Aromatic Plants of Kenya III: Volatile and some Non-volatile Constituents of *Croton sylvaticus* Hochst

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The essential oil isolated by hydrodistillation from leaves of *Croton sylvaticus* Hochst (0.14 %) was analyzed by GC and GC-MS. Over 52 components, constituting about 86.3 % of the oil, were separated. The major constituents were β-caryophyllene oxide (35.1 %) and α-humulen1,2-epoxide (12 %).

The petroleum ether extract of the stem bark yielded (-)-hardwickic acid, β-sitosterol, stigmasterol and *ent*-8β- 15,labd- 13-en (E)-diol.

Key Words: *Croton sylvaticus,* Euphobiaceae, essential oil, non-volatiles, GC-MS, β–caryophyllene oxide, hardwickic acid.

INTRODUCTION

Croton sylvaticus Hochst Euphobiaceae) is a tree 3.5-24 m, whose bark smells of black pepper and is greyish and smooth. Leaves (broadly) ovate, base cuneate, rounded or subordate, apex acuminate, margin glandular, crenate-serrate, 6-14 by 3-11 cm densely stellate-pubescent at first, later becoming subglabrous or sparsely pubescent beneath. Flowers are greenish-cream, to 3 mm long monoecious, in racemes 10-30 cm long. Fruit orange or red, trilobed-subglobose or ovoid 7-11 by 5-10 mm, stellate pubescent. The plant is found in various places in Kenya [1].

A decoction of leaves and root bark of *Croton sylvaticus* is traditionally used in treatment of tuberculosis, inflammation and as a purgative, while some parts of the plant are used in treatment of malaria [1, 2]. Pharmacological work on aqueous extract of the stem bark showed that it prolongs duration of ether anaesthesia, reduced exploratory activity, exhibited muscle relaxant activity as measured by chimney test and showed significant analgesic activity on two mouse models, i.e. hot plate and acetic acid writhing tests [3]. We report, in the present study, the composition of essential oil of *Croton sylvaticus* leaves and some non-volatile compounds from its stem bark.

EXPERIMENTAL

Plant material

Croton sylvaticus leaves and stem bark were collected near Kwale Forest Station, Mombasa, in April 1989 and identified by Mr. G. Mungai of the East African Herbarium, Nairobi - Kenya. Voucher specimens were deposited at the Faculty of Pharmacy, University of Nairobi.

Essential oil Isolation

Semi-dried leaves (100 g) were subjected to hydrodistillation for 3 hours in a Clevenger-like apparatus to give the essential oil which was dried over anhydrous sodium sulphate and stored at about 4°C.

Gas chromatography

The essential oil was analyzed by Hewlett-Packard 5890 gas chromatography equipped with FID and coupled to a Hewlett-Packard 3393A integrator. Analytical conditions were: a fused silica column (51 m x 0.22 mm i.d.) coated with methylsilicone (film thickness 0.12 mm; Hewlett-Packard); helium as the carrier gas; the oven temperature was held at 40°C for 5 min, then programmed from 40°C to 180°C at 5°C/min then from 180°C to 280°C at 20°C/min, then isothermal at 280°C for 10 min. Detector temperature was 250°C and the samples were injected on the GC column in the splitless mode.

Gas Chromatography-Mass Spectrometry

The GC-MS analysis was carried out on a VG Masslab 12-250 instrument equipped with a Hewlett-Packard 5790 GC and a data system at an ionization voltage of 7.0 eV and in the electra impact mode using helium as carrier gas and the other GC analytical conditions were identical to those mentioned above.

The identity of the constituents was established by computer mass spectral library search, comparison of MS with those published in the literature, comparison of retention times with those of reference compounds, and by peak enhancement [4].

Isolation of non-volatiles

Room dried and pulverized material of the stem bark (1195 g) of *Croton sylvaticus* was Soxhlet extracted

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with petroleum ether (60 - 800) for 48 hrs and the solvent removed *in vacuo*. The dark brown syrup obtained was dissolved in $CHCl_3$ and chromatographed on a normal silica gel column to obtain 12 fractions using increasing ratios of benzene, chloroform to methanol.

The second fraction was evaporated to dryness, redissolved in methanol and left in the refrigerator for about a week to yield an oily crystalline material. Several re-crystallizations in ethanol yielded pure white crystals. The eighth and eleventh fractions yielded white needle-like crystals and white cluster-like crystals, respectively, from ethyl acetate.

RESULTS AND DISCUSSION

Table 1 shows results of the analysis of essential oil of *Croton sylvaticus* leaves. Over 52 components constituting about 86.3 % of the oil were separated, many of which were identified. The main constituents of this oil were β-caryophyllene oxide (35.1 %) and α-humulen-1,2-epoxide (12.0 %). The essential oil composition indicates that the major ingredients are sesquiterpenoids. While this has been reported to be so for some *Croton* species in Brazil, some of these species have also been found to contain monoterpenoids or phenylpropanoids as major constituents [5]

Structures of the isolated compounds were elucidated by using melting points, proton NMR, 2-D NMR, ^{13}C NMR, MS and published literature [6-9]. They were found to be known compounds which had previously been isolated from other plants. Fraction 2 contained (-)-hardwickic acid (0.6%) [6-9] and fraction 8 a mixture of β-sitosterol and stigmasterol (0.004%). Fraction 11 contained ent-8β-15,labd-13-en E)-diol (0.05%) [8].

The fact that (-)-hardwickic acid has also been isolated from *Croton aromaticus* and *Croton sonderianus* [9] indicates that this compound may be of chemataxonomical importance for this genus. (-)-Hardwickic acid has also been reported to have antimicrobial [9] and insecticidal activity against aphids [10]. There is a need for more pharmacological and biological work on this compound.

TABLE 1: Constituents of the Essential oil of *Croton sylvaticus* leaves

Component	%	Component	%
α-phellandrene	t	*trans*-calamenene	0.20
p-cymene	t	δ-cadinene	3.20
limonene	t	unknown	t
α-terpinene	t	kessane	0.30
cis-linalool oxide	t	α-calacorene	0.80
linalool	0.60	2 unknowns	t
bomeol	0.07	β-calacorene	0.08
cis-pinocamphone	0.20	mint ether	0.20
bornyl acetate	0.30	spathulenol	2.60
a-cubenene	0.40	β-caryophyllene oxide	35.10
unknown	t	unknown	0.40
α-copaene	4.40	salvial-4(14)-en-i-one	0.90
β-bourbonene	1.10	unknown	0.40
unknown	0.90	α-humulen- 1 ,2-epoxide	12.00
thymoquinol dimethyl ether	t	unknown	0.40
β-caryophyllene	1.70	isospathulenol	2.70
geranyl acetate	0.40	unknown	0.80
α-humulene	0.70	unknown	1.00
allo-aromadendrene	0.40	unknown	0.70
β-ionone	t	caryophyllenol?	1.70
amorphene?	0.70	unknown	2.80
germacrene-D	t	unknown	1.10
tridecan-2-one	t	unknown	0.05
unknown	0.90	unknown	0.30
α-muurolene	0.10	hexahydrofamesyl acetate	1.30
unknown	3.50	(E,E)-farnesyl acetate	0.70

t = trace

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