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Chemical Composition and Biological Activities of Essential Oils from the Oleogum Resins of Three Endemic Soqotraen *Boswellia* Species

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Abstract: The chemical composition, antioxidant and anticholinesterase activity of three essential oils (EOs) obtained from the oleogum resin of three endemic Sqotraen *Boswellia* species, *Boswellia socotrana* Balf. f, *Boswellia ameero* Balf. f, and *Boswellia elongata* Balf. f were determined. GC-MS technique was used for the analysis of the oils. Oils of *B. socotrana* and *B. ameero* were characterized by a high content of monoterpenes. The main constituens of *B. socotrana* and *B. ameero* were (E)-2,3-epoxycarene (51.8%), 1,5-isopropyl-2-methylbicyclo[3.1.0]hex-3-en-2-ol (31.3%), and α-cymene (7.1%); (3E,5E)-2,6-dimethyl-1,3,5,7-octatetraene (34.9%), 1-(2,4-Dimethylphenyl)ethanol (20.3%), 3,4-dimethylstyrene (17.3%), α-campholenal (13.4%) and α-terpineol (12.4%) respectively. The composition of *B. elongata* oil was dominated by the diterpene verticiol (52.4%), the sesquiterpene caryophellene (39.1%) and methylcycloundecanecarboxylate (7.8%). The oils were screened for their antioxidant activity by using the DPPH free radical scavenger assay and their anticholinesterase activity on acetylcholinesterase enzyme by using in vitro Ellman method. The antioxidant activity of EOs from *B. socotrana* (IC_{50} =121.4 µg/mL) appeared to be more potent than that of *B. elongata* (IC_{50} =211.2 µg/mL) and *B. ameero* (IC_{50} =175.2 µg/mL). EO of *B. socotrana* showed the higher AChE inhibitory activity with 59.3% at concentration of 200 µg/mL in comparison to EOs of *B. elongata* and *B. ameero* (29.6, 41.6 enzyme inhibition) respectively.

Key words: Essential oils, *Boswellia*, GC-MS, antioxidant, AchE inhibitor, Soqotra

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1. Introduction

The genus *Boswellia* (family Burseraceae) consists of many species widespread throughout the world. It includes approximately 23 species of small trees that grow mainly in Arabia, on eastern coast of Africa and in India. Olibanum is a natural oleo-gum-resin that exudes from tappings in the bark of *Boswellia* trees [1]. The genus is represented in Soqotra Island by 8 species. The oleogum resin is used traditionally by the native inhabitants for relieving the pain in cavities, sweetening the breath, or to sooth a disturbed stomach [2].

Considerable work on the composition of the essential oils (EOs) from different species of *Boswellia* is reported in literature. Monoterpenoids seem to be the dominant class of compounds found in the oils. The main compounds of *B. dalzielii* EO from Nigeria were α-pinene (45.7%), γ-terpinene (11.5%) and trans-sabinene hydrate [3]. The EO of *B. frereana* from Somalia was dominated by α-thujene (10.10%), p-cymene (4.3%) [4]. The EO of *B. serrata* from India was found to contain mainly α- thujene (61.0%), α-pinene (7.7%), and sabinene (5.1%) [5]. Since the chemical composition of EOs depends on various environmental factors, three studies on the EOs composition of *B. carteri* from Somalia revealed this composition variety: α-thujene (19.2%), sabinene (9.4%), limonene (7.8%) and α-pinene (7.2%) [4];octyl acetate(60.0%), octanol (12.7%) and p-cymene (8.7%)[6]; α-pinene (41.0%), limonene (12.8%) [7]

Several studies investigated the anti-inflammatory, immunomodulatory, anti-leukotriene, antiacetylcholinesterase, and anticancer activity of the resin and especially its major components, boswellic acid derivatives [8-18]. In addition, the EO showed antibacterial, antifungal and immunostimmulating activity [7, 19, 20].

To the best of our knowledge, no literature information is available on the chemistry and biology of the EOs of Soqotraen *Boswellia* species. In the present paper, we wish to report the chemical composition, antioxidant, and antiacetylcholinesterase activities of the EOs obtained from the olegum resins of three endemic Soqotraen *Boswellia* species.

2. Materials and Methods

2.1 Plant material

The plant material was collected in March 2006 from different locations in Soqotra Island. The plants were taxonomically identified at the Centre of Environment and Biodiversity, Aden University, Yemen. Species names are according to International Plant Name Index (IPNI) (http://www.ipni.org). Voucher specimens (*B. socotrana* (SP-Bu-03) *B. elongata* (SP-Bu-02) and *B. ameero* (SP-Bu-01) of the plant material are deposited at the Pharmacognosy Department, Aden University, Yemen.

2.2 Volatile oil extraction

Oleogum resins (12 g each) of *B. socotrana, B. elongata, B. ameero,* were hydrodistilled for 2-3 h in a Clevenger type apparatus according to European Pharmacopia to yield three oils. The obtained volatile oils dried over anhydrous Na_2SO_4 and stored at 4 °C until testing.

2. 3 Determination of antioxidant activity

Estimation of a radical scavenging effect was carried out by using a DPPH free radical scavenger assay in 96 well micro titre plates (MTP) according to the modified method given in refs. [21, 22]. 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 samples

were prepared at 4 mg/mL and diluted. 5 µl of methanolic DPPH solution was added to each well. The plate was shaken to ensure thorough mixing before being wrapped with aluminium 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 micro titre plate reader Tecan GeniosPro (Germany). A methanolic solution of DPPH served as control. All tests were carried out in duplicates. Ascorbic acid (Sigma Aldrich, Germany) was used as positive control.

2.4. Microplate assay for AChE activity

2.4.1. Chemicals

Acetylthiocholine iodide (ATCI), 5,5' –dithiobis-(2-nitrobenzoic acid) (DTNB), galanthamine, bovine serum albumine (BSA) and acetylcholinesterase (AChE) from horse serum (lyophilized, 500 U/vial solid, 65 U/mg) were purchased from Sigma (Germany). The following buffers were used: Buffer A: 50 mM Tris-HCl, pH 8, containing 0.1% BSA; Buffer B: 50 mM Tris-HCl, pH 8 containing 0.1 M NaCl, 0.02 M MgCl2×6H2O; Galanthamine (Sigma Aldrich, Germany).

2.4.2.AChE inhibitory activity

AChE inhibitory activity was detected by a microtiter plate assay based on Ellman's method [23, 24] by using acetylthiocholine as substrate. In 96–well plates, 25 µl of 15 mM ATCI (43 mg/10 mL Millipore water), 125 µl of 3 mM DTNB (11.9 mg/10 mL buffer B), 50 µl of buffer A, 25 µl of EO at concentration of 2 and 0.5 mg/mL (final concentration in the assay: 0.2, 0.05 mg /mL DMSO) were added and the absorbance was measured at 405 nm every 13 s for five times. After adding 25 µl of 0.22 U/mL enzyme (0.34 mg AChE dissolved in 100 mL buffer A), the absorbance was read again every 13 s for five times. The absorbance was measured using a Tecan GeniosPro micro plate reader. Percentage of inhibition was calculated by comparing the rates for the sample to the blank (DMSO), control contained all components except the tested EOs. Galanthamine was used as positive control. All treatments were performed in tetraplicate on each well, and was done twice. (n= 2)

2.5. Gas chromatography-mass spectrometry

Analytical GC-MS system consisted of an Agilent 6890N gas chromatograph and a mass selective detector (Agilent®5973 Network MSD). 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 110ºC; pressure rise: 6 kPa/min). The MS operated in the electron impact mode with an ionization energy of 70eV. The oven program started with 1min 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/s and with a 5.00 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 times and mass spectra of standard compounds. Relative amounts of detected compounds were calculated based on GC peak areas.

Table 1. Main Components of EOs from Oleogum resins of *Boswellia socotrana* (A), *Boswellia elongata* (B), *Boswellia ameero* (C)

RI	Compounds[a]	A (%)	B (%)	C (%)
1020	α-Cymene	7.1	-	-
1039	1,5-Isopropyl-2-methylbicyclo[3.1.0]hex-3-en-2-ol	**31.3**	-	-
1082	4-Terpinenylacetate	3.9	-	-
1111	α-Campholenal	-	-	**13.4**
1130	(3E,5E)-2,6-Dimethyl-1,3,5,7-octatetraene	-	-	**34.9**
1153	(E)-2,3-Epoxycarene	**51.8**	-	-
1170	3,4-Dimethylstyrene	-	-	**17.3**
1177	α-Terpineol	-	-	12.4
1196	1-(2,4-Dimethylphenyl)ethanol	-	-	**20.3**
1213	4-methyl-Benzoic acid	2.7	-	-
1238	p-Menth-1(7)-en-2-one	**2.6**	-	-
1350	α-Terpinylacetate	0.1	-	-
1432	Caryophyllene	-	**39.1**	-
1536	Methylcycloundecanecarboxylate	-	**7.9**	-
2106	Verticiol	-	**52.4**	-
	Total identified	**98.5**	**99.4**	**98.3**

[a]Compounds listed in order to their elution on the DB-5 column
Retention indices on the DB-5 column relative to C_{10}-C_{20} *n*-alkanes

3. Results and Discussion

The yields of EOs were obtained by hydrodistillation of the crushed oleogum resins derived from *B. socotrana, B. ameero* and *B. elongata* (1.2%, 1.8%, and 2.3%) on dry weight basis respectively. The reported average yields of some *Boswellia* species were ranging from 1.3% to 3% [3, 20].

There have been no reports on GC-MS analysis of the three EOs. The GC-MS analysis of EOs from *B. socotrana, B. ameero* and *B. elongata* allowed the identification of 7, 5, and 3 components which accounted for 98.5%, 98.3% and 99.4% of the three EOs respectively (Table 1) .
Oil of *B. socotrana* was characterized by a higher content of oxygenated monoterpenes (89.9%): (E)-2,3-Epoxycarene (51.8%), 1,5-isopropyl-2-methyl bicyclo[3.1.0]hex-3-en-2-ol (31.3%), 4-Terpinenylacetate (3.9%) and p-Menth-1(7)-en-2-on (2.6%), while the oil of *B. ameero* contained (46.2%) phenylpropan derivates and oxygenated monoterpenes: 1-(2,4-Dimethylphenyl)ethanol (20.3%), α-campholenal (13.4%) and α-terpineol (12.4%). Both oils were characterized by bicyclo[3.1.0]hexane and bicyclo[3.1.1]heptane.

EO of *B. elongata* possessed a different terpenic composition from the two aforementioned oils. The oil was poor in monoterpenes, but the diterpene verticiol (52.4%) predominated over the sesquiterpene caryophellene (39.1%). The presence of such a high content of a cembranoid diterpene, verticiol, was detected for the first time in the EO of frankincense derived from *B. elongata.* The comparison of our results with those of the literature showed that the main components of chemical composition of the three endemic Soqotraen *Boswellia* EOs were markedly different from that of other known *Boswellia* species such as *B. carteri*, *B. sacra*, *B. frereana, B. serrata,* and *B. dalzielii*. [3-7, 25]

Table 2. Antioxidant, anticholinesterase activity of EOs of *B. socotrana, B. elongata and B. ameero*

	DPPH	Enzyme inhibition in %	
Sample	IC_{50} (µg/mL)	200 µg/mL	50 µg/mL
B. socotrana	121.4 (± 7.6)	59.3 (±7.8)	19.1 (±6.6)
B. elongata	211.2 (± 12.8)	29.6 (± 6.7)	11.5 (±8.1)
B. ameero	175.2 (± 10.3)	41.6 (±6.0)	10.7 (±6.7)
Ascorbic acid	96.5 (± 5.9)		
Galanthamine (8 µg/mL)		87.8 (±7.7)	

The free- radical scavenging activity of *Boswellia* EOs evaluated using the DPPH method is presented in Table 2. The model of scavenging stable DPPH free radicals can be used to evaluate the antioxidative activities in a relatively short time. The absorbance decreases as a result of a color change from purple to yellow as the radical is scavenged by antioxidants through donation of hydrogen to form the stable DPPH-H molecule [26]. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen-donating ability. The EOs were able to reduce the stable free radical DPPH to the yellow colored 1, 1 –diphenyl-2-picrylhydrazyl with an IC_{50} value of. 121.4 µg/mL, 211.2 µg/mL and 175.2 µg/mL for *B. socotrana*, *B. elongata* and *B. ameero* respectively. The free- radical scavenging activity of *B. socotrana* EO showed higher activity than those of *elongata* and *B. ameero*. The positive factor for *B. socotrana* EO was the higher concentration of oxygenated monoterpenes [27, 28] but there are no data on the antioxidant activity of the oxygenated monoterpenes (E)-2,3-epoxycarene that was the main constituent in this oil. *Boswellia* EOs showed lower free- radical scavenging activity in comparison to other reported essential oils rich in oxygenated monoterpes, such as *Melissa officinalis,* and *M. piperita* with IC50= 7.58 and 2.53 µg/mL, respectively [29, 30].

Inhibition of the acetylcholinesterase, which is responsible for the hydrolysis of acetylcholine, represents the most effective approach for finding new AChE inhibitors from natural sources for treating Alzeihmer's disease. Currently no AChE inhibitory activity has been reported from EOs of *B.* species. *B.* species contain in addition to essential oils, triterpenic boswellic acids, a group of compounds reported to have AChE inhibitory activity [17]

In the present study, some AChE inhibitory activity was detected in EOs of *Boswellia species* (Table 2). At a concentration of 200 µg/mL, EO of *B. socotrana* (59.3% enzyme inhibition) exhibited higher AChE inhibitory activity than the EOs of *B. elongata* and *B. ameero* (29.6 and 41.5% enzyme inhibition, respectively). The AChE inhibitory activity of *B. socotrana* oil may be due to the presence of (E)-2,3-epoxycarene, and to p-menth-1(7)-en-2-one, a group of monoterpenoid skeletons reported to have AChE inhibitory activity [31, 32]. Pulegone, a monoterpene with p-menthane skeleton in *Mentha* spp showed AChE inhibitory activity with IC_{50} 890 µM [32]. The reported AChE inhibition for oils of *Melissa officinalis* and *Rosmarinus officinalis* [33, 34] was stronger than the inhibitory activity of *B. socotrana* oil. This may be due to the additive or may be even synergistic effect among the components of the oils, as it was reported for 1.8 cineole, (+) –α-pinene, camphor and bornyl acetate (IC_{50}: 670, 630, >4000, >4000 µM respectively) in *Salvia lavandulaefolia* [31].

In conclusion, based on the chemical profile of the EOs, the three oleogum resins represent a new brands which can be named Soqotraen brands that are specific in their main volatile components in comparison to the old brands such as Eritrean (*B. carteri*), Omani (*B. sacra*), Somali (*B. frereana*) and Indian brands (*B. serrata*) [1]. Bioactivity guided fractionation is in progress for isolating the active compound (s) from *B. socotrana* oil.

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