Available online at www.sciencedirect.com
SCIENCE DIRECT®
PERGAMON
Phytochemistry 62 (2003) 1251–1255
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Muscanone: a 3-*O*-(1″, 8″, 14″-trimethylhexadecanyl)naringenin from *Commiphora wightii*

Majekodunmi O. Fatope[a,*], Suad Khamis S. Al-Burtomani[a], John O. Ochei[b], Abdulrahman O. Abdulnour[a], Salma M.Z. Al-Kindy[a], Yoshio Takeda[c]

[a]*Department of Chemistry, College of Science, Sultan Qaboos University, PO Box 36, Al-Khod-123, Muscat, Sultanate of Oman*
[b]*Department of Microbiology, College of Medicine, Sultan Qaboos University, PO Box 35, Al-Khod-123, Muscat, Sultanate of Oman*
[c]*Faculty of Integrated Arts and Sciences, University of Tokushima, Minamijosanjimacho 1-Chome, Tokushima 770-8502, Japan*

Received 19 March 2002; received in revised form 20 November 2002

Abstract

A new antifungal flavanone, muscanone (**1**), was isolated along with known naringenin (**2**) from *Commiphora wightii* (Arn.) Bhandari (Burseraceae) by directing the fractionation of an EtOH extract of the air-dried trunk of *C. wightii* with microbial sensitivity assay. The structures of **1** and **2** were determined from EIMS, HREIMS, DEPT, ^{1}H–^{1}H COSY, HSQC and HMBC spectral data. Muscanone (**1**) was identified as 3-*O*-(1″, 8″,14″-trimethylhexadecanyl)naringenin and was found to be active against *Candida albicans*. The isolation, structure elucidation, NMR spectral assignments, and bioactivities of **1** and **2** are reported.
© 2003 Elsevier Science Ltd. All rights reserved.

Keywords: *Commiphora wightii*; Burseraceae; Flavanone; Antifungal activity; Brine shrimp lethality test; Structure elucidation

1. Introduction

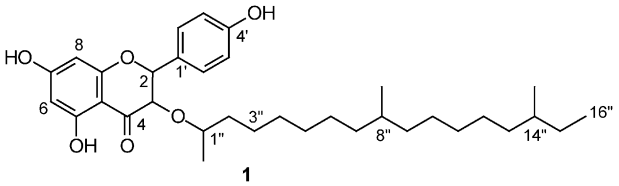
Commiphora wightii (Arn.) Bhandari (Syn. *C. mukul* Hook ex Stocks) Engl. (Burseraceae) is a prostrate and spiny shrub with a whitish bark. It has serated, non-hairy and trifoliate leaflets. It is thus distinct from *Commiphora foliacea, Commiphora habessinica* and *Commiphora gileadensis* (Miller and Morris, 1988), which are also found in the Sultanate of Oman. The Dhofaris in the southern part of Oman use *Commiphora* plants to disinfect wounds and also as an anthelmintic and hair shampoo (Miller and Morris, 1988). The application of a lotion prepared from the bark of *Commiphora* plants to treat skin conditions such as impetigo, eczema and shingles motivated us to investigate *C. wightii* for antifungal components. Many groups have studied the phytochemistry of the genus *Commiphora* and found dammarane triterpenes (Dekebo et al., 2002; Waterman and Ampoto, 1985), ferulates (Zhu et al., 2001), furanosesquiterpenes (Brieskorn and Noble, 1983; Manguro et al., 1996; Maradufu, 1982; Ubillas et al., 1999), mansumbinane derivatives (Provan et al., 1992), steroids (Bajaj and Dev, 1982), lanostenols (Provan and Waterman, 1988), sesquiterpenes (Andersson et al., 1997; Dolara et al., 2000), oxygenated alkanes (McDowell et al., 1988), guggulsterones (Swaminathan et al., 1987), guggutetrols (Kumar and Dev, 1987), and lignans (Provan and Waterman, 1985). We report here the isolation of a new flavanone, muscanone (**1**), and known naringenin (**2**) from the air-dried trunk of *C. wightii,* and their bioactivity on *Candida albicans*. Previously, flavonoids with antimicrobial properties have been isolated from the Leguminosae (Harborne, 1999) and Cactaceae (Pare et al., 1991) families.

2. Results and discussion

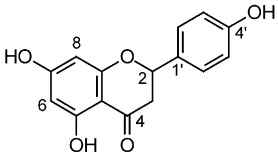
A portion of the EtOH extract of air-dried trunk of *C. wightii* was fractionated by solvent–solvent partition between $CHCl_3$ and H_2O, followed by further fractionation of the $CHCl_3$ soluble residue between 10% aqueous MeOH and hexane. The residue obtained on evaporation of the aqueous MeOH fraction constituted ca. 80% of the EtOH extract. It was lethal to brine

* Corresponding author. Tel.: +968-515491; fax: +968-513415.
E-mail address: majek@squ.edu.om (M.O. Fatope).

0031-9422/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0031-9422(02)00686-6



1



2

shrimp larvae (BST $LC_{50} = 196$ μg/ml) but inactive against *Candida albicans*, *Staphylococcus aureus*, and *Streptococcus pyogenes* at 1000 μg/ml. The separation achieved by this procedure did not lead to the enrichment of the antifungal component in the aqueous methanol fraction. As a result, another portion of the EtOH extract was submitted to CC over silica gel to give seven combined fractions. Fractions 4 and 5, eluted with $CHCl_3$–EtOAc (1:1) and EtOAc, were active against *Candida albicans*, *Staphylococcus aureus*, and *Streptococcus pyogenes* at 500 μg/ml. The active fractions were combined and further separated on silica gel column to give **1** and **2**.

Muscanone (**1**) was obtained as a brownish solid. The EIMS spectrum of **1** gave a molecular ion at *m/z* 554 $[M]^+$, which was established as $C_{34}H_{50}O_6$ on the basis of HREIMS. This also suggested the presence of 10 unsaturated equivalents in the structure of **1**. The IR spectrum shows absorptions due to hydroxyl, carbonyl and aromatic groups. The presence of a naringenin substructure in **1** was demonstrated by UV absorptions at 214, 292, 225, and 337 nm, respectively (Harborne, 1998). A comparison of the ^1H NMR and ^{13}C NMR data of **1** and **2** (Table 1) showed some similarities and differences. The pattern of the aromatic signals of the two compounds are similar, thus further suggesting a 4′,5,7-trihydroxyflavanone nucleus for muscanone (**1**). The resonance of H-3 in compound **1** was observed at δ 5.09 (1H, *d*, $J = 11.6$ Hz) but the H-3 atoms of **2** resonated as two signals at δ 3.17 (1H, *dd*, $J = 17.0$, 13.0 Hz), δ 2.70 (1H, *d*, $J = 17.0$ Hz), respectively. The multiplicity and the chemical shift of H-3 in **1** as compared with **2** indicated the presence of an alkoxy substituent at C-3 of **1**. A cluster of overlapping signals was observed at δ 0.80–1.10, 1.20–1.40, 1.60–1.90, and 5.13 in the ^1H NMR of **1**. These signals were attributed to the C_{19} ether side chain. The ^{13}C NMR spectrum of **1** indicated the presence of 13 sp^2 carbons (one of which is a ketone carbon), 12 methylene, four methyl, three methinoxy and two methine groups. The DEPT and HSQC NMR spectra partially established a saturated substructure for the C_{19} ether side chain. The signal at δ_c 69.8 (C-1″) showed cross-peaks to the signals at δ 5.13 (1H, *m*, H-1″), and the signals at δ_c 34.0 (C-8″) and 34.5 (C-14″) also showed cross-peaks to the overlapping signals of H-8″ and H-14″ between δ 1.20 and 1.40. The presence of a 9,15-dimethyl-2-heptadecanoxy moiety at C-3 was further supported by the fragmentation pattern (Fig. 1) of **1** in the EIMS, and by the connectivity observed in the HMBC spectrum. Compound **1** gave a molecular ion peak at *m/z* 554 $[M]^+$ in the EIMS. The cleavage of the oxygen-alkyl ether bond and the protonation of the resulting ion gave *m/z* 288. The removal of ring B, corresponding to 93 amu, from the molecular ion and the truncation of the side chain led to a mass spectral fragment at *m/z* 434. The presence of the peaks at *m/z* 402, 401, 153, 152 and 94 unambiguously showed that the C_{19} moiety is not tethered to ring A or B in compound **1**. The peaks at *m/z* 401 and 153 were formed by retro

Table 1
^1H and ^{13}C NMR of compounds **1** and **2**

Entry	**1**			**2**		
	δ_H (*J* in Hz)[a]	δ_C DEPT[b]		δ_H (*J* in Hz)[a]	δ_C DEPT[b]	
1						
2	4.66	(11.6, *d*) 72.2	CH	5.45(13.0, *d*)	79.0	CH
3	5.09	(11.6, *d*) 83.4	CH	3.17(17.0, 13.0, *dd*) 2.70(17.0, *d*)	42.6	CH_2
4		197.3			196.3	
5		164.1			164.4	
6	5.99	(*s*) 96.2	CH	5.96(*s*)	95.9	CH
7		166.9			166.4	
8	5.95	(*s*) 95.1	CH	5.96(*s*)	95.9	CH
9		163.3			163.5	
10		100.6			102.3	
1′		128.2			128.1	
2′	7.43	(8.1, *d*) 129.3	CH	7.40(7.6, *d*)	129.9	CH
3′	6.91	(8.1, *d*) 115.0	CH	6.91(7.6, *d*)	115.3	CH
4′		157.9			157.8	
5′	6.91	(8.1, *d*) 115.0	CH	6.91(7.6, *d*)	115.3	CH
6′	7.43	(8.1, *d*) 129.3	CH	7.40(7.6, *d*)	129.9	CH
1″	5.13	(*m*) 69.8	CH			
2″	1.30–1.40	(*m*) 36.8	CH_2			
3″	0.80–1.10	(*m*) 24.3	CH_2			
4″	0.80–1.20	(*m*) 31.1	CH_2			
5″	0.80–1.20	(*m*) 31.1	CH_2			
6″	0.80–1.20	(*m*) 27.4	CH_2			
7″	1.30–1.40	(*m*) 35.5	CH_2			
8″	1.20–1.40	(*m*) 34.0	CH			
9″	1.30–1.40	(*m*) 35.4	CH_2			
10″	0.80–1.20	(*m*) 27.2	CH_2			
11″	0.80–1.20	(*m*) 31.5	CH_2			
12″	0.80–1.20	(*m*) 26.9	CH_2			
13″	1.30–1.40	(*m*) 35.0	CH_2			
14″	1.20–1.40	(*m*) 34.5	CH			
15″	1.20–1.40	(*m*) 30.3	CH_2			
16″	0.88	(6.3, *t*) 17.8	CH_3			
1″-CH_3	1.62	(6.7, *d*) 24.9	CH_3			
8″-CH_3	1.00	(9.6, *d*) 20.3	CH_3			
14″-CH_3	0.97	(9.6, *d*) 19.9	CH_3			

[a] Assigned by ^1H–^1H COSY.
[b] Protonated carbons assigned by HSQC.

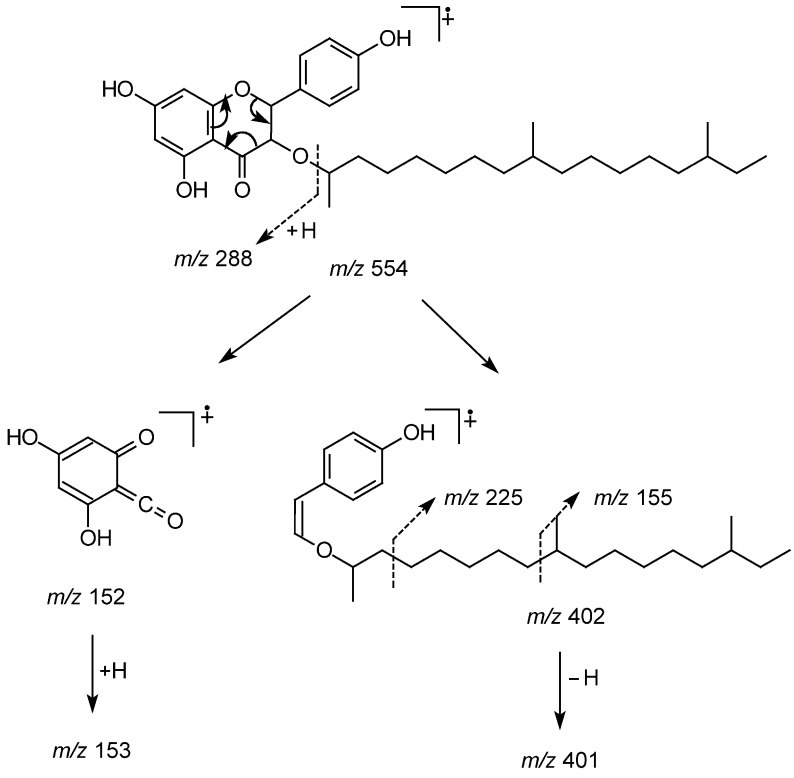


Fig. 1. EIMS fragmentation pattern of compound **1**.

Diels-Alder cleavage of ring C (Fig. 1), and the peak at *m*/*z* 94 was derived from a cleavage of the carbon–carbon bond between ring B and C followed by a protonation of the resulting phenoxyl ion. The ion at *m*/*z* 402 lost 93 amu or ring B and two 42 amu to give peaks at *m*/*z* 309, 267 and 225. Further fragmentation of the ion at *m*/*z* 225 by successive lost of five methylene groups resulted in peaks at *m*/*z* 211, 197, 183, 169 and 155, respectively. Long-range connectivity of δ 5.09 (H-3) with δ_c 69.8 (C-1″) and that of δ 5.13 (H-1″) with δ_c 24.3 (C-3″) were discerned in the HMBC NMR. In addition, connectivity of signals at δ 1.62 (1″-CH_3) with δ_c 69.8 (C-1″), at δ 1.00 (8″-CH_3) with δ_c 35.5 (C-7″) or δ_c 35.4 (C-9″), at δ 0.97 (14″-CH_3) with δ_c 35.0 (C-13″) or δ_c 30.3 (C-15″), and also at δ 0.88 (16″-CH_3) with δ_c 35.4 (C-14″) were observed. Therefore, **1** was identified as 3-*O*-(1″, 8″, 14″-trimethylhexadecanyl)naringenin, and its complete ^{1}H NMR and ^{13}C NMR data are given in Table 1.

Compound **2**, $C_{15}H_{12}O_5$, had ^{13}C NMR characteristics that are consistent with those of naringenin (Breitmaier and Voelter, 1990). It gave the expected molecular ion at *m*/*z* 272 $[M]^+$ in the EIMS. Table 1 shows the ^{1}H and ^{13}C NMR spectral data of **2**. After the assignments of the UV, IR, 1D and 2D NMR spectral data, it was possible to establish that **2** is a 4′,5,7-trihydroxyflavanone.

The two isolates were tested for antifungal activity against *Candida albicans*. The new flavanone, muscanone (**1**) inhibited the growth of *C. albicans* at 250 μg/ml. Naringenin (**2**) did not. Flavanones with stronger antifungal properties have previously been isolated from plants (Miles et al., 1991). Our result is of interest to us from structure–activity considerations and medicinal uses of *C. wightii*.

3. Experimental

3.1. General

Melting points were determined on a Gallenkamp apparatus and were uncorr. UV was recorded on a UV–visible HP-8453 spectrophotometer. IR was measured on a Nicolet FT-IR spectrometer. ^{1}H and ^{13}C NMR (DEPT, ^{1}H–^{1}H COSY, HSQC and HMBC) were recorded on Brüker Avance 400 spectrometer at 400 and 100.58 MHz, respectively with TMS as internal standard. EIMS (70 eV) and HREIMS (3.0 KV) were obtained with Jeol JMS-SX102A. Kieselgel (Riedel-deHaen) 70-230 mesh, was used for CC and Whatman precoated silica gel (60A K6F) plates were used for TLC. TLC bands were visualized under UV lamp or by spraying with vanilline-H_2SO_4 followed by heating.

3.2. Plant material

Thick and spiny branches or trunks of *C. wightii* were collected from a hill opposite the gate of PDO Staff

Club, Qurum, Muscat in September 1999. A specimen of the trunk is preserved at Sultan Qaboos University Herbarium, Oman.

3.3. Extraction and isolation

Air-dried trunks of *C. wightii* (800 g) were milled and extracted at room temperature by maceration with EtOH (5.0 l) for 2 weeks. The extract was concentrated under reduced pressure to give a residue (62 g). A portion of the extract (25 g) was fractionated between water (400 ml) and chloroform (400 ml). The residue from the chloroform fraction was partitioned between hexane (400 ml) and 10% aqueous MeOH (400 ml). On evaporation, the aqueous methanol fraction gave a residue of 20.6 g, which was inactive at 1000 μg/ml against *Candida albicans*. A second portion of the the EtOH extract (24 g) was chromatographed on silica gel (130 g) and eluted successively with solvents to give fractions and residues shown in parenthesis: hexane (0.33 g); $CHCl_3$–hexane, 1:1, (0.17 g); $CHCl_3$ (0.96 g); $CHCl_3$–EtOAc, 1:1, (3.11 g); EtOAc, (0.22 g); EtOAc–EtOH, 3:1, (12.53 g); and EtOAc–EtOH, 1:1, (0.27 g). Fractions 4 and 5 eluted with $CHCl_3$–EtOAc, (1:1) and EtOAc were active against *Staphylococcus aureus*, *Streptococcus pyogenes* and *Candida albicans* at 500 μg/ml. Fractions 4 and 5 were combined to give a residue (3.33 g), which was further chromatographed on silica gel (50 g) and eluted with increasing polarity of hexane–EtOAc mixtures to give 13 fractions. Fraction 5, eluted with Hexane–EtOAc (4:1), gave Naringenin (**2**) (330 mg) with R_f value of 0.75 (Eluent EtOAc±hexane, 2:1). Fraction 7 eluted with Hexane–EtOAc (6.5:3.5), gave muscanone (**1**) (144 mg) with R_f value of 0.63 (Eluent EtOAc–hexane, 2:1).

3.4. Muscanone (3-O-(1″, 8″, 14″-trimethylhexadecanyl) naringenin) (*1*)

Brownish solid. mp 124–126 °C; IR ν_{max}^{KBr} cm^{-1}: 3424, 2926, 1731, 1645, 1473, 1259, 1164, 829; UV λ_{max}^{EtOH} nm (log ε): 214 (3.4), 225 (2.4), 292 (2.2), 337 (1.6); EIMS (rel. int.): 554 $[M]^+$ (13); 434 (62); 418 (30); 402 (12); 401 (25); 309 (10); 267 (10); 288 (25); 259 (53); 225 (19); 197 (13); 183 (22); 169 (22); 157 (25); 155 (22); 153 (100); 131 (40); 94 (55); 53 (12); 18 (20). HREIMS *m/z* 554.3585 (calc. for $C_{34}H_{50}O_6$ 554.36074). ^1H and ^{13}C NMR spectral data in acetone-d_6 are given in Table 1.

3.5. Brine shrimp lethality test (BST)

Fractions were evaluated for lethality to brine shrimp using standard protocols (Meyer et al., 1982; McLaughlin, 1991). In this test a drop of DMSO was added to vials of the test and control substances to enhance the solubility of test materials.

3.6. Antifungal assay

Antimicrobial sensitivity test was performed using a standard agar-dilution-streak method (Mitscher et al., 1972, 1984). *C. albicans*, *S. aureus*, and *S. pyogenes* clinical isolates used were from laboratory stock cultures. After culturing one or two microbiological loop of the organisms in tryptone soya broth (10 ml) (Oxoid) at 37 °C for 24 h, 1 ml of a suspension of *C. albicans* and 100 μl of other organisms were separately diluted in 10 ml sterile saline and used to streak tryptone soya agar (Oxoid) plates containing test samples, negative controls and positive controls containing streptomycin and ketoconazole, respectively. All extracts were dissolved in DMSO. The concentration of test sample that inhibited each of the organisms, after incubation at 37 °C for 24 h was determined by serial 2-fold dilutions.

Acknowledgements

Grant IG/SCI/CHEM/99/01 from College Research Committee, Sultan Qaboos University, to MOF is gratefully acknowledged.

References

Andersson, M., Bergendorff, O., Shan, R., Zygmunt, P., Sterner, O., 1997. Minor components with smooth muscle relaxing properties from scented myrrh (*Commiphora guidotti*). Planta Med. 63, 250–254.

Bajaj, A.G., Dev, S., 1982. Chemistry of ayurverdic crude drugs—V. guggulu (resin from Commiphora mukul)—5. Some new steroidal components and stereochemistry of guggulsterol-I at C-20 and C-22. Tetrahedron 38, 2949–2954.

Breitmaier, E., Voelter, W., 1990. Carbon-13 NMR Spectroscopy: High Resolution Methods and Applications in Organic Chemistry and Biochemistry, third ed. VCH Publishers, New York.

Brieskorn, C.H., Noble, P., 1983. Furanosesquiterpenes from the essential oil of myrrh. Phytochemistry 22, 1207–1211.

Dekebo, A.A., Dagne, E.E., Lars, L.K., Hansen, K., Od, O.R., Gautum, R., Arne, A.J., Aasen, J., 2002. Two octanordammarane triterpenes from *Commiphora kua*. Phytochemistry 59, 399–403.

Dolara, P., Corte, B., Ghelardinic, C., Pugliese, A.M., Cerbai, E., Menichetti, S., Nostro, A.L., 2000. Local anaesthetic, antibacterial and antifungal properties of sesquiterpenes from myrrh. Planta Med. 66, 356–358.

Harborne, J.B., 1998. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, third ed. Chapman and Hall, London.

Harborne, J.B., 1999. The comparative biochemistry of phytoalexin induction in plants. Biochem. Syst. Ecol. 27, 335–367.

Kumar, V., Dev, S., 1987. Chemistry of ayurvedic crude drugs-VII: absolute stereochemistry of guggultetrols. Tetrahedron 43, 5933–5948.

Manguro, L.O.A., Mukonyi, K.M., Githiomi, J.K., 1996. Bisabolenes and furanosesquiterpenoids of Kenya *Commiphora kua* resin. Planta Med. 62, 84–85.

Maradufu, A., 1982. Furanosesquiterpenoids of *Commiphora erythraea* and *C. myrrh*. Phytochemistry 21, 677–680.

McDowell, P.G., Lwande, W., Deans, S.G., Waterman, P.G., 1988. Volatile resin exudates from stem bark of *Commiphora rostrata*: potential role in plant defence. Phytochemistry 27, 2519–2522.

McLaughlin, J.L., 1991. Crown gall tumours on potato discs and brine shrimp lethality. Two simple bioassays for higher plant screening and fractionation. In: Hostettmann, K. (Ed.), Methods in Plant Biochemistry, Volume 6. Academic Press, London, pp. 1–33.

Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E., McLaughlin, J.L., 1982. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 45, 31–34.

Miles, D.H., Meideros, J., Chen, L., Chittawong, V., Swithenbank, C., Lidert, Z., Payne, A.M., Hedin, P.A., 1991. A search for agrochemicals from Peruvian plants. In: Hedin, P.A. (Ed.), Naturally Occurring Pest Bioregulators, 1991. (Ed.), ACS Symposium Series 449Chapter 28. American Chemical Society, Washington, DC, pp. 399–406.

Miller, A.G., Morris, M., 1988. Plants of Dhofar—The Southern Region of Oman: Traditional Economic and Medicinal Uses. The Office of the Adviser for Conservation of the Environment, Diwan of Royal Court, Sultanate of Oman, pp. 82–28.

Mitscher, L.A., Leu, R.P., Bathala, M.S., Wu, W.N., Beal, J.L., White, R., 1972. Antimicrobial agents from higher plants I. Introduction, rationale and methodology. Lloydia 35, 157–166.

Mitscher, L.A., Rao, R.G.S.. In: Krogsgaard-Larsen, P., Christensen, B.S., Kofold, H. (Eds.), Natural Products and Drug Development. Munksgaard, Copenhagen, pp. 193–209.

Pare, P.W., Dmitrieva, N., Mabry, T.J., 1991. Phytoalexin aurone induced in *Cephalocereus senilis* liquid suspension culture. Phytochemistry 30, 1133–1135.

Provan, G.T., Waterman, P.G., 1985. Picropolygamain: a new lignan from *Commiphora incisa* resin. Planta Med. 271–272.

Provan, G.T., Waterman, P.G., 1988. Major triterpenes from the resins of *Commiphora incisa* and *C. kua* and their potential chemotaxonomic significance. Phytochemistry 27, 3841–3844.

Provan, G.T., Gray, A.T., Waterman, P.G., 1992. Mansumbinane derivatives from stem bark of *Commiphora kua*. Phytochemistry 31, 2065–2068.

Swaminathan, S., Bakshi, R.K., Dev, S., 1987. Higher isoprenoids-XIX. Guggulsterones to dexamethasone. Tetrahedron 43, 3827–3838.

Ubillas, R.P., Mendez, C., Jolad, S.D., Luo, J., King, S.R., Carlson, T.J., Fort, D.M., 1999. Antihyperglycemic furanosesquiterpenes from *Commiphora myrrh*. Planta Med. 65, 778–779.

Waterman, P.G., Ampofo, S., 1985. Dammarane triterpenes from the stem bark of *Commiphora dalzielli*. Phytochemistry 24, 2925–2928.

Zhu, N., Rafi, M.M., DiPaola, R.S., Xin, J., Chin, C.-K., Badmaev, V., Ghai, G., Rosen, R.T., Ho, C.-T., 2001. Bioactive constituents from gum guggul (*Commiphora wightii*). Phytochemistry 56, 723–728.