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Antiplasmodial activity of sesquiterpene lactones and a sucrose ester from *Vernonia guineensis* Benth. (Asteraceae)

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ABSTRACT

Ethnopharmacological relevance: Aqueous preparations of *Vernonia guineensis* Benth. (Asteraceae) are used in Cameroonian folk medicine as a general stimulant and to treat various illnesses and conditions including malaria, bacterial infections and helminthic infestations.
Materials and methods: Ten gram samples of the leaf and tuber powders of *Vernonia guineensis* were extracted separately using dichloromethane, methanol and distilled water. The extracts were dried *in vacuo* and used in bioassays. These extracts and three compounds previously isolated from *Vernonia guineensis* [vernopicrin (**1**), vernomelitensin (**2**) and pentaisovalerylsucrose (**3**)] were screened for antiplasmodial activity against chloroquine (CQ)-sensitive (Hb3) and CQ-resistant (Dd2) *Plasmodium falciparum* lines.
Results: Crude extracts and pure compounds from *Vernonia guineensis* showed antiplasmodial activity against both Hb3 and Dd2. The IC_{50} values of extracts ranged from 1.64 to 27.2 μg/ml for Hb3 and 1.82–30.0 μg/ml for Dd2; those for compounds **1**, **2** and **3** ranged from 0.47 to 1.62 μg/ml (1364–1774 nM) for Hb3 and 0.57–1.50 μg/ml (1644–2332 nM) for Dd2. None of the crude extracts or pure compounds was observed to exert toxic effects on the erythrocytes used to cultivate the *Plasmodium falciparum* lines.
Conclusion: In Cameroonian folk medicine, *Vernonia guineensis* may be used to treat malaria in part due to the antiplasmodial activity of sesquiterpene lactones (**1**, **2**), a sucrose ester (**3**) and perhaps other compounds present in crude plant extracts. Exploring the safety and antiplasmodial efficacy of these compounds *in vivo* requires further study.

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1. Introduction

Malaria remains endemic in most tropical countries, especially those in sub-Saharan Africa (Snow et al., 2005; Alonso et al., 2011). The emergence of *Plasmodium falciparum* parasites with reduced susceptibility to artemisinins and partner drugs (*e.g.*, mefloquine, lumefantrine and piperaquine) that comprise artemisinin-based combination therapies (ACTs) are worrisome and challenge existing efforts to control, treat and eliminate *Plasmodium falciparum* malaria (Nyunt and Plowe, 2007, Fairhurst et al., 2012). The discovery and development of new antimalarial drugs thus remains a priority. Such discoveries may be made by identifying lead compounds derived from medicinal plants used by traditional healers to treat malaria or febrile illnesses in general.

The carrot-like tubers of *Vernonia guineensis* are commonly used in ethnomedicine as an adaptogen (to combat stress), a stimulant, an aphrodisiac, general poison antidote, a treatment for jaundice and prostate-related problems, as well as an antibacterial, anthelmintic and antimalarial agent (Iwu, 1993; Tchinda et al., 2002; Noumi, 2010). Antitrypanosomal and anti-cancer compounds have been isolated from the root extract of this plant (Tchinda et al., 2003; Toyang et al., 2012a). Antibacterial and anthelmintic activities have been found in crude extracts and pure compounds isolated from *Vernonia guineensis* (Donfack et al., 2012, Toyang et al., 2012b). To date, *Vernonia guineensis* extracts and compounds have not been tested *in vitro* for potential antiplasmodial activities. In this context, we explored the *in-vitro* antiplasmodial activity of crude extracts and pure compounds from *Vernonia guineensis*.

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2. Materials and methods

2.1. Plant collection

The tubers and leaves of *Vernonia guineensis* Var. *cameroonica* C. D. Adams were collected in Baicham, Boyo Division, North West Region, Cameroon, in 2009. A voucher specimen was authenticated at the Limbe Botanic Garden, South West Region, Cameroon, and a voucher specimen (No. SCA 12431) deposited at the Limbe Botanic Garden Herbarium.

2.2. Preparation of crude extracts, isolation of pure compounds, and thin layer chromatography (TLC) analysis

Samples of *Vernonia guineensis* were extracted as described (Toyang et al., 2012b). Briefly, 10-g samples of dried *Vernonia guineensis* leaf and tuber powders were separately extracted with 100 ml dichloromethane, methanol or distilled water twice at 37 °C for 24 h. The samples were filtered (dichloromethane and methanol extracts) or centrifuged (water extracts) to separate the extract from the marc. The extracts were dried *in vacuo* to obtain solvent-free crude extracts for use in bioassays. Two sesquiterpene lactones (vernopicrin-**1** and vernomelitensin-**2**) and one sucrose ester (pentaisovalerylsucrose-**3**) were available from recent isolations (Toyang et al., 2012a, 2013) and also used in this study. The sesquiterpene lactones and sucrose ester (Fig. 1) were isolated from the leaves and roots of *Vernonia guineensis*, respectively. TLC analysis was carried out using normal phase silica plates coated with UV254 fluorescence indicator. The solvent system used was *n*-hexane/ethyl acetate (1:1). The developed plates were visualized under a UV station at 254 nm and 365 nm and any bands detected. The plates were further treated with H_2SO_4 spray and the R_f values of bands found in the crude extracts were compared with those of compounds **1**, **2** and **3**.

2.3. Bioassays

2.3.1. In-vitro antiplasmodial assay

The 50% inhibitory concentrations (IC_{50}) of *Vernonia guineensis* extracts and compounds were measured using a SYBR Green I-based DNA detection method (Smilkstein et al., 2004). Briefly, two laboratory-adapted *Plasmodium falciparum* lines, chloroquine (CQ)-sensitive Hb3 and CQ-resistant Dd2, were cultured as described (Moll et al., 2004). Stock solutions of crude extracts and pure compounds were diluted in cell culture water to 100 and 10 μg/ml, respectively. Two-fold serial dilutions of extracts (0.0977–100 μg/ml), compounds (0.00977–10 μg/ml), chloroquine (0.00078–0.8 μg/ml), and artesunate (0.0000469–0.0481 μg/ml) were added to 96-well plates. The plates were dried overnight in a dark sterile hood and stored for up to 1 week at 4 °C. Suspensions of *Plasmodium falciparum*-infected erythrocytes (2% hematocrit, 1% parasitemia) were added to the drug-coated plates and incubated in an atmosphere of 5% CO_2, 5% O_2 and 90% N_2 at 37 °C for 72 h. The assay was terminated by freezing the plates at −20 °C for 24 h. Parasite growth was evaluated using SYBR-Green I DNA dye fluorescence. Fluorescence was measured on a BMG Labtech FLUOstar Optima instrument (Ortenberg, Germany) at excitation and emission wavelengths of 485 and 530 nm, respectively. Analysis of data was performed with Graphpad Prism (Graphpad Software, La Jolla, CA). Normalized fluorescence was plotted against the logarithm of the drug concentration to yield the concentration of drug that produced 50% of the observed decline from the maximum fluorescence in the drug-free wells (IC_{50}).

2.3.2. Chemical injury to erythrocytes

To determine if the crude extracts and pure compounds caused chemical injury to erythrocytes, these cells (2% hematocrit) were incubated in the highest concentration of each drug under the same conditions of the drug response assay. Thin blood smears were stained with Giemsa and erythrocytes observed by light microscopy (100 ×) for any gross morphological changes.

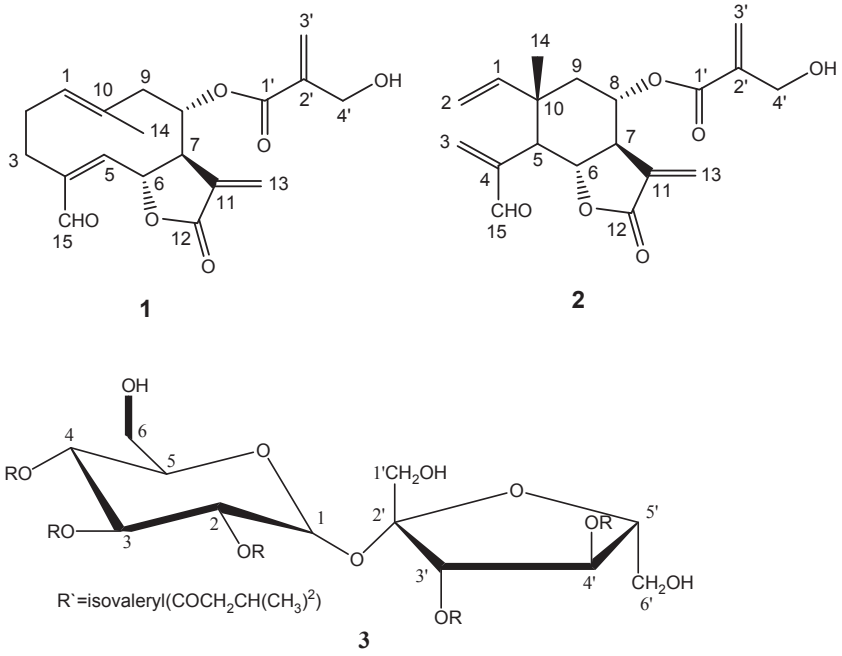

Fig. 1. Chemical structures of vernopicrin (**1**), vernomelitensin (**2**) and pentaisovalerylsucrose (**3**).

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