL for AChE and BChE, respectively. The higher activity against BChE is of a certain interest since, in the late stage AD, levels of AChE have declined by up to 85% and BChE represents the predominant ChE in brain[9]. For this reason, recently, studies have targeted BChE as a new approach to intercede in the progression of AD. Previously, Perry *et al.*[10], reported the AChE inhibitory activity of 1,8-cineole and α-pinene with IC_{50} of 0.67 and 0.63 mM, respectively. Camphor was less potent (IC_{50} >10 mM). Our investigation on BChE inhibitory activity reveled that main compounds founded in *S. lerifolia* oil such as camphor, camphene and β-pinene did not inhibited the enzyme at maximum concentration tested 10 mM. On the contrary α-pinene and 1,8-cineole showed IC_{50} of 0.87 and 0.93 mM, respectively, aginst BChE. The structural diversity of the active anticholinesterase terpenoids complicates the prediction of potential structure-activity relationships.

S. leriifolia essential oil inhibited the production of inflammatory mediators probably through oxidative degradation of products of phagocytes, such as O^{2-} and HOCl. Incubation of RAW 264.7 cells with essential oil of *S. leriifolia* induced a significant inhibitory effect on the LPS-induced nitrite production (IC_{50} 165 μg/mL). This activity may be done to the presence of monoterpenes. Different previous studies demonstrated that essential oils and some constituents, such as 1,8-cineole exerted anti-inflammatory activity[11]. *S. leriifolia* essential oil did not show any cytotoxicity up to 1000 μg/mL concentration.

For many of the plants and compounds that have demonstrated activities anticholinesterase activity relevant to AD therapy, the clinical data are very limited. Clinical efficacy and potential toxicity of active plants and compounds in larger trials requires further assessment, before recommendations concerning their routine use can be identified.

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Table 1 Essential Oil Composition of *Salvia leriifolia* Benth.

Compound	I^a	I^b	%	ID method[c]
Thujene	926	1035	0.8	*I*, MS
α-Pinene	936	1032	4.7	*I*, MS, Co-GC
Camphene	953	1076	6.2	*I*, MS, Co-GC
β-Pinene	978	1118	2.7	*I*, MS, Co-GC
β-Myrcene	986	1174	0.9	*I*, MS, Co-GC
α-Phellandrene	1005	1186	0.6	*I*, MS
δ-3-Carene	1012	-	0.5	*I*, MS, Co-GC
α-Terpinene	1016	1188	1.2	*I*, MS, Co-GC
1,8-Cineole	1035	1213	8.6	*I*, MS, Co-GC
(*Z*)-β-Ocimene	1047	1245	0.9	*I*, MS
γ-Terpinene	1059	1255	0.4	*I*, MS
Terpinolene	1089	1290	0.7	*I*, MS, Co-GC
Linalool	1098	1553	tr	*I*, MS
n-Nonanal	1100	1400	1.3	*I*, MS
α-Campholene aldehyde	1128	1499	0.8	*I*, MS
trans-Pinocarveol	1138	1664	0.5	*I*, MS
Camphor	1147	1532	10.5	*I*, MS
Terpinen-4-ol	1178	1611	0.9	*I*, MS
α-Terpineol	1189	1683	0.7	*I*, MS
Myrtenol	1196	1804	0.6	*I*, MS, Co-GC
Isoborneol	1203	-	1.5	*I*, MS
n-Decanal	1205	1506	1.2	*I*, MS
trans-2-Caren-4-ol	1216	-	0.5	*I*, MS
Nerol	1232	1797	0.5	*I*, MS
Geraniol	1255	1857	0.6	*I*, MS
Neral	1258	1694	tr	*I*, MS
Phellandral	1286	-	0.9	*I*, MS
α-Cubebene	1351	1466	1.1	*I*, MS
Isocaryophyllene	1412	1666	1.0	*I*, MS
β-Caryophyllene	1418	1612	2.4	*I*, MS, Co-GC
β-Gurjunene	1432	1610	4.9	*I*, MS
Aromadendrene	1436	1628	0.2	*I*, MS
α-Humulene	1454	1690	1.1	*I*, MS, Co-GC
allo-Aromadendrene	1461	1661	1.7	*I*, MS
Calarene	1482	-	1.7	*I*, MS
Valencene	1488	1740	2.6	*I*, MS
α-Muurolene	1499	1742	2.9	*I*, MS
γ-Cadinene	1515	1765	2.1	*I*, MS, Co-GC
δ-Cadinene	1524	1772	2.0	*I*, MS, Co-GC
Spathulenol	1579	2150	3.7	*I*, MS
Epiglobulol	1580	-	0.6	*I*, MS
Globulol	1582	2098	4.0	*I*, MS
Torreyol	1587	-	1.3	*I*, MS
Fonenol	1590	-	2.4	*I*, MS
1,2,3,4,4a,5,6,8a-Octahydro-7-methyl-4-methylene-1-(1-methylethyl) naphthalene	1592	-	2.8	*I*, MS
Viridiflorol	1594	2104	4.1	*I*, MS
Eicosane	2000	2000	0.7	*I*, MS, Co-GC
Tricosane	2300	2300	2.7	*I*, MS, Co-GC
Pentacosane	2500	2500	3.1	*I*, MS, Co-GC
Eptacosane	2700	2700	1.4	*I*, MS, Co-GC
Identified compounds			97.3	

[a] SE-30 MS column. [b] HP-Innowax MS column. [c] *I*, Retention index; MS, mass spectrum; Co-GC: co injection with authentic compound. tr: trace, < 0.1%.

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