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Essential Oil Composition from Oleogum Resin of Soqotraen Commiphora kua

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Abstract: The major constituents of the essential oil obtained by hydrodistillation from the oleogum resin of *Commiphora kua* Vollesen were identified by GC-MS. Sixteen constituents were detected from the essential oil, which constituted about (90.5%) of the total amount. Major constituents of the oil were α- cadinol (33.0%), γ-cadinene (22.5%), δ-cadinene (17.0%), isocaryophyllene (3.7%), allo-aromadendrene (2.8%), α-muurolene (2.7%), and α-humulene (2.4%). The Oil of *Commiphora kua* showed moderate antifungal activity against *Cladosporium cucumerinum*.

Keywords: Essential oil; *Commiphora kua*; Soqotra; GC-MS; α- cadinol; *Cladosporium*.

1. Introduction

The genus *Commiphora* (Burseraceae) includes over 200 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. kua*,(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

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[1, 2]. Phytochemical investigations on the resin of *C. kua* led to the isolation of dammarane triterpenes, furanosesquiterpenoids and bisabolenes, some of which possessed anti-inflammatory and phytofungicidal activity against *Cladosporium cucumerinum* [3-6]. Moreover, the essential oil from *C. kua* grown in Kenya was found to contain mainly monoterpenes [5].

The reported chemical composition of the essential oils of several *Commiphora* species was characterized mainly by monoterpenes, oxygenated sesquiterpenes and sesquiterpene hydrocarbons, which invariably differ from species to species [5, 7-15].

Many *Commiphora* species were known for their medicinal properties, and exhibited interesting biological activities such as anti-inflammatory, antibacterial, antimicrobial, antioxidant, hepatoprotective, smooth muscle relaxing, antimalarial, anticandidal, antimycobacterial, antischistosomal, larvicidal, molluscicidal, anticancer, antiulcer and hypolipidemic [16-27] effects. Some of these biological effects may be due to the presence of oil components.

In this study, the essential oil obtained from *C. kua* was analyzed for its chemical composition, and then the antifungal activity against the phytopathogenic fungus *Cladosporium cucumerinum* by using a microbioassay on TLC plates was investigated.

2. Materials and Methods

2.1. Plant Material

The plant material was collected in March 2006 from Soqotra Island. The plant was taxonomically identified at the Centre of Soqotra Archipelago Conservation and Development Program (SCDP), Yemen. Species name is according to International Plant Name Index (IPNI) (http://www.ipni.org). A voucher specimen (SMP-Bu-11) of the plant material is deposited at the Pharmacognosy Department, Aden University, Yemen.

2.2 Essential Oil extraction

Oleogum resin (20g) of C. kua was subjected to hydrodistillation for 3 h in a Clevenger-type apparatus. The obtained oil was subsequently dried over anhydrous Na_2SO_4 and stored under refrigeration until analyzed and tested. The oil yield was calculated on a dry weight basis as 0.9 %.

2.3 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 μ l; 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 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 μ m 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 the peak areas of the total ion chromatograms (TIC).

2.4 Antifungal assay

Initial tests of fungicidal activity were carried out by the method of Gottstein *et al* [28]. 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 essential oil was 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. Three independent tests were performed and an average of the observations was calculated (n= 3).

3. Results and Discussion

The essential oil obtained after hydrodistillation of oleogum resins of *C. kua* gave an average yield of 0.9 % on dry weight basis. The obtained average yield of Soqotraen *C. kua* oil was less than the yield reported for Kenyan *C. kua* oil [5].

To identify the chemical constituents, the essential oil from C. kua was subjected to GC/MS analysis (Table 1). Among the 16 compounds identified, the major components included α- cadinol (33.0%), γ-cadinene (22.5%), δ-cadinene (17.0%), isocaryophyllene (3.7%), allo-aromadendrene (2.8%), α -muurolene (2.7%), and α -humulene (2.4%). The oil was characterized by a high content of sesquiterpene hydrocarbons (56.3%), and oxygenated sesquiterpenes (34.2%), and devoid of monoterpenes, which represented the most major compounds in some reported *Commiphora* oils (7-9). α- Cadinol (33.0%), as the first major compound in the oil exhibited cytotoxic activity against human lung carcinoma cell line A-549 and human colon adenocarcinoma cell line DLD-1, antitermitic and antifungal activities [29, 30]. γ-Cadinene as the second major compound was detected in the essential oils of C. shaerocarpa and C. myrrha [10, 11], whilst δ -cadinene (17.0%), as the third major compound was reported in C. shaerocarpa (2.1%), C. holtziana (1.1%), C. kataf (1.0%)[11], and C. terebinthina (1.6%)[9]. Essential oils rich in α - cadinol, γ -cadinene, δ -cadinene were found to exhibit antiplasmodial activity [31]. Besides, cadinene- containing oils are able to produce induction of hepatic P450s enzymes and therefore increase the drug metabolism in liver [32]. α-Muurolene was identified in the essential oil of Ethiopian C. terebinthina (3.7%) [9], and showed antifungal activity against the phytopathogenic fungus Cladosporium cucumerinum [33]. While minor amounts of α humulene were identified in C. shaerocarpa, C. holtziana, C. kataf, C.myrrha from Ethiopia [11] and C. africana from Benin [13], isocaryophyllene was reported for the first time in the essential oil of Commiphora species, and both compounds showed anticancer activity [29, 34, 35].

Our results thus appeared to be quite different from previously reported data on the chemical composition of Kenyan *C. kua* oil, which consisted mainly of the monoterpenes: α -pinene (44.3%), pcymene (28.7%), α -thujene (22.4%) and β -pinene (10%) [5]. Sesquiterpene hydrocarbons with

monocyclic (α -humulene, elemene) and tricyclic (α -bourbonene, α -gurjunene) skeletons were found only in small quantities in the oil.

The fungicidal potential of the essential oil from C. kua was evaluated against the phytopathogenic fungus Cladosporium cucumerinum by using a microbioassay on TLC plates. At concentration of 400 μ g, moderate antifungal activity with inhibition zones of 9.2 \pm 1.2 mm was observed. The antifungal activity of the essential oil may well be due to the presence of synergy between the major components of the oil. Considering the fact that C. kua oil contained as cadinene derivative, α -muurolene which possessed antifungal activity against Cladosporium cucumerinum [33].

The following conclusions could be drawn from this study: The chemical composition of the essential oil of C. kua differed drastically from that of the reported oil of Kenyan C. Kua. The composition of the essential oil of C. kua from Soqotra was characterized by high content of α -cadinol and other sesquiterpene hydrocarbons which acquired the oil marked pharmacological properties.

Table 1. Main components of essential oil from the oleogum resins of *Commiphora kua*

RI	^a Compounds ^a	(%)
1341	δ-Elemene	0.8
1379	α-Ylangene	0.2
1384	α-Copaene	0.9
1394	β-Elemene	0.7
1402	β-Bourbonene	0.6
1425	β-Gurjunene	0.5
1432	Isocaryophyllene	3.7
1457	α-Humulene	2.4
1476	allo-Aromadendrene	2.8
1512	unidentified	4.3
1518	α-Muurolene	2.7
1533	δ- Cadinene	17.0
1534	γ-Cadinene	22.5
1577	Elixene	0.5
1611	Lendene	1.0
1662	α- Cadinol	33.0
1670	Patchoulol	1.2
	Total identified	94.8

^aCompounds 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

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