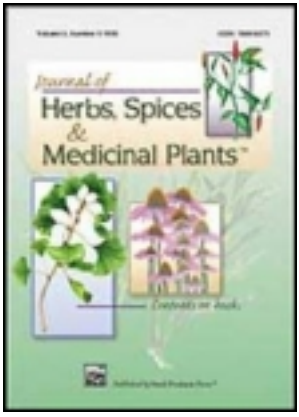

Journal of Herbs, Spices & Medicinal Plants

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/whsm20

Chemical Composition and Biological Evaluation of the Essential Oil of Commiphora opobalsamum L.

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Published online: 11 Oct 2008.

To cite this article: Shaza M. Al-Massarany, Fawkeya A. Abbas, Betul Demirci, Kemal H. C. Baser, Shabana I. Khan, Adnan J. Al-Rehaily, Jaber S. Mossa & Ehab A. Abourashed (2008) Chemical Composition and Biological Evaluation of the Essential Oil of Commiphora opobalsamum L., Journal of Herbs, Spices & Medicinal Plants, 13:4, 111-121, DOI: 10.1080/10496470801946133

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ABSTRACT. The chemical composition of three essential oil samples (stored aerial parts, fresh aerial parts, and fresh flowering tops) of *Commiphora opobalsamum* L., obtained by hydrodistillation, was determined using GC-MS analysis. The identified constituents represented 69.5 to 84.4 percent of the total chemical compounds of the three samples. The major components were α-cadinol in the stored aerial parts, α-calacorene

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Received: November 16, 2006.

Journal of Herbs, Spices & Medicinal Plants, Vol. 13(4) 2007
Available online at http://jhsmp.haworthpress.com

doi:10.1080/10496470801946133

in the fresh aerial parts, and terpinen-4-ol in the fresh flowering tops. The essential oil from the fresh aerial parts exhibited antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Candida glabrata*, *C. krusei*, *Cryptococcus neoformans*, and *Mycobacterium intracellulare*. The same oil sample was non-selectively cytotoxic to four tumor cell lines: SK-MEL, KB, BT549 and SK-OV3. Weak antioxidant activity of the oil from the fresh aerial sample was demonstrated in a DPPH free-radical scavenging assay.

KEYWORDS. Antimicrobial, antitumor, antioxidant, DPPH, GC/MS, medicinal plant, terpenes

INTRODUCTION

The genus *Commiphora* (Burseraceae) consists of about 150 species of shrubs and small trees that are aromatic and mostly confined to Eastern Africa, although a few species growing in Arabia and India (11). The flora of Saudi Arabia has six species of the genus *Commiphora* that are especially prominent in the west and southwest regions of the kingdom (9). *Commiphora opobalsamum* L., known locally as balsam or Bisham, is a strong-smelling, green, non-thorny tree or large shrub that reaches 10 to 12 feet in height (18). In traditional Arabian medicine, *C. opobalsamum* extract is used to treat headache, urinary retention, and constipation (20). Local people use the dried, under-bark of the plant to treat infected wounds and the twigs to brush teeth (9).

A total extract of *C. opobalsamum* has been shown to possess hypotensive, antiulcerogenic, and hepatoprotective effects in rats (1,3,4), and preliminary phytochemical screening of the aerial parts of *C. opobalsamum* has revealed the presence of flavonoids, sterols, triterpenes, saponins, volatile bases, and volatile oil (5). A recent investigation by our group isolated three triterpenes (fridelin, canophyllal, and oleanonic acid), two flavonoids (mearnsetin and quercetin) and a phenolic acid (syringic acid) from *C. opobalsamum* (13). The essential oils isolated from other *Commiphora* species have been reported to contain furanosesquiterpenes (6–8,11) and monoterpenes (17). The chemistry of genus *Commiphora* has also been the subject of a recent review article by Hanus et al. (14).

The purpose of this work was to study chemical composition and biological activity of essential oil obtained from different parts of *C. opobalsamum* using in-vitro assays.

MATERIALS AND METHODS

Plant material: Fresh cut aerial parts (63 g) and fresh flowering parts (570 g) of *C. opobalsamum* were collected in March, 2004, near the city of Makkah, located in the western region of Saudi Arabia. Stored powdered aerial parts (350 g), obtained from an earlier collection from the same region in March 2003, was also used. The plant material was identified by Dr. Ateeq-ur-Rahman of the Research Center for Medicinal, Aromatic and Poisonous Plants, College of Pharmacy, King Saud University, and a voucher specimen was deposited in the University herbarium.

Essential oil extraction and analysis. The oils were separately isolated from all collected plant parts by water distillation for 3 h, using a Clevenger-type apparatus according to the method described in the Egyptian pharmacopoeia (12). Extracted oil samples were dried over anhydrous Na_2SO_4 and kept at 4°C in sealed vials until analysis.

The chemical composition the essential oil was analyzed by gas chromatography using a Hewlett Packard 6890 system. The chromatograph was equipped with an HP-Innowax FSC column (60 m × 0.25 mm, 0.25 μm film thickness), a flame ionization detector (FID), and used nitrogen as carrier gas (1 mL/min). Oven temperature was kept at 60°C for 10 min and was programmed to 220°C at a rate of 4°C/min, kept constant at 220°C for 10 min, and programmed to 240°C at a rate of 1°C/min. The injector and FID temperature were set at 250°C and the injection volume was 1 μL (10% oil in *n*-hexane). Individual component composition percentages were obtained by electronic integration of the chromatograph measurements, using *n*-alkanes as reference points in the calculation of relative retention indices (RRI).

GC-MS analysis was done with a Hewlett Packard GCD system using an Innowax FSC column (60 m × 0.25 mm, 0.25 μm film thickness) with helium as carrier gas. The GC oven and injector temperature conditions were as described above. Split flow was adjusted at 50 mL/min. Electron impact ionization mass spectra (EIMS) were recorded at 70 eV. Mass ranged from *m*/*z* 35 to 425. For constituent identification, a library search was done using the in-house "Başer Library of Essential Oil Constituents."

Biological assays: Antimicrobial activity of the essential oils was determined using a panel of bacteria (methicillin resistant *Staphylococcus aureus* and *Mycobacterium intracellulare*) and fungi (*Candida albicans*, *C. glabrata*, *C. krusei*, *Cryptococcus neoformans* and *Aspergillus fumigatus*) following procedures described earlier (15). Antimalarial activity was

tested against two strains of *Plasmodium falciparum* (D6 strain: chloroquine-sensitive & W2 strain: chloroquine-resistant) based on the determination of parasitic lactate dehydrogenase (LDH) activity (15). Antitumor activity was tested against four human solid tumor cell lines: malignant melanoma (SK-MEL), oral epidermal carcinoma (KB), breast ductal carcinoma (BT549), and ovary carcinoma (SK-OV3) according to a procedure described earlier (19).

Estrogenic activity was tested in a cell based assay that utilized recombinant yeast cells expressing human estrogen receptor (21). Cyclooxygenase-2 (COX-2) inhibition was evaluated by the ability of cultured mouse macrophages to convert exogenous arachidonic acid to PGE_2 (16). Antioxidant activity was determined through the inhibition of reactive oxygen species (ROS) generation in myelomonocytic HL-60 cells using the DCFH-DA (2′,7′-dichlorofluorescin diacetate) method (10).

Free radical scavenging activity, based on the scavenging of the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) by antioxidants, was determined using optical densitometry as described earlier (2). All bioassays were run in duplicates.

RESULTS AND DISCUSSION

After hydrodistillation, the essential oil yield (volume/weight) was 0.10 percent for stored powdered plant, 0.13 percent for fresh aerial parts, and 0.17 percent for fresh flowering tops. The oil obtained from the stored powder had a dark yellow color, while the oils obtained from the fresh plant were colorless to a pale yellow color. All oil samples had a pleasant characteristic aroma. A total of 72 compounds were detected in the stored whole powdered plant sample, representing about 69.5 percent of the total composition of the oil (Table 1). The main constituents were α-cadinol (10.1%), spathulenol (5.8%), viridiflorol (4.9%), β-eudesmol (3.9%), T-muurolol (3.7%), calamenene (3.5%), 1-epi-cubenol (2.6%), T-cadinol (2.8%), δ-cadinene (2.3%), humulene epoxide-II (2.1%) and α-calacorene (2%).

In the essential oil of fresh aerial parts, 81 components representing 84.4 percent of the total oil were identified. The major compounds were α-calacorene (9.4%), terpinen-4-ol (8.5%), δ-cadinene (5%), viridiflorol (4.6%), T-muurolol (4.5%), cadalene (4.3%) and 1-epi-cubenol (2.9%). In the essential oil of fresh flowering tops, 89 compounds were identified representing about 79.3 percent of the total oil composition. The oil was characterized by the presence of terpinen-4-ol (9.8%), cadalene (5.4%),

TABLE 1. Essential oil composition of *Commiphora opobalsamum* L

RRI[1]	Compound	Stored powder	Fresh aerial part	Fresh flowering tops
			(%)[2]	
1450	*trans*-Linalool oxide (Furanoid)	–	0.1	tr[3]
1466	α-Cubebene	0.1	0.1	0.1
1483	4-Nonanol	0.2	–	–
1496	3-Nonanol	0.3	–	–
1497	α-Copaene	tr	0.5	0.5
1521	2-Nonanol	0.2	–	–
1528	α-Bourbonene	–	tr	–
1535	β-Bourbonene	0.2	tr	0.2
1541	Benzaldehyde	–	0.1	tr
1544	α-Gurjunene	–	–	0.1
1549	β-Cubebene	–	–	0.1
1553	Linalool	0.1	0.4	0.8
1562	Octanol	0.1	0.1	
1571	*trans-p*-Menth-2-en-1-ol	–	0.3	0.4
1583	Terpinen-1-ol	–	0.1	
1594	*trans*-b-Bergamotene	–	0.1	0.1
1600	β-Elemene	0.2	0.2	0.2
1602	6-Methyl-3,5-heptadien-2-one	–	0.1	–
1611	Terpinen-4-ol	0.5	8.5	9.8
1612	β-Caryophyllene	–	tr	0.1
1638	*cis-p*-Menth-2-en-1-ol	–	0.3	0.5
1651	Sabinaketone	–	0.2	–
1657	Umbellulone	–	0.2	–
1659	γ-Gurjunene	–	0.1	–
1662	(*Z*)-3-Hexenyl hexanoate	–	–	0.2
1664	Nonanol	0.2	0.1	0.1
1677	*epi*-Zonarene	tr	0.1	–
1682	δ-Terpineol	0.1	–	–
1687	α-Humulene	0.1	0.1	0.1
1688	Selina-4,11-diene[4]	0.1	–	–
1689	*trans*-Piperitol[5]	–	–	0.1
1690	Cryptone	–	0.2	–
1700	*p*-Mentha-1,8-dien-4-ol[6]	–	–	0.3
1704	γ-Muurolene	0.4	0.2	–
1706	α-Terpineol	0.5	1.6	3.4
1708	Ledene	–	0.3	–
1726	Germacrene D	0.2	–	0.4
1740	α-Muurolene	1.0	1.1	1.2
1742	β-Selinene	–	0.3	–

(Continued)

TABLE 1. *(Continued)*

RRI[1]	Compound	Stored powder	Fresh aerial part	Fresh flowering tops
			(%)[2]	
1744	α-Selinene	0.1	–	–
1744	Phellandral	0.2	0.1	–
1748	Piperitone	–	0.2	0.1
1758	*cis*-Piperitol	–	0.2	0.3
1758	(*E,E*)-α-Farnesene	–	–	tr
1762	Pentanoic acid	0.1	–	–
1773	δ-Cadinene	2.3	5.0	4.8
1776	γ-Cadinene	0.5	0.4	0.3
1785	7-*epi*-α-Selinene	–	0.1	–
1786	*ar*-Curcumene	–	0.1	–
1799	Cadina-1,4-diene (=*Cubenene*)	0.3	0.4	–
1802	Cumin aldehyde	0.1	0.1	0.1
1808	Nerol	–	–	0.1
1810	Guaia-3,7-diene	0.2	0.1	0.2
1814	*p*-Mentha-1,5-dien-7-ol	–	0.3	0.4
1827	(*E,E*)-2,4-Decadienal	0.4	–	–
1849	Calamenene	3.5	0.9	0.6
1857	Geraniol	–	0.3	0.7
1864	*p*-Cymen-8-ol	0.1	2.6	1.8
1870	Hexanoic acid	0.5	–	tr
1875	*trans*-2-Hydroxy-1,8-cineole	–	0.3	–
1878	Guaiacol	0.2	0.1	–
1898	1,11-Oxidocalamenene	0.2	tr	–
1900	*epi*-Cubebol	–	–	0.2
1918	β-Calacorene	0.1	0.1	–
1937	Phenyl ethyl alcohol	0.2	0.1	0.2
1941	α-Calacorene	2.0	9.4	3.8
1945	1,5-Epoxy-salvial(4)14-ene	0.3	–	–
1949	Piperitenone	–	–	0.3
1953	Palustrol	0.3	–	–
1957	Cubebol	0.2	–	0.2
1981	Heptanoic acid	1.1	0.1	0.3
1984	γ-Calacorene	0.4	0.3	0.5
1992	(*E*)-2-hexenoic acid	–	–	0.1
2008	Caryophyllene oxide	1.2	–	–
2029	Perilla alcohol	0.3	–	–
2037	Salvial-4(14)-en-1-one	0.6	0.1	–
2041	Pentadecanal	–		0.6

(Continued)

TABLE 1. *(Continued)*

RRI[1]	Compound	Stored powder	Fresh aerial part	Fresh flowering tops
			(%)[2]	
2050	(*E*)-Nerolidol	–	0.1	tr
2051	Gleenol	0.5	0.5	0.6
2057	Ledol	tr	–	0.1
2065	γ-Nonalactone	0.3	–	–
2071	Humulene epoxide-II	2.1	–	–
2073	*p*-Mentha-1,4-dien-7-ol	–	0.3	0.2
2080	Cubenol	–	0.7	0.7
2084	Octanoic acid	0.9	–	–
2088	1-*epi*-Cubenol	2.6	2.9	2.6
2095	Hexyl benzoate	–	–	0.6
2098	Globulol	0.6	–	0.1
2104	Viridiflorol	4.9	4.6	4.4
2113	Cumin alcohol	0.4	0.5	0.5
2131	Hexahydrofarnesyl acetone	–	–	0.3
2144	Spathulenol	5.8	1.7	1.8
2148	(*Z*)-3-Hexen-1-yl benzoate	–	–	0.8
2170	(*E*)-2-Hexen-1-yl benzoate	–	–	1.4
2179	Tetradecanol	–	–	0.4
2186	Eugenol	0.7	0.4	0.5
2187	T-Cadinol	2.8	1.5	1.5
2192	Nonanoic acid	1.5	0.1	0.3
2209	T-Muurolol	3.7	4.5	3.5
2209	Cembrene	–	0.3	–
2219	δ-Cadinol (=alpha-muurolol)	1.7	0.5	0.7
2219	Dimyrcene II-a	tr	–	tr
2226	Methyl hexadecanoate[7]	0.2	–	0.3
2228	Pogostol	1.4	0.9	1.1
2239	Carvacrol	–	0.7	0.8
2250	α-Eudesmol	1.2	0.8	0.8
2255	α-Cadinol	10.1		
2256	Cadalene	–	4.3	5.4
2257	β-Eudesmol	3.9		
2273	Selin-11-en-4α-ol	0.5	–	0.6
2278	Neocembrene A	–	0.5	0.7
2298	Decanoic acid	0.6	0.4	0.6
2369	(2*E*,6*E*)-Farnesol	–	0.1	0.8
2369	Eudesma-4(8),7-dien-4β-ol	1.5	0.4	0.5
2389	Caryophylla-2(12),6-dien-5α-ol[8]	0.5	–	–
2392	Vertisilla-4(20),7,11-triene	–	0.7	0.7

(Continued)

TABLE 1. *(Continued)*

RRI[1]	Compound	Stored powder	Fresh aerial part	Fresh flowering tops
			(%)[2]	
2418	VOC-1[9]	–	4.2	1.5
2419	4-Isopropyl-6-methyl-1,2,3,4-tetrahydronaphthalen-1-one	0.8	tr	tr
2438	VOC-2	–	3.6	1.8
2500	Pentacosane	–	–	0.5
2503	Dodecanoic acid	0.2	0.4	0.1
2600	Hexacosane	–	–	0.3
2622	Phytol	–	0.1	0.3
2670	Tetradecanoic acid[10]	–	–	0.1
2700	Heptacosane	–	–	0.4
2724	VOC-3	–	7.1	3.1
2822	Pentadecanoic acid	–	0.2	–
2921	VOC-4	–	2.8	2.4
2931	Hexadecanoic acid	0.2	0.6	0.7
2946	VOC-5	–	1.3	0.5
	Total	69.5	84.4	79.3

[1]RRI = Relative retention indices calculated against n-alkanes.
[2]% calculated from FID data.
[3]Trace (< 0.1 %).
[4]4,11-Eudesmadiene.
[5]Trans-p-Menth-1-en-3-ol.
[6]Limonen-4-ol.
[7]Methyl palmitate.
[8]Caryophyllenol I.
[9]VOC-1 to 5 are unknown constituents.
[10]Myristic acid.

δ-cadinene (4.8%), viridiflorol (4.4%), α-calacorene (3.8%), T-muurolol (3.5%), α-terpineol (3.4%) and 1-epi-cubenol (2.6%). In addition to the known compounds, five unknown constituents (VOC 1-5) were detected in the fresh aerial samples (19% of total) and the fresh flowering tops (9.3%), but could not be identified as they could not be isolated in a pure form (Table 2).

The yield of essential oil, the diversity of compounds, and the level of identified constituents favored the use of the fresh aerial parts and fresh flowering tops in oil extraction. The number of identified compounds (125 total) in the analyzed samples far exceeded the maximum number

TABLE 2. Electron impact mass spectrometry of unknowns

VOC unknowns[1]	Relative intensity of mass to charge ratio (m/z)
VOC-1	m/z (rel. int.): 272[M]$^+$(10), 257(35), 229(6), 189(30), 173(12), 161(39), 147(33), 134(100), 133(92), 119(94), 107(66), 93(60), 91(69), 81(61) and 79(53).
VOC-2	m/z (rel. int.): 272[M]$^+$(11), 257(73), 229(22), 201(18), 189(15), 175(26), 161(41), 147(32), 133(53), 121(96), 107(61), 93(100), 91(73), 79(78), 67(60) and 55(48).
VOC-3	m/z (rel. int.): 288[M]$^+$(3), 273(4), 245(16), 227(9), 201(23), 188(17), 159(44), 145(61), 133(32), 119(49), 105(69), 91(60), 82(59), 69(85), 43(92) and 41(100).
VOC-4	m/z (rel. int.): 272[M]$^+$(7), 257(20), 229(5), 189(6), 161(9), 147(13), 135(20), 123(100), 121(28), 109(28), 93(26), 81(31), 55(22) and 43(42).
VOC-5	m/z (rel. int.): 290[M]$^+$(12), 272(3), 257(2), 191(15), 173(19), 163(22), 151(7), 135(69), 122(67), 109(57), 95(93), 81(85), 67(56) and 43(100).

[1]Unidentified volatile organic compounds.

identified in any other *Commiphora* species studied to date. Some identified components, common to all studied species, include δ- and γ-cadinene, T-cadinol, α-copaene, β-elemene, β-eudesmol, α-gurjunene, α-humulene, and α- and β-selinene. The review by Hanus et al. (14) can be used for comparison of the currently reported results with those obtained for other species of *Commiphora* species.

Only the essential oil of fresh aerial parts provided sufficient material for preliminary biological evaluation for in-vitro assays for antimicrobial, antimalarial, antitumor, antiinflammatory, antioxidant, and estrogenic activity. Compared with respective biologically active controls, the essential oil of the fresh aerial parts exhibited weak antimicrobial activity against *C. glabrata*, *C. krusei*, *C. neoformans*, and *M. intracellulare* with IC_{50} = 80, 90, 150 and 15 μg/mL, respectively, as compared with. amphotericin B at 0.15, 0.7 and 0.85 μg/mL, and ciprofloxacin at 0.3 μg/mL, respectively. The essential oil was cytotoxic to the four tested tumor cell lines: SK-MEL, KB, BT549, and SK-OV3, but the cytotoxicity was nonselective and almost 50-times less active than doxorubicin (Table 3).

Although inactive in the DCFH-DA antioxidant assay in a cellular system, the same essential oil sample exhibited weak activity in the DPPH assay (mean IC_{50} = 892 μg/mL vs. silymarin at 61 μg/mL), suggesting antioxidant activity of the essential oil may be mediated through a

TABLE 3. Anticancer activity and cytotoxicity of the essential oil of *C. opobalsamum*

Sample	Cancer cells				Non cancer cells	
	SK-MEL[1]	KB[2]	BT-549[3]	SK-OV3[4]	VERO[5]	LLC-PK1[6]
	IC_{50} (µg/ml)					
Essential oil	97	70	48	82	90	57
Doxorubicin[7]	1.7	1.5	1.1	1.7	>5	1.7

[1]Human malignant melanoma.
[2]Human oral epidermal carcinoma.
[3]Human breast ductal carcinoma
[4]Human ovary carcinoma.
[5]Monkey kidney fibroblast.
[6]Pig kidney epithelial cell.
[7]Positive control.

free-radical scavenging mechanism. The antioxidant activity, however, was not strong enough to inhibit ROS-catalysed oxidation of DCFH in HL-60 cells. Whether the lack of antioxidant activity in HL-60 cells is due to reduced permeability or to other intrinsic properties of the essential oil constituents is unclear. No antimalarial, antiinflammatory, or estrogenic activity was observed for the fresh aerial parts (data not shown). Results of the preliminary antimicrobial evaluation of *C. opobalsamum* essential oil may partially substantiate the folkloric use of its aerial parts for treating infected wounds, and for oral hygiene. Further evaluation in an extended microbial panel is thus warranted.

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