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Original Article

Chemistry and Antifungal Activity of the Essential oils from Oleogum resins of Two Soqotraen Commiphora Species

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Abstract

The chemical composition of two essential oils obtained from oleogum resins of Commiphora ornifolia (Balf.f.) Gillett, and Commiphora kau Vollesen, collected from Soqotra Island was analyzed by GC-MS. While the diterpenes (91.4%): retinol (54.2%), verticiol (33.1%) and cembrene (4.1%) were the major compounds of C. ornifolia oil, sesquiterpene hydrocarbons (87.4%) were the predominant portion of Commiphora kau oil with ε-muurolene (33.0%), τ-cadinene (22.5%), □-cadinene (17.0%), α-guaiene (4.3%), isocaryophyllene (3.7%), allo-aromadendrene (2.8%), α-muurolene (2.7%), and α-caryophyllene (2.4%). The Oil of C. kau showed moderate antifungal activity against Cladosporium cucumerinum

Key Words: *Essential oils, Commiphora, Soqotra, Verticiol, GC-MS*

Introduction:

The genus Commiphora (Burseraceae) includes about 150 species, distributed mostly around Red Sea in East Africa, and with few species also occurring in Arabia and India. Commiphora species are small trees or shrubs with short, thorny branches. The genus is represented in Soqotra island by five species: C. kau,(Royle) vollesen, C. ornifolia (Balf.f.) Gillett, C. parvifolia (Balf.f.) Engl., C. planifrons (Balf.f.) Engl. and C. socotrana (Balf.f.) Engl., four of which are endemic to Soqotra. In Soqotraen folk medicine, Commiphora species are among the most important medicinal plants. Powdered resin of C. kua is given in warm milk or water to a toddler or young child with sore stomach. Fresh sap or resins of C. ornifolia is used to treat a variety of sores and skin ailments. The resin is not chewed, but can be inserted into a cavity to relieve toothache and to burn out the diseased area of the tooth1.

The chemical composition of several reported EOs from Commiphora species was characterized by high content of α-pinene, limonene, sabinene, α–thujene, β-pinene, p-cymene, terpinen-4-ol, myrcene, 3-carene and (E) β-ocimene2,3,4,5. On the other hand, some Commiphora species yielded oils rich in sesquiterpenes and oxygenated sesquiterpenes such as furanoeudesma-1,3-diene, furanodiene, indestrene, and β-elemene6,7,8, α –oxobisabolene, γ-bisabolene9, curzerennone, α-selinene, β-selinene, Germacrene D and germacrene B 7, 10.

Many Commiphora species are known for their medicinal properties , and exhibit interesting biological activities such as anti-inflammatory11, antibacterial12, antimicrobial13, antioxidant, hepatoprotective14 smooth muscle relaxing15, anticancer16, antimalarial, anticandidal, antimycobacterial17, antischistosomal18, phytofungicidal19, larvicidal20 and molluscicidal21 effects. Some of these biological properties of Commiphora species may be due to the presence of oil components.

In the framework of our investigation on the EOs composition and biological activities of the Soqotraen plants 22, we report here the chemical composition and the antifungal activity of the EOs of two species of Commiphora, which have not been investigated so far.

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Materials and Methods

Plant materials

The plant material was collected in March 2006 from Soqotra Island. The plants were taxonomically identified at the Centre of Soqotra Archipelago Conservation and Development Program (SCDP), Yemen. Species names are according to International Plant Name Index (IPNI) (http://www.ipni.org). Voucher specimens, Commiphora ornifolia, (SMP-Bu-08) Commiphora kau (SMP-Bu-11) of the plant material are deposited at the Pharmacognosy Department, Aden University, Yemen.

Volatile oil extraction Oleogum resins (20 g, each) of C. ornifolia and C. kau were subjected to hydrodistillation for 3 h using a Clevenger type apparatus.

The obtained EOs were dried over anhydrous Na2SO4. The EOs were stored at 4 °C in bottles covered with aluminium foil to avoid photodegradation and loss of volatile compounds, until tested and analyzed. The oils were analyzed by GC-MS-analysis.

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 from mass selective detector as a TIC (Total Ion Chromatogram).

Antifungal assay Initial tests of fungicidal activity were carried out by the method of Gottstein et al 23. This semiquantitative test allows a relative estimation of the activity of compounds with similar diffusion characteristics.

The phytopathogenic fungus Cladosporium cucumerinum Ell. et Arth. was used as test organism. Antifungal tests were performed on TLC plates (glass plates, 20 x 20 cm, silica gel 60 HF254, thickness 0.5 mm (Merck). The EOs were applied by using microsyringes on the TLC plate at concentrations of (50µg, 100µg, 200µg und 400µg) as individual spots (diameter 1 cm, corresponding to a surface of 78 mm2). Subsequently, the plates were dried in a warm air stream, in order to evaporate remaining solvents. Each plate was covered with approx. 10 ml spore suspension of C. cucumerinum (approx. 2.5 × 106 spores/ml). Afterwards the plates were dried at room temperature for some minutes, placed into a TLC chamber lined with water soaked filter paper and covered. After 48 h incubation at 25 °C in an incubator a dark grey mycelium had developed. Benomyl (Riedel-de-Haen, Germany) was used as positive control. The evaluation of the antifungal effect was based on the area of the white spots corresponding to fungus growth inhibition. Two independent tests were performed and an average of the observations was calculated (n= 3).

Results and Discussion

The EOs obtained after hydrodistillation of oleogum resins (20 g, each) of C. ornifolia and C. kau gave an average yield of 1.2 and 0.9 % on dry weight basis respectively. The obtained average yield of EO from Soqotraen C. kau was less than that EO reported for C. kau species from Kenya 5.

The chemical composition of the EOs from both species, C. ornifolia and C. kau, were essentially composed of diterpenes (91.4%) and sesquiterpenes (87.4%) respectively (Tab. 1). The main compounds of the oil of C. ornifolia, in order of their abundance, were retinol (54.2%), verticiol (33.1%) and cembrene (4.1%). Diterpenes were identified in the EO of Commiphora species, especially monocyclic diterpenes such as α-camphorene (dimyrcene) and cembrene from C. mukul [10]. In our study, the oxygenated diterpenes retinol (54.2%) and verticiol (33.1%) were identified for the first time in Commiphora species. The high content of retinol in the EO from the oleo gum resin of C. ornifolia shows that this plant could have a medicinal potential as a source for vitamin A topical preparations.

On the other hand, the most prevalent compounds detected in the oil of C. kau were the sesquiterpene hydrocarbons: ε-muurolene (33.0%), τ-cadinene (22.5%), □-cadinene (17.0%), α-guaiene (4.3%),

isocaryophyllene (3.7%), allo-aromadendrene (2.8%), α-muurolene (2.7%), and α-caryophyllene (2.4%). Interestingly, there were significant differences between the main components of the EO of Soqotraen C. kau and those previously determined in EO of Kenyan C. kau5. Thus, monoterpene hydrocarbons such as α-pinene (44.3%), p-cymene (28.7%), α-thujene (22.4%) and β-pinene (10%) were quantitatively abundant in the EO of Kenyan C. kau, whilst they were absent in EO of C. kau collected from Soqotra. To our knowledge this is the first report of the presence of such high content of the bicyclic sesquiterpene hydrocarbons (87.4%) in oil of C. kau, compared to the reported Commiphora EOs that were rich in sesquiterpenes 6-10 . Sesquiterpene hydrocarbons with monoyclic (elemene) and tricyclic (α-bourbonene, α-gurjunene) skeletons were found only in small quantities in the oil of C. kau. This difference in the chemical composition of EOs from Soqotraen and Kenyan C. kau species may be due to the several ecological factors such as climate, altitude, latitude, geographical source, harvest conditions etc.

The fungicidal potential of the oils from both species were evaluated against the phytopathogenic fungus Cladosporium cucumerinum by using a microbioassay on TLC plates. The results of the bioassay are summarized in Table 2.

At concentration of 400 µg, moderate antifungal activity with inhibition zones of 15 mm was observed for EO of C. kau. Oil of C. ornifolia showed no activity at the same concentration. The antifungal activity of 2-Methyl-5-(4'(S)-hydroxy-1'(R),5'-dimethylhex-5'-enyl)-phenol isolated from C. kau from Kenya was reported19.

The antifungal activity of EO of C. kau may well be due to the presence of synergy between the major components of the oil. Considering the fact that C. kau contained □-muurolene (33.0%), τ-cadinene (22.5%), ε-cadinene (17.0%), the antifungal results obtained may be attributed to the presence of these compounds24 . Bioactivity guided fractionation is in progress for isolating the active compound (s) from C. Kau oil.

The following conclusions could be drawn from this study: The chemical composition of EO of the endemic Soqotraen C. ornifolia (diterpenes 91.4%) differed drastically from that of the reported oils of Commiphora genus2-10. The composition of EO of C. kau from Soqotra was characterized by high content of sesquiterpene hydrocarbons (87.4%), which may be responsible for the fungicidal activity against C. cucumerinum.

Table 1. Main Components of EOs from the oleogum resins of Commiphora ornifolia (A) and Commiphora kau (B)

RI	[a]Compounds[a]	A (%)	B (%)
1341	δ-Elemene	-	0.78
1379	α-Ylangene	-	0.17
1384	α-Copaene	0.19	0.9
1394	α-Bourbonene	-	0.59
1402	β-Elemene	0.61	0.65
1432	**Isocaryophillene**	-	**3.72**
1448	α-Bergamotene	-	0.38
1455	α-Gurjunene	-	0.49
1456	ε-Guaiene	0.64	-
1457	**α-Caryophyllene**	1.59	**2.36**
1476	**allo-Aromadendrene**	-	**2.82**
1518	**α-Muurolene**	-	**2.68**
1533	**τ-Cadinene**	-	**22.51**
1534	**δ-Cadinene**	1.65	**17.04**
1577	Elixene	-	0.48
1611	Lendene	-	1.03
1662	**ε-Muurolene**	-	**33.03**
1670	Azulene	-	1.24
1672	**α-Guaiene**	-	**4.34**
2010	Cembrene	**4.07**	-
2018	Verticiol	**33.11**	-
2218	Retinol	**54.23**	-
	Total identified	**96.09**	**95.21**

[a]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

Table 2: antifungal activity of EOs from the oleogum resins of Commiphora ornifolia and Commiphora kau against Cladosporium cucumerinum (n = 3)

Sample	Inhibition zone in mm (diameter)		
	400 µg	200 µg	100 µg
C. ornifolia EO	**nd**	nd	nd
C. kau EO	**8.5** (±0.6)	**4.2 (± 0.3)**	**1.5 (± 0.3)**
Benomyl (5 µg)		**28**	

nd: not detected

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