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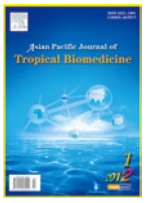

Document heading

Chemical composition analysis and antioxidant activity evaluation of essential oil from *Orthosiphon thymiflorus* (Roth) Sleesen

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ABSTRACT

Objective: *Orthosiphon thymiflorus* (Roth) Sleesen (*O. thymiflorus*) is an aromatic plant widely distributed in the Western Ghats of South India. The present study investigates the chemical composition and antioxidant activities of *O. thymiflorus* essential oil isolated from the leaves. **Methods:** The essential oil was hydrodistilled from the leaves by using Clevenger apparatus. Isolated essential oil was chemically analyzed by gas chromatography (GC) and gas chromatography- mass spectrometry (GC-MS). *In vitro* antioxidant activity of the isolated essential oil was determined. **Results:** Chemical composition analysis of essential oil reported to the presence of thirty three compounds. The major compound was 2-isopropyl-5-methyl-9-methylene-bicyclo-1-decene (4.4.0) (42.62%) followed by carotol (16.48%), α -cadinol (6.14%) and δ -3-carene (5.15%). *In vitro* antioxidant activity of the isolated oil showed dose dependent free radical scavenging activity against DPPH (IC_{50} 437.12±0.02 μg/mL) and hydroxyl radicals (IC_{50}317.88 ±0.02 μg/mL). Metal chelating and prevention of deoxyribose degradation activities of the oil was observed with the IC_{50} of 339.78±0.01 μg/mL and 158.33±0.02 μg/mL respectively. Antioxidant activity of the oil was low when compared with the positive controls such as BHT and ascorbic acid. **Conclusion:** The present study shows various chemical components present in the *O. thymiflorus* essential oil and with it's significant antioxidant activity thus has great potential to be used as natural food supplement.

1. Introduction

The genus *Orthosiphon* belongs to the family Lamiaceae. The species are annual or perennial herbs mainly distributed in Eastern and Western Ghats of India. Members of this genus produce many useful secondary metabolites including flavones, glycosides, volatile oil, betulinic acid, oleanolic acid, ursolic acid, flavonoids and rosmarinic acid [1, 2]. Earlier studies reported that Orthosiphon species exhibit dynamic pharmacological properties such as, antioxidant, antibacterial, hepatoprotective, antiinflammatory, cytotoxic, diuretic, antihypertensive and vasodialative properties [3-5].

Orthosiphon thymiflorus (Roth) Sleesen is a common annual aromatic erect herb that grows on the slopes, in crevices of rocks and as colonizer along the road, characterized by the hair that covers all parts of the plant. Only few reports are available about its medicinal uses. Aqueous extract of leaves is reported to have diuretic activity in rats [6] and acetylcholine antagonistic activity in frog skeletal muscle contraction [7]. To our knowledge, there are no published reports on the chemical composition and antioxidant activities of the essential oil from O.thymiflorus. Therefore we focused our study on the analysis of chemical composition and antioxidant activity of this essential oil.

2. Materials and methods

2.1. Plant material

The plant material *Orthosiphon thymiflorus* (Roth) Sleesen was collected from Aliyar which is located at the foot hills of Valparai, Coimbatore, Tamil nadu, India. An authentic sample was identified by Botanical Survey of India (BSI), Southern Circle Coimbatore, and voucher specimen has been deposited in BSI (BSI/SRC/5/23/10-11/Tech.-1228). To

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avoid causalities some of the plants were maintained under green house condition at Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore.

2.2. Essential oil extraction

Freshly collected leaves of O.thymiflorus were extracted by hydrodistillation for 3h using a Clevenger apparatus. The extracted essential oil was dried over anhydrous sodium sulphate and the purified oil was filled in small vials, tightly sealed and stored in a refrigerator (4℃) until further analysis.

2.3. Chemical composition analysis

2.3.1. Gas chromatography (GC) analysis

Gas chromatography (GC) analysis was carried out using Varian 3800 gas chromatography equipped with mass selective detector coupled to front injector type 1079. The chromatograph was fit with DB 5 column (30 m×0.25 mm). The injector temperature was set at 280℃, and the oven temperature was initially maintained at 45℃ then set to 300 ℃ at the rate of 10℃/min and finally held at 200℃ for 5 min. Helium was used as a carrier gas with the flow rate of 1.0 mL/min. The percentage of composition of the essential oil was calculated by the GC peak areas.

2.3.2. Gas chromatography/ mass spectrometry (GC/MS) analysis

Gas chromatography coupled with mass spectroscopy was performed using Varian 3800 gas chromatography equipped with Varian 1200L single quadrupole mass spectrometer. The GC condition was the same as described earlier. The mass spectrometer was operated in the electron impact mode at 70 eV. Ion source and transfer line temperature was maintained at 250℃. The compounds were identified based on the comparison of retention indices (RI), retention time (RT) and mass spectra.

2.4. Antioxidant activity

2.4.1. DPPH radical scavenging activity

Radical scavenging activity was determined according to Blois [8]. Different concentrations of essential oil was dissolved in DMSO and mixed individually with 0.1 mM DPPH and 50 mM Tris-HCl buffer (pH 7.4). Reaction mixture was incubated at 37 ℃ for 30 min and then absorbance was measured at 517 nm. The percentage of DPPH scavenging activity was calculated using the following equation: % Inhibition = [(AB - AA)/AB]×100, where AB, absorption of blank sample, AA, absorption of test sample.

2.4.2. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of essential oil was measured by the method of Zhao *et al.* [9]. Reaction mixture includes 7.5 mM $FeSO_4$, 7.5 mM 1, 10-phenanthroline, 0.2 M phosphate buffer (pH7.8), 30 mM H_2O_2 and different concentrations of essential oil. The reaction was started by adding H_2O_2. After incubation at room temperature for 5 min, the absorbance of the mixture was read at 536 nm was measured. % Inhibition = [(AB - AA)/AB]×100, where AB, absorption of blank sample, AA, absorption of test sample.

2.4.3. Metal chelating activity

Chelation of ferrous ions by essential oil was estimated by method of Dinis *et al.* [10] Briefly, 2 mM $FeCl_2$ was added to different concentrations of essential oil. Further, reaction was initiated by the addition of 5 mM ferrozine. The mixture was vigorously shaken and left to stand at room temperature for 10 min. Absorbance was measured read at 562 nm after 10 min. % Inhibition = [(AB - AA)/AB] × 100, where AB, absorption of blank sample, AA, absorption of test sample.

2.4.4. Prevention of deoxyribose degradation activity

The ability of essential to prevent Fe^{2+}/ H_2O_2-induced decomposition of deoxyribose was carried out using the method of Halliwell and Gutteridge [11].

Different concentration of oil was added to a reaction mixture containing 20mM deoxyribose, 0.1M phosphate buffer, 20mM hydrogen peroxide and 500 μM $FeSO_4$.

The reaction mixture was incubated at 37℃ for 30 min, and the reaction was then stopped by the addition of 2.8% TCA. This was followed by the addition of 0.6% TBA solution. The tubes were subsequently incubated in boiling water for 20 min. The absorbance was measured at 532 nm. % Inhibition = [(AB - AA)/AB]×100, where AB, absorption of blank sample, AA, absorption of test sample.

2.5. Statistical analysis

Data obtained from the *in vitro* experiments were analysed using SPSS (10.00) for IC_{50} calculation.

3. Results

3.1. Chemical composition of essential oil

Reddish orange color oil (1mL from 500g of fresh leaves) was obtained by hydrodistillation of *Orthosiphon thymiflorus* leaves, of which 33 compounds were identified. 2-isopropyl-5-methyl-9-methylene-bicyclo-1-decene (4.4.0) was identified as major compound which accounted for 42.62%. The other major components were carotol (16.48%), α -cadinol (6.14%) δ -3-carene (5.15%), α -humulene (3.61%), γ - cadinene (3.34%) and cis -caryophyllene (1.76%) (Table 1).

3.2. Antioxidant activity

The essential oil was subjected to screening for the possible

antioxidant activity by four complementary test systems namely DPPH free radical, hydroxyl radical scavenging, metal chelating and prevention of deoxyribose degradation. The antioxidant activity of the essential oil was considerably significant in all test systems studied and at the same time activity of the oil was lower than that of positive controls such as ascorbic acid and BHT. The results are presented in Table 2.

Table 1.
Chemical composition of the essential oil of *Orthosiphon thymiflorus* leaves.

S.No.	RI*	Compounds Name	%
1	939	α - pinene	0.54
2	976	Sabinene	0.05
3	980	α - pinene	0.52
4	1011	δ -3-carene	5.15
5	1022	o-cymene	0.23
6	1023	Menthene <1-para>	0.61
7	1026	p-cymene	0.65
8	1031	α -phellandrene	0.66
9	1040	cis- α -ocimene	0.01
10	1088	Terpinolene	0.13
11	1098	Linalool	0.33
12	1143	Camphor	0.04
13	1179	Naphthalene	0.38
14	1189	α - terpineol	0.02
15	1235	trans -chrysanthenyl acetate	0.30
16	1351	α - cubebene	0.18
17	1376	α -copaene	0.35
18	1391	α - elemene	0.26
19	1397	Cedrene <1,7-di-epi-alpha>	0.23
20	1401**	2- isopropyl-5-methyl-9- methylene-bicyclo-1-decene(4.4.0)	42.62
21	1404	cis -caryophyllene	1.76
22	1409	α - cedrene	0.01
23	1454	α -humulene	3.61
24	1461	Allo-aromadendrene	0.40
25	1491	Valencene	0.01
26	1499	α -muurolene	0.12
27	1513	γ - cadinene	3.34
28	1524	α -sesquiphellandrene	0.31
29	1574	GermacreneD-4-ol	0.14
30	1594	Carotol	16.48
31	1653	α -cadinol	6.14
32	1661	Lyral	0.40
33	1683	α -bisabolol	0.83
		Total identified	86.81

*Retention Indices obtained on DB 5 column
** Ayse Yusufoglu et al. (2004) - DB 5 HT column

Table 2.
Antioxidant activity of *O. thymiflorus* essential oil.

Assays	IC50 (μg mL^{-1})a
DPPH free radical scavenging	437.12±0.02
Hydroxyl radical scavenging	317.88±0.02
Metal chelation	339.78±0.01
Prevention of deoxyribose degradation	158.33±0.02
Ascorbic acid	12.49±0.02
BHT	30.14±0.01

[a] IC_{50} values were expressed as the mean ± SD of three replicates

4. Discussion

4.1. Chemical composition of essential oil

Plant based secondary metabolites such as essential oil and extracts are widely used in the pharmaceutical industries and considered Generally Recognized as Safe (GRAS). Very little information is available in the literature on the chemical composition of Orthosiphon essential oils. Sesquiterpene, 2-isopropyl-5-methyl-9-methylene-bicyclo-1-decene (4.4.0) was identified as major compound. Similar result was reported in the essential oil of Lavandula [12]. In addition *O.thymiflorus* essential oil reported to the presence of various chemical components like α -humulene, cis -caryophyllene, linalool, pinenes, elemene, cymene,

valencene and camphor. This result is in agreement with the report of Amzad Hossain *et al*. [13].

4.2. Antioxidant activity

Essential oils of lamiaceae members such as ocimum, salvia, oregano and thyme have proved to be potential radical scavengers [14]. It is assumed that observed antioxidant activity of the *O. thymiflorus* essential oil might be influenced by the presence of bioactive compounds such as monoterpenes, sesquiterpene alcohols and sesquiterpenes The essential oil of lemon balm (Melissa officinalis L.) showed an antioxidant and free radical scavenging activity [15] with the most powerful scavenging constituents comprising of monoterpenes, sesquiterpene alcohols and sesquiterpenes such as neral geranial, citronellal, isomenthone and menthone.

Conflict of interest statement

We declare that we have no conflict of interest.

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