Brine Shrimp Lethality of a Glutarimide Alkaloid from *Croton sylvaticus* Hochst

M.C. KAPINGU[1*] Z.H. MBWAMBO[1], M.J. MOSHI[1] AND J.J. MAGADULA[1],

[1]*Muhimbili University College of Health Sciences, Institute of Traditional Medicine, P.O. Box 65476, Dar es Salaam, Tanzania.*

Cytotoxic properties of pure compounds from the leaves of *Croton sylvaticus* (Euphorbiaceae) against brine shrimp (*Artemia salina*) larvae were investigated. A glutarimide alkaloid, julocrotine (1) showed very high cytotoxic activity with a LC_{50} (95 % CI) value of 0.074 (0.052-0.105) µg/ml when tested *in vitro* while lupeol (2) and penduliflaworisin (3) were inactive in brine shrimp lethality test. The structures of the isolated compounds were determined by modern spectroscopic methods.

Key Words: *Croton sylvaticus*, Euphorbiaceae, Julocrotine, Lupeol, Penduliflaworosin, Cytotoxic

INTRODUCTION

Croton sylvaticus Hoschst. ex Krauss (Forest fever-berry) is a medium-sized deciduous tree, 3.5-24 m high, which is found in different parts of Tanzania including the coastal area, Mbeya district on the Southern Highlands, Kilimanjaro, Arusha, Amani (Tanga), Mahale (Kigoma region) and in various places in Kenya [1]. It is commonly known as 'msandusi', 'msindusi' or 'msunduzi' among the Digo in Tanga region, Tanzania and Mombasa, Kenya [2]. A decoction of the leaves and root bark is used traditionally for the treatment of tuberculosis, inflammation, as a purgative, as a wash for body swelling caused by kwashiorkor or by tuberculosis and for the treatment of malaria [1-2]. Work done on mice showed that an aqueous extract of the stem bark prolonged ether anaesthesia, reduced exploratory activity, exhibited muscle relaxant activity and analgesic activity [3]. Essential oils extracted from the leaves by hydrodistillation showed the presence of over 52 components, among which β-caryophyllene oxide and α-humulen-1,2-epoxide were the major constituents. In addition, β-sitosterol, stigmasterol and (-)-hardwickic acid were isolated as the non-volatile constituents [3]. Apart from already documented traditional uses, traditional healers claim to be using the leaf extracts for treating cancer, although it was difficult to establish the type of cancer being treated. Preliminary study of the ethanolic extract of the leaves of *Croton sylvaticus* showed mild cytotoxic activity with LC_{50} (95 % CI) of 29.73 (21.1-41.92) µg/ml against *Artemia salina* when tested *in vitro*. This indicated that the plant might contain potentially useful cytotoxic/anticancer compounds. However, there is little phytochemical or pharmacological work done on this plant. Therefore in this study we present further phytochemical work on the leaves together with results of the test for cytotoxicity on brine shrimp (*Artemia salina*) larvae.

MATERIALS AND METHODS

PLANT MATERIAL

The leaves of *Croton sylvaticus* were collected in January 2002 from Pugu forest, Dar-es-Salaam region, Tanzania. A botanist, Mr Frank Mbago, confirmed the identity of the plant. A voucher specimen (No. FM-56) has been deposited at the Herbarium of the Institute of Traditional Medicine, Muhimbili University College of Health Sciences, Tanzania.

EXTRACTION AND ISOLATION

The air-dried and powdered leaves (1 kg) of *Croton sylvaticus* were soaked with 5000 ml of 80 % ethanol for 72 h with occasional shaking. The extract was concentrated to dryness under reduced pressure at 40 °C to give 65 g of dry extract, which was chromatographed over a silica gel (60-120 mesh) column under a gradient elution using petroleum ether-ethyl acetate as eluents in different solvent systems. This afforded three pure compounds, *viz.* **I** (10 mg), **II** (6 mg) and **III** (5 mg).

BRINE SHRIMP LETHALITY (BST)

The brine shrimp lethality test (BST) was used to predict the presence, in the extracts, of cytotoxic activity [4]. Both the crude extract and isolated compounds were tested for brine shrimp lethality. Solutions of the extract and the pure compounds were made in DMSO and incubated in duplicate vials with the brine shrimp larvae. Ten brine shrimp larvae were placed in each of the duplicate vials. Control brine shrimp larvae were placed in a third vial which contained seawater and DMSO only. After 24 h the *nauplii* were examined against a lighted background, and the average number of surviving larvae in each vial was determined. The mean percentage mortality was plotted against the logarithm of concentrations and the concentration killing fifty percent of the larvae (LC_{50}) was determined from the graph by taking the antilogarithm of the concentration corresponding to 50 % mortality rate of the test organisms. Cyclophosphamide was used as a standard test drug.

DATA ANALYSIS

The mean results of brine shrimp mortality against the logarithms of concentrations were plotted using Kaleida Graph computer program, which also gives the regression equations. The regression equations were used to calculate LC_{16}, LC_{50} and LC_{84} values. Confidence intervals (95 % CI) were calculated according to the method of Litchfield and Wilcoxon [5]. An LC_{50} value greater than 100 µg/ml was considered to represent an inactive compound or extract.

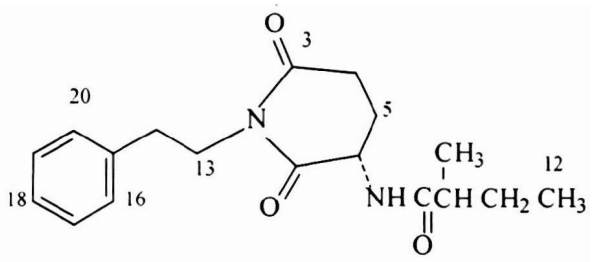
RESULTS AND DISCUSSION

The three compounds were identified from their chemical and spectroscopic characteristics as julocrotine, 2-[N-(2-methylbutanoyl)]-N-phenyl-ethylglutarimide (**I**), [6], lupeol, [lup-20 (29)-en-3ß-ol] (**II**) [7] and penduliflaworosin, [(ent-(12*R*)-methyl-15,16-epoxy-9,10-friedolabda-5(10),13 (16), 14-trien-19-oate 20,12 lactone] (**III**) [8]. Determination of compound **III** was also done by comparing with spectral data of neoclerodan-5,10-en-19,6β,20,12-diolide which was isolated previously from *Croton macrostachys* [9]. In Brine Shrimp Lethality test, julocrotine exhibited brine shrimp cytotoxicity with an LC_{50} (95 % CI) value of 0.074 µg/ml, while lupeol and penduliflaworosin were inactive. Their LC_{50} values were 308 and 312 µg/ml, respectively (Table 1).

Croton sylvaticus is claimed to be of medicinal value in treating cancer or related diseases. The 20 % aqueous ethanol extract of the leaves showed a cytotoxic effect on brine shrimp larvae, LC_{50} 29.73 µg/ml, indicating a possibility that the extract may contain a cytotoxic compound. Julocrotine (**I**) that was isolated from this extract exhibited high brine shrimp toxicity. To the best of our knowledge the occurrence of julocrotine (**I**), lupeol (**II**) and penduliflaworisin (**III**) in *Croton sylvaticus* is being reported for the first time. Similarly we are reporting for the first time the toxicity of julocrotine (**I**) to brine shrimps. The two known compounds, lupeol (**II**) and penduliflaworisin (**III**) were inactive in the brine shrimps test. There is need to test this compound on cancer cell lines and to carry out other tests in order to establish its toxicity.

Table 1: Cytotoxic tests of compounds isolated from *Croton sylvaticus*

Compound	LC_{16} (µg/ml)	LC_{50} (µg/ml)	LC_{84} (µg/ml)	95 % Confidence Interval (µg/ml)
Julocrotine	0.021	0.074	0.259	0.052-0.105
Lupeol	54.26	308	1749	190-498
Penduliflaworosin	61.48	312	1588	199-489



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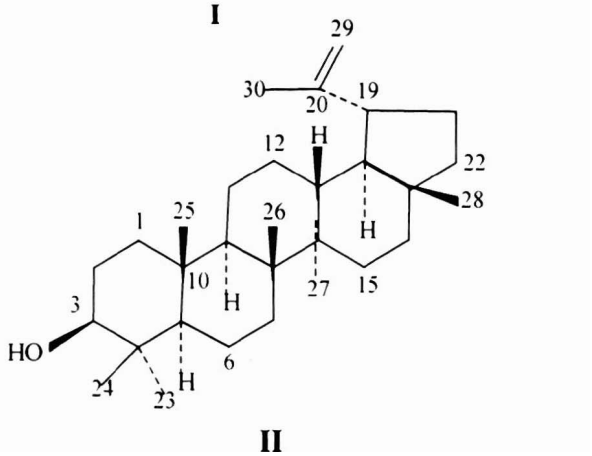

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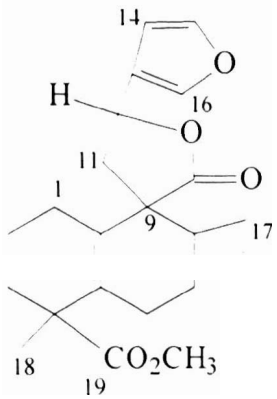

III

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