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Chemical composition and antioxidant activity of the essential oil of *Stachys yemenensis* Hedge: Endemic in Yemen

Amina Al-Shaibani[1]*, Martina Wurster[2], Mehdi A.Alhaj[3], Al-Husein M.R.Al-Othman[1], Ulrike Lindequist[2]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Sana'a University, (YEMEN)

[2]Department of Pharmaceutical Biology, Institute of Pharmacy, Ernst-Moritz-Arndt-University, Greifswald, Friedrich-Ludwig-Jahn-Str. Greifswald, (GERMANY)

[3]Department of Medicinal Chemistry, Faculty of Pharmacy, Aden University, (YEMEN)

E-mail: aminaalshaibani@yahoo.com

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ABSTRACT

The essential oil from leaves of *Stachys yemenensis* Hedge endemic to Yemen (Lamiaceae), was analysed by using GC-MS. Twelve components showed represented 99.3% of the total oil. The major components of the oil were α- phellandrene (34.7%), β-phellandrene (11.2%), limonene (10.8%), elemol (8.5%), α-eudesmol (8.4%), bicyclogermacrene (6.4%), p-cymene (5.4%), spathulenol (3.4%), myrcene (3.2%), β-eudesmol (3.1%), and δ-cadinene (2.7%). The antioxidant activity of the oil was investigated by DPPH method and showed antiradical effect, with IC_{50} value of 1.12 mg/ml.

KEYWORDS

Stachys yemenensis;
Lamiaceae;
Essential oils;
GC-MS;
α-Phellandrene.

INTRODUCTION

The genus *Stachys* is one of the largest genera of the Lamiaceae family and distributed in the Mediterranean region and South-West Asia. About 300 Stachys species are recorded. Two species of this genus are found in Yemen, *S. yemenensis* (indigenous) and *S. aegyptiaca*[1,2].

Stachys species have been reported in folk medicine to treat genital tumors, sclerosis of the spleen, inflammatory tumors and cancerous ulcers[3]. Antimicrobial activity of *Stachys* oils was reported[3-7]. The chemical composition of essential oils from *Stachys* species was extensively investigated by various authors and the major constituent groups, monoterpenes, oxygenated sesquiterpenes and sesquiterpene hydrocarbons were found to vary from species to species[1,8-14].

In the present work we report for the first time the chemical composition and the antioxidant activity of the essential oil from the endemic Yemeni plant *Stachys yemenensis*.

MATERIALS AND METHODS

Plant materials

The leaves of *S. yemenensis* were collected from Ashmor-district, Hajah province, Yemen, in February 2009. The plant was identified by Mr. Hassan M. Ibrahim of the Botany Department, Faculty of Sciences, and Sana'a University. Voucher specimen (*S. yemenensis* (YMP-La 13) of the plant material has been deposited at the Pharmacognosy Department, Sana'a

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Volatile oil extraction

Dried leaves (20 g) of *S. yemenensis* were hydrodistilled for 3 h in a Clevenger type apparatus according to European Pharmacopoeia (2008). The obtained oil was subsequently dried over anhydrous Na_2SO_4 and analyzed. The oil yield was calculated on a dry weight basis.

GC/MS analysis

Analytical GC-MS system consisting of an Agilent 6890N Gas Chromatography and a Mass Selective Detector (Agilent®5973 Network MSD) was used. Injection was done with Agilent®7683 Series Injector (Split 1:40 at 250°C, 2.0 µl, carrier gas: helium 1.1 mL/min (60 kPa) at 110°C; pressure rise: 6 kPa/min). The MS operated in the electron impact mode with ionization energy of 70 eV. The oven program started with 1 min at 70°C, the oven temperature was increased at 3°C/ min to 220°C. Full scan mass spectra were acquired from 45-650 m/z at a rate of 4.5 scans/ sec and with a 5.0 min solvent delay. Chromatography was performed using a 30 m DB-5 column (J&W Scientific, Folsom, USA) with 0.25 mm i.d. and 0.25 µm film thickness.

The detected compounds were identified by processing of the raw GC-MS data with ChemStation G1701CA software and comparing with NIST mass spectral database 2.0 d (National Institute of Standards and Technology, Gaithersburg, USA) and from retention indices and mass spectra of standard compounds. Relative amounts of detected compounds were calculated based on the peak areas of the total ion chromatograms (TIC).

Determination of antioxidant activity

For the preliminary test, analytical TLC on silica gel plates were developed with appropriate conditions after application of 5 ìL of oil solution (5 mg/mL, ethyl ether), dried and sprayed with DPPH solution (0.2%, MeOH). Five minutes later active compounds appeared as yellow spots against a purple background.

Estimation of a radical scavenging effect was carried out by using a DPPH free radical scavenger assay in 96 well microtitre plates (MTP) according to the modified method[15,16]. A solution of DPPH (Sigma Aldrich, Germany) was prepared by dissolving 5 mg DPPH in 2 mL of methanol, and the solution was kept in the dark at 4 °C until use. Stock solutions of the sample was prepared at 4 mg/mL and diluted at different concentrations (from 0.20 - 4 mg/ml). 5 µl of methanolic DPPH solution was added to each well. The plate was shaken to ensure thorough mixing before being wrapped with aluminum foil and stored in the dark. After 30 min the optical density (OD) of the solution was measured at the wavelength of 517 nm using a microtitre plate ELISA reader (Thermo, Finnland). A methanolic solution of DPPH served as control. All tests were carried out in triplicates. Ascorbic acid (Sigma Aldrich, Germany) was used as positive control. Decreasing of DPPH solution absorbance indicates an increase of DPPH radical scavenging activity.

RESULTS AND DISCUSSIONS

The yield of the oil was 0.45% (w/w) of a pleasant smelling Essential Oil. Water distilled Essential Oil from leaves of *S. yemenensis* was analyzed by GC-MS. The composition of the Essential Oil is presented in TABLE 1, where compounds are listed in order of their elution on DB-5 column. Twelve compounds were characterized in the oil of *S. yemenensis* representing 99.3%

TABLE 1: Main components of the essential oil of *S. yemenensis*.

Compounds	RI	%	M^+ peak	Base peak	Major peaks
Myrcene	990	3.2	136	93	41.2,53,69.2,79,109
α- Phellandrene	1004	34.7	136	93	40.3,53,65.2,77.2,91,119
p-Cymene	1016	5.4	134.	119	41,51,55, 65, 77,91, 97, 103
Limonene	1026	10.8	136	68	41,53,79,93,107,121
β-Phellandrene	1029	11.2	136	93	41.2,53,69.2,77.1,91,121
Bicyclogermacrene	1498	6.4	204	93	39, 41,53, 67, 79,,107,121,161
δ-Cadinene	1522	2.7	204	161	41,55, 81, 91,105, 119, 134, 189
Elemol	1546	8.5	222	59	41, 55, 67, 79, 81, 93, 107, 121, 135, 161, 189
Spathulenol	1582	3.4	220	43	41,55, 67, 69, 79, 91, 105, 119,159,187,202, 205
β-Eudesmol	1650	3.1	222	59	43,, 67, 79, 93, 108, 149, 164, 189, 204
α Eudesmol	1661	8.4	222	59	41, 55,81, 93, 107,149,161, 189, 204
Total		97.8			

Compounds listed in order to their elution on the DB-5 column Retention indices on the DB-5 column relative to C10-C20 n-alkanes

with 65.3% monoterpene hydrocarbons, 23.4% oxygenated sesquiterpenes, and 9.1% sesquiterpene hydrocarbons. The main constituents of the oil were α-phellandrene (34.7%), β-phellandrene (11.2%), limonene (10.8%), elemol (8.5%), α-eudesmol (8.4%), bicyclogermacrene (6.4%), p-cymene (5.4%), spathulenol (3.4%), myrcene (3.2%), β-eudesmol (3.1%), δ-cadinene (2.7 %) and heptadecane (1.5%).

When the chemical profile of the essential oil studied was compared to previously investigated essential oils from *Stachys* species, it appeared somewhat similar. α-phellandrene (34.7%) as the first major compound in the oil of *S. yemenensis* was detected in the oil of *S. schtschegleevii* (4.7%)[17]. β–phellandrene (11.2%) as the second main compound in the *S. yemenensis* oil was also found in the oil of *S. lavandulifolia* (37.5%)[18,19], *S. laxa* (5.5%)[20], *S. schtschegleevii* (14.7%)[17] from Iran, *S. lavandulifolia* (2.2%) from Turkey[21], *S. glutinosa* (6.8%) from Corsica-France[22] and *S. germanica* (4.8%) from Solvakia[23]. Limonene (10.8%) was reported also in the oil of *S. inflata* (11.6%) (Omidbaigi et al. 2006), *S. schtschegleevii* (8.8%)[17], *S. obliqua* (6.2%)[24], and *S. aegyptaica* (5.45%)[25]. While bicyclogermacrene (6.4%) was identified in the oil of *S. pubescens* (11.6%)[13], *S. inflata* (5.1%), *S. laxa* (6.7%)[26], *S. aleurites* (14.5%)[12], p-cymene (5.4%) was identified in the *S. annua* (8.4%), *S. inflata* (2.5%)[17], *S. aegyptaica* (3.9%)[25]. Spathulenol (3.4%) was reported in the oil of *S. atherocalyx* (22.1%)[27], *S. inflata* (2.3%)[28,29], *S. obtusicrena* (11.5%)[30], *S. pubescens* (5.2%)[31], *S. byzantina* (16.1%)[14], *S. ixodes* (5.6%)[33]. Myrcene (3.2%) was found in the oil of *S. lavandulifolia* (23.9%) (18; 19), *S. inflata* (6.5%)[28] from Iran as well as *S. lavandulifolia* (3.3%) from Turkey[21] and *S. aegyptaica* (3.8%) from Egypt[25]. δ Cadinene (2.7%) was also one of the major compounds of *S. atherocalyx* (5.0%)[27], *S. schtschegleevii* (3.3%), *S. balansae* (2.0%)[34].

The antioxidant activity of the essential oil was evaluated through their ability for free radical scavenging against the stable 2,2-diphenyl-1- picrylhydrazyl radical (DPPH). The sample gave a positive result in the preliminary assay on TLC. Then, a spectrophotometric assay was carried out and the percentage DPPH reduction was calculated for the oil. The antioxidant effect of essential oil on DPPH was examined at different concentrations (0.250-4 mg/ml). The absorbance decreases as a result of a colour change from purple to yellow as the radical is scavenged by antioxidants. The essential oil was able to reduce the stable free radical DPPH to the yellow-coloured 1,1-diphenyl- 2-picrylhydrazyl with IC_{50} of 1.12 mg/ml. Various results were obtained from radical scavenging activity evaluation of other Stachys species oils. Oils of *S. palustris, S. cretica and S. hydrophila* from different regions of the Mediterranean area showed considerable DPPH radical scavenging activity with IC_{50} values of 0.482, 0.652 and 0.664 mg/ml respectively[35]. The high percentage of monoterpene hydrocarbons could explain the lower DPPH radical scavenging activity for *S. yemenesis* oil compared to oils obtained from *S. palustris, S. cretica and S. hydrophila,* which contain oxygenated compounds *as* main components of oils even though essential oils are, from the chemical point of view, quite complex mixtures and this complexity makes it often difficult to explain the biological activities[36].

CONCLUSION

The data reported in our study showed that the oil composition of *S. yemenensis* was characterized mainly by monoterpene hydrocarbons 65.3%, oxygenated sesquiterpenes 23.4%, and showed among the *Stachys* oils, the highest percentage in elemol (8.5%), α-eudesmol (8.4%), heptadecane (1.5%). The oil possessed lower antiradical activity compared to the recorded Stachys oils.

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