



PHYTOCHEMICAL INVESTIGATION OF RESINS FROM KENYAN *Commiphora holtziana*

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ARTICLE INFO

Article History:

Received 28<sup>th</sup> April, 2013  
Received in revised form  
11<sup>th</sup> May, 2013  
Accepted 16<sup>th</sup> June, 2013  
Published online 18<sup>th</sup> July, 2013

Key words:

*Commiphora holtziana*,  
Kenyan,  
Phyto-chemical,  
Resins.

ABSTRACT

*Commiphora holtziana* gum resins when solvent extracted followed by a combination of chromatographic separation techniques on hexane extract of the Wajir sample, led to the isolation and characterization of a new compound, 11-hydroxy- $\gamma$ -muurolene **1**. In addition, two known compounds, (1E)-2-methoxy-8,12-epoxygermacra-1(10),7,11-triene-6-one **2** and (1E)-3-methoxy-8,12-epoxygermacra-1,7(8),10(15),11-tetraen-6-one **3** were also characterized. A total of 14 compounds were identified by the comparison of the mass spectra with data available in the GC – MS library. Both dichloromethane and hexane extracts from both Isiolo and Wajir populations showed antibacterial activity. In addition the hexane extract from Wajir population showed antifungal properties. The acetone extract from Wajir population showed antibacterial properties. Activities were observed against Fungi, Gram (+) bacteria and Gram (-) bacteria. Pure compounds did not show any activity.

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INTRODUCTION

Most *Commiphora holtziana* is found in Eastern Africa, with a few species occurring in Arabia and India. It is valued highly for its medicinal properties both in Kenya and the Far – East (Beentje, 1994; Gillet, 1991). The most commercialized species is *C. myrrh* with *C. holtziana* commercialized either as an adulterator or as the Kenyan myrrh and going for a lower price. Previous Phytochemical studies on the *Commiphora* species have shown that the resins contain polysaccharides, proteins, steroids, sterols and terpenes (Hanus *et al.*, 2005). The terpenes are mainly sesquiterpenes notably furanosesquiterpenes based on eudesmane, 4, elemene 5 and germacrene 6 (Provan *et al.*, 1987). In this paper, we report the isolation of a new compound along with other known compounds. The generation of knowledge on the chemistry and biological activity of the resins is also reported.

MATERIALS AND METHODS

**General:** CC on Merck Silica gel 60 (70 – 230 mesh) and Sephadex LH-20. Analytical TLC: Merck pre-coated silica gel 60 PF<sub>254</sub>. PTLC: silica gel (Merck). Chromatographic zones were detected under UV (254, 366 nm) and/or iodine vapor in some cases. GC-MS analyses was performed on an Agilent HP 7890A GC system using a fused Silica capillary column (30 m x 0.25mm i.d., thickness 0.25 $\mu$ m, DB-5), fitted with an on – column injector, which was directly coupled to a magnetic sector 5975C triple axis MSD, (Thermo-Finnigan MAT95 XP, Bremen, Germany). Ionization was by electron impact (70eV, source temperature 285°C). The oven temperature was maintained at 35°C for 10 min, and then programmed at 10°C/min to 280°C which was maintained for 10.5 minutes. The carrier gas was helium, with a

flow rate of 1.2ml/min. The total run time was 40 minutes. Tentative identifications were given by the GC–MS library. <sup>1</sup>HNMR (200 MHz) and <sup>13</sup>C NMR (50 MHz).

**Plant material:** The *Commiphora holtziana* gum resin was collected from Isiolo and Wajir districts and identified by a KEFRI taxonomist. Samples were picked by hand and packed in clear poly-ethene paper bags and coded.

**Extraction and isolation:** Air dried and ground resin (400g) of the Wajir sample was first steam distilled and oil collected using an essential oil extractor. The volume of the oil was determined by reading from the graduated collector. For isolation of compounds, another 400g of the Wajir sample was extracted first with hexane, followed by dichloromethane, acetone and finally with methanol. The hexane extract (24 g) was subjected to column chromatography with column size (4.4 cm x 20 cm) on Silica gel (200 g) eluting with hexane containing increasing amounts of ethyl acetate. The fraction eluted with 10% ethyl acetate in hexane (1.3 g) was subjected to further CC Silica gel (1.8 cm x 20 cm) eluting with hexane containing increasing amounts of ethyl acetate and then Sephadex LH-20 (eluting with CH<sub>2</sub>Cl<sub>2</sub> /MeOH; 1:1) and PTLC (eluent, n-hexane/acetone, 10:0.5) on Silica gel to give compounds **1** (102.8 mg), **2** (334.6 mg) and **3** (131.9 mg). The DCM extract (21 g) was chromatographed as above. The fractions from the elution with 100% hexane gave a mixture which was separated by PTLC (eluent, n-hexane/acetone, 10:0.5) to give compound **3** (132.1 mg).

RESULTS AND DISCUSSION

Separation of the oleo gum resins showed three components (Table 1). Extraction and Isolation from hexane extract from Wajir revealed compounds **1**, **2** and **3** while Dichloromethane extract gave **3** from the same population. Compound **1** was isolated as colorless oil, retention time 20.18 in hexane solvent. EIMS showed a [M<sup>+</sup>] peak at

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m/z 220.  $^{13}\text{C}$ NMR-15 C-atoms, DEPT: 4(-CH), 5(-CH<sub>2</sub>), 3(-CH<sub>3</sub>) & 3 quaternary.  $^1\text{H}$ NMR &  $^{13}\text{C}$ NMR (Table 2) showed that this compound is of terpene skeleton and together with the chemotaxonomic data indicated that it is a sesquiterpene derivative similar to  $\gamma$ -muurolene with a difference at C-11.

### 11-Hydroxy- $\gamma$ -muurolene (1)

Colorless oil,  $^1\text{H}$  (CDCl<sub>3</sub>, 200 MHz): 5.55 *br s* (H-4), 4.75, 4.70 *s* (H-15), 1.23 *s* (H-14), 0.99 *s* (H-13), 0.97 *s* (H-12),  $^{13}\text{C}$  (CDCl<sub>3</sub>, 50 MHz): 154.1 (C-9), 149.9 (C-3), 121.6 (C-4), 106.7 (C-15), 80.9 (C-11), 55.1 (C-6), 47.5 (C-10), 40.4 (C-8), 37.3 (C-2), 37.7 (C-5), 30.2 (C-1), 25.0 (C-14), 24.3 (C-7), 21.7 (C-13), 21.5 (12). EIMS (70ev): 220 (20) [M]<sup>+</sup>, 205 (27) [M-Me]<sup>+</sup>, 202 (41) [M-H<sub>2</sub>O]<sup>+</sup>, 187 (38), 177 (31), 122 (18), 159 (91), 147 (45), 119 (100), 105 (39), 91 (55), 79 (20), 43 (24).

**Table 1. Quantification of *C. holtziana* components from Wajir and Isiolo populations**

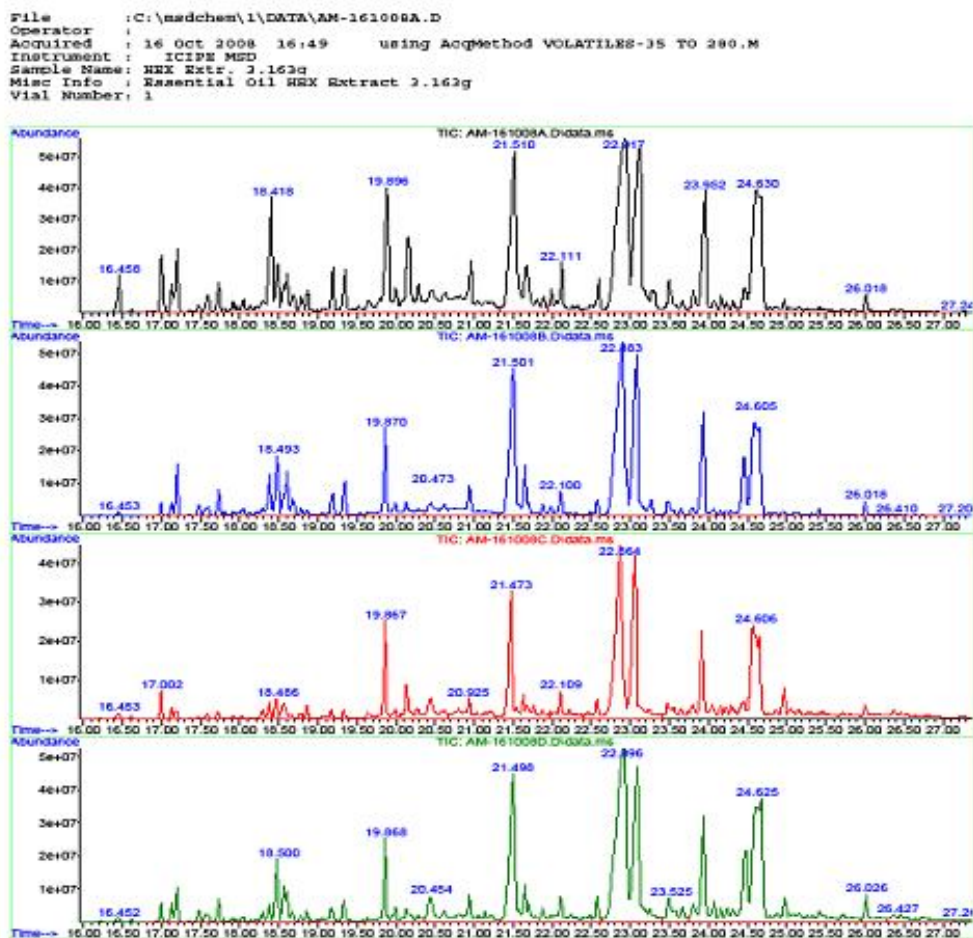
Component	Yield Obtained (%)		Literature Yield (%) [Tucker, 1986]
	Wajir	Isiolo	
Essential oil	9.2	9.1	2 – 10
Solvent soluble resin	41.0	44.0	25 – 40
Water soluble gum	40.2	39.8	30 – 60

The yield of essential oil and the water soluble gum obtained from *C. holtziana* were within range (Tucker, 1986), but the solvent soluble resin was above the range reported in literature. Tracings from the two extracts from two populations show negligible variation (Fig.1)

**Table 2.  $^1\text{H}$ -NMR (200 MHz, CDCl<sub>3</sub>) and  $^{13}\text{C}$ -NMR (50 MHz, CDCl<sub>3</sub>) data of compound 39**

No.	$^1\text{H}$ $\delta_{\text{H}}$ (int, mult, j in Hz)	$^{13}\text{C}$ $\delta_{\text{C}}$
1		30.2
2		37.3
3	5.55 (1H, <i>br s</i> )	149.9
4		121.6
5		37.7
6		55.1
7		24.3
8		40.4
9		154.1
10		47.5
11		80.9
12	0.99/0.97 (3H, <i>s</i> )	21.5
13	0.97/0.99 (3H, <i>s</i> )	21.7
14	1.23 (3H, <i>s</i> )	25.0
15	4.75 (1H, <i>br s</i> ), 4.70 (1H, <i>s</i> )	106.7

Antimicrobial activity (Table 3) of the extracts was evaluated by the agar diffusion assay method (Jorgensen *et al.*, 1999). Crude methanolic extract of *Commiphora holtziana* was active against all Gram positive bacteria, *Bacillus pumilis*, *Bacillus subtilis* and *Staphylococcus aureus* with inhibition zones of 9.5, 9.2 and 10.7mm respectively for the Wajir methanol extract at 5mg/well. Similar results were reported by Musa (2008) where the methanolic extract of *C. kerstingii* Engl. inhibited the growth of several bacteria with *S. aureus* giving the highest inhibition zone of 30mm at 5mg/well. Inactivity was exhibited by methanol extracts from both populations against the fungus *S. cerevisiae* and *E. coli*.



A-Dichloromethane extract and B- hexane extract from Isiolo collection  
C- Dichloromethane extract and D- hexane extract from Wajir collection

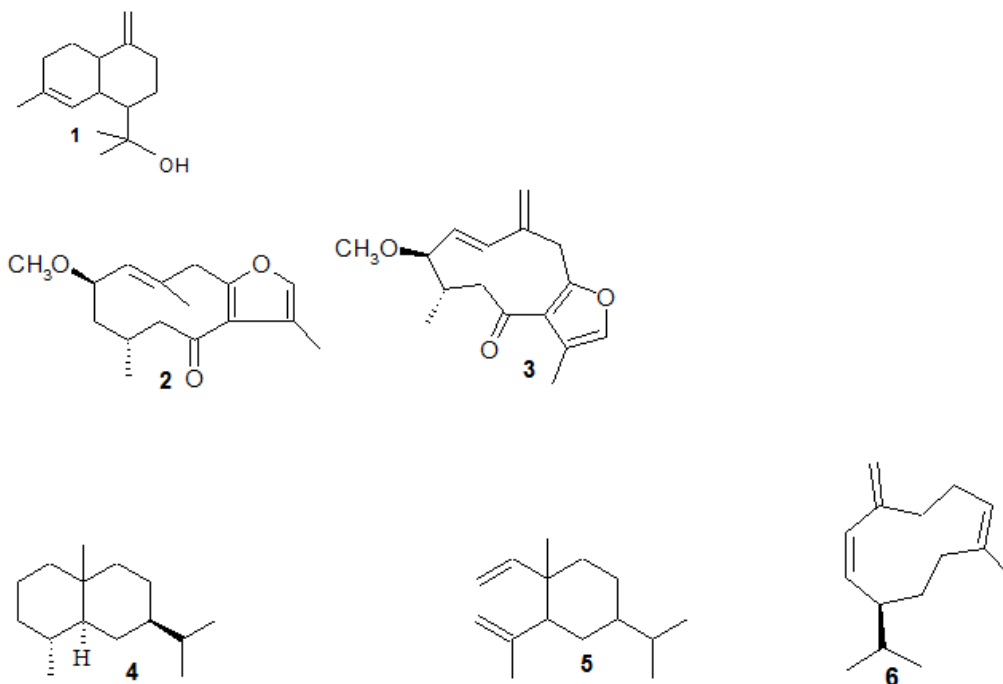
**Figure 1. GC tracings of *Commiphora holtziana* from Wajir and Isiolo collections**

Table 3. Antimicrobial activities of *Commiphora holtziana* crude extracts

Sample (5mg/ml)	1	2	3	4	5
Dichloromethane extract from Isiolo	7.85	8.54	10.32	8.66	11.36
Methanol extract from Isiolo	8.38	8.58	-	8.56	-
Hexane Extract from Isiolo	7.84	8.07	-	8.37	-
Dichloromethane extract from Wajir	8.60	8.75	9.58	9.31	10.62
Acetone Extract from Wajir	8.31	8.64	-	8.83	-
Hexane Extract from Wajir	8.25	8.54	-	8.36	10.54
Methanol Extract from Wajir	9.46	9.16	-	10.66	-
Nystatin	N/A	N/A	N/A	N/A	12.79
Gentamycin	24.30	24.38	10.60	25.00	N/A

Micro organisms: 1= *Bacillus pumilus* (Clinical isolate), 2 = *Bacillus subtilis* (Clinical isolate), 3 = *Escherichia coli* (Clinical isolate), 4 = *Staphylococcus aureus* (Clinical isolate), 5 = *Sacharomyces cerevisiae* (Clinical isolate).

N/A = Not Applicable "-" = Not active



There was also no activity in the Hexane extract from Isiolo and acetone extract from Wajir against the above micro-organisms.

### Conclusion

Three components of *C. holtziana* namely essential oils, resins and gums were separated and quantified. Three compounds, namely 11-hydroxyl- $\gamma$ -muurolene (1(10)*E*, 2*R*\*, 4*R*\*)-2-methoxy-8,12-epoxygermacra-1(10),7,11-trien-6-one and (1*E*)-3-methoxy-8,12-epoxygermacra-1,7(8),10(15),11-tetraen-6-one were isolated and characterized from this plant of which compound appeared to be new. Comparative study by GC – MS analysis of two populations of *Commiphora holtziana* showed appearance of similar compounds in both populations with a variation only in their abundance. There was some anti-microbial activity, probably suggesting why the plant is used traditionally for wound healing.

### Acknowledgements

We acknowledge support by the Government of Kenya through the Kenya Forestry Research Institute (KEFRI). Mr. Francis Gachathi is thanked for identification of the plant material.

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