

Brine Shrimp Lethality of Alkaloids from *Croton sylvaticus* Hoechst

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Three compounds were isolated from the leaves of *Croton sylvaticus* (Euphorbiaceae) and evaluated for their brine shrimp lethality. Julocrotine, a glutarimide alkaloid, was very toxic *in vitro* with a LC₅₀ (95% confidence interval) value of 0.074 (0.052-0.105) µg/ml. Lupeol and penduliflaworosin were not toxic. The structures of the isolated compounds were determined by spectroscopic methods.

Key words: *Croton sylvaticus*, Euphorbiaceae, julocrotine, lupeol, penduliflaworosin, brine shrimp lethality

INTRODUCTION

Croton sylvaticus Hoechst 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-humulene-1,2-epoxide were the major constituents [3]. Apart from already documented traditional uses, traditional healers claim to be using the leave 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 toxicity with LC₅₀ (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

anticancer compounds. However, up to now there is little phytochemical or pharmacological work done on this plant.

Therefore in this study we present further phytochemical work on the leaves of *Croton sylvaticus* together with results of the brine shrimp lethality test (BST) of pure compounds isolated from this plant.

MATERIALS AND METHODS**Plant material**

The leaves of *Croton sylvaticus* were collected in January 2002 from Pugu Forest, Dar es Salaam, 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 column chromatographed over silica gel (60-120 mesh) under a gradient elution using petroleum ether - ethyl acetate mobile phase solvent system. This afforded three

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pure compounds, viz. 1 (10 mg), 2 (6 mg) and 3 (5 mg).

Brine shrimp lethality test (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 sea water and DMSO only. After 24 h the nauplii were examined against a lighted background, and the average number of survived larvae in each triplicate 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 $\mu\text{g/ml}$ was considered to represent an inactive compound or extract.

RESULTS

Characterization of compounds

The three compounds were identified from their chemical and spectroscopic characteristics as julocrotine (2-[*N*-(2-methylbutanoyl)]-*N*-phenylethyl-glutarimide (1)) [6], lupeol [lu-20 (29)-en-3 β -ol] (2) [7] and penduliflaworosin [(ent-(12*R*)-methyl-15,16-epoxy-9,10-friedo-labda-5(10),13(16),14-trien-19-oate 20,12 lactone] (3) [8]. Identification of compound 3 was further confirmed by comparing with spectral data of neoclerodan-5,10-en-19,6 β ,20,12-diolide which was isolated previously from *Croton macrostachys* [9].

Brine shrimp lethality test

In the brine shrimp lethality test, julocrotine was very toxic with an LC_{50} (95% CI) value of 0.074 $\mu\text{g/ml}$, while lupeol and penduliflaworosin were inactive. Their LC_{50} values were 308 and 312 $\mu\text{g/ml}$ respectively (Table 1).

Table 1: Cytotoxicity of compounds isolated from *Croton sylvaticus*

Compound	LC_{16} ($\mu\text{g/ml}$)	LC_{50} ($\mu\text{g/ml}$)	LC_{84} ($\mu\text{g/ml}$)	95% Confidence Interval ($\mu\text{g/ml}$)
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

DISCUSSION

Croton sylvaticus is claimed to be of medicinal value in treating cancer or related diseases. The 20% aqueous ethanol extract of the leaves was

mild toxic with LC_{50} (95% CI) of 29.73 (21.1-41.92) $\mu\text{g/ml}$ against *Artemia salina* when tested *in vitro*, indicated a possibility that the extract may contain a toxic compounds. Julocrotine exhibited high brine shrimp toxicity. To the best

of our knowledge, the occurrence of julocrotine, lupeol and penduliflaworosin (3) in *Croton sylvaticus* is being reported for the first time. Similarly we are reporting for the first time the brine shrimp lethality test of julocrotine (1). The two known compounds, lupeol (2) and penduliflaworosin (3), were inactive in the brine shrimps test. These results support the traditional healers claim, but it could also mean that this compound is potentially toxic, since brine shrimp lethality activity can also be used as an indicator for toxicity. There is need to test this compound on cancer cell lines and other tests in order to establish its safety and the possibility of developing an anticancer agent.

ACKNOWLEDGEMENT

This study was supported by the government grant through Tanzania Commission for HIV/AIDS (TACAIDS). We are very grateful to the Chemistry Department, University of Botswana, for spectral analysis. Mr. Abdul Waziri, of Chemistry Department, University of Dar es Salaam, Tanzania, is also acknowledged for brine shrimp lethality tests.

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