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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—10-g samples of the leaf and tuber powders of *V. 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 *V. 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 *V. guineensis* showed antiplasmodial activity against both Hb3 and Dd2. The IC_{50} values of extracts ranged from 1.64 – 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 – 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 *P. falciparum* lines.

Conclusion—In Cameroonian folk medicine, *V. 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.

Keywords

Vernonia guineensis; antiplasmodial activity; sesquiterpene lactone; sucrose ester; Cameroon

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1.0 Introduction

Malaria remains endemic in most tropical countries, especially those in sub-Saharan Africa (Alonso et al., 2011; Snow et al., 2005). 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 *P. 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 *V. guineensis* (Donfack et al., 2012, Toyang et al., 2012b). To date, *V. 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 *V. guineensis*.

2.0 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 *V. guineensis* were extracted as described (Toyang et al., 2012b). Briefly, 10-g samples of dried *V. 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 and c) and also used in this study. The sesquiterpene lactones and sucrose ester (Fig. 1) were isolated from the leaves and roots of *V. 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 Rf 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 *V. guineensis* extracts and compounds were measured using a SYBR Green I-based DNA

detection method (Smilkstein et al., 2004). Briefly, two laboratory-adapted *P. falciparum* lines, chloroquine (CQ)-sensitive Hb3 and CQ-resistant Dd2, were cultured as described (Moll et al., 2008). 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 *P. 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 nm 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 (100X) for any gross morphological changes.

2.4. Statistical analysis

The antiplasmodial assay experiments were run at least in duplicate with each experimental drug concentration duplicated during each run. The IC_{50} values of the different experiments were calculated and the data represented as mean ± SD. Comparison of activity against CQ-sensitive *vs.* CQ-resistant *P. falciparum* strains was carried out. P-values <0.05 were deemed significant.

3.0 Results

Compounds **1** and **2** were detected by TLC in the dichloromethane and methanol extracts, but not in the aqueous extract of the leaves of *V. guineensis* using (not shown). Compound **3** was detected by TLC in the dichloromethane extract but not in the methanol and aqueous extracts of the root biomass of *V. guineensis* (chromatogram not shown).

These pure compounds and crude extracts from *V. guineensis* inhibited the growth of Hb3 and Dd2 (Table 1). The differential drug susceptibility of Hb3 (CQ-sensitive) and Dd2 (CQ-resistant) to CQ was confirmed, with the former parasite line showing an IC_{50} = 28 nM and the latter an IC_{50} = 219 nM (Table 1). Both Hb3 and Dd2 were highly susceptible to artesunate (IC_{50} = 4–5 nM), as expected. The IC_{50} values of compounds **1**, **2** and **3** were also similar for Hb3 and Dd2 and ranged from 1364 – 2130 nM for Hb3 and 1664 – 2332 nM for Dd2. The IC_{50} values of compounds **1**, **2** and **3** were thus at least an order of magnitude higher than those for CQ and artesunate.

The IC_{50} values of extracts were similar for Hb3 and Dd2, and ranged from 1.64 – 27.2 μg/ml for Hb3 and 1.82 – 30.0 μg/ml for Dd2. The IC_{50} values of root extracts ranged from 3.162 – 29.98 μg/ml, while those of leaf extracts ranged from 1.635 – 11.35 μg/ml and were generally higher (P<0.05) (Table 1). The dichloromethane root and leaf extracts were generally more potent than the methanol (P<0.05) and aqueous (P<0.05) extracts of the plant. The methanol and aqueous root extracts exhibited similar antiplasmodial activity at

concentrations 8–10 times less than the potency of the dichloromethane root extract. The difference between the antiplasmodial activity of the dichloromethane and methanol leaf extracts, however, was not significant ($P>0.05$) compared to that between the dichloromethane and aqueous leaf extracts ($P<0.05$).

Microscopic observation of uninfected erythrocytes incubated for 72 h with the crude extracts of *V. guineensis* and compounds **1**, **2** and **3** at the highest concentrations used in the drug response assay showed no morphological differences compared to positive control and CQ and artesunate negative controls.

4.0 Discussion

Plants remain the most frequently used source of antimalarial remedies in developing countries where access to relatively-expensive, modern chemotherapeutics may be limited. The possibility that plant-based remedies exert antiplasmodial effects has already been realized with *Cinchona officinalis* and *Artemisia annua*, source plants for quinine and artemisinin – two of the most effective antimalarial drugs ever developed. These plants have and continue to receive much attention as the demand for their associated compounds has often outweighed their supply (Meshnick and Dobson, 2001; de Ridder et al., 2008). Plants are thus likely to remain an important component of the world's antimalarial arsenal. Here we have explored the putative antiplasmodial properties of *V. guineensis*.

The different solvent extracts of *V. guineensis* leaves and roots demonstrated variable activity against two *P. falciparum* lines, with the dichloromethane leaf and root extracts showing greater antiplasmodial activity than the other extracts. Vernopicrin (**1**) and vernomelitensin (**2**), two sesquiterpene lactones previously isolated from the acetone extract of *V. guineensis* (Toyang et al., 2012c), showed antiplasmodial activity with IC_{50} values of 0.614 and 0.807 μg/ml, and 0.472 and 0.569 μg/ml, respectively, against the CQ-sensitive and CQ-resistant *P. falciparum* lines. The *Vernonia* genus is known to be a rich source of sesquiterpene lactones and many species of this genus have yielded antiplasmodial sesquiterpenes with and without the lactone functionality (Kraft et al., 2003; Chea et al., 2006; Pillay et al., 2007; Chukwujekwu et al., 2009; Buskuhl et al., 2010). The sesquiterpenes identified from other *Vernonia* species have ranged in antiplasmodial activity with IC_{50} values of 0.2 – 4.8 μg/ml. Hirsutinolide, a sesquiterpene lactone isolated from *V. scorpioides*, exhibits the most potent antiplasmodial activity with an IC_{50} = 0.2 μg/ml (Buskuhl et al., 2010), whereas vernodalol isolated from *V. colorata* was the least active of the sesquiterpenes with an IC_{50} = 4.8 μg/ml (Kraft et al., 2003). Schmidt et al. (2009) reported the comparative antiprotozoal activity of 35 sesquiterpene lactones and found IC_{50} values ranging from 0.329 – 27.47 μg/ml against *P. falciparum*. With IC_{50} values <1 μg/ml against *P. falciparum*, compounds **1** and **2** appear to be among the most potent sesquiterpene lactones; by comparison, only 8.6% (3/35) of the sesquiterpene lactones assayed by Schmidt et al. (2009) showed IC_{50} values <1 μg/ml.

Pentaisovalerylsucrose (**3**), a sucrose ester isolated from the roots of *V. guineensis*, also demonstrated activity against the CQ-sensitive and CQ-resistant *P. falciparum* lines. To the best of our knowledge, this is the first report on the antiplasmodial activity of a sucrose ester. The activity of the dichloromethane extract of the roots and leaves of *V. guineensis* might be linked to the presence of compounds 1, 2 and 3 (Table 1) as evidenced by their detection in TLC analysis (Figure not shown), as well as their previous isolation from nonpolar fractions of the roots and leaves of *V. guineensis* (Toyang et al., 2012a, 2012c). The activity of the methanol extract of the plant's leaves may also be linked to the presence of compounds 1 and 2 as these were detected by TLC in this extract (chromatogram not shown). The antiplasmodial activity of the aqueous extracts may be due to the presence of

other metabolites yet to be identified as none of the three compounds tested were detected by TLC in the aqueous extracts. It is also unlikely that any of the three compounds were present to any significant degree in the aqueous extracts given that compounds 1, 2 and 3 in concentrations as low as 1 mg/ml were insoluble in aqueous solutions. Previous studies of *V. guineensis* roots also afforded two stigmastane derivatives, vernoguinosterol and vernoguinoside, with antitrypanosomal activity (Tchinda et al., 2002). It is unknown whether these compounds also possess antiplasmodial activities as they have not yet been investigated.

5.0 Conclusion

The *in-vitro* antiplasmodial activity of crude extracts and purified compounds from *V. guineensis* may underlie the use of this plant in Cameroonian folk medicine to treat malaria. To the best of our knowledge, this is the first report on the antiplasmodial activity of extracts and compounds isolated from this plant. Further studies are needed to assess the safety and antiparasitic efficacy of vernopicrin, vernomelitensin and pentaisovalerylsucrose in *in-vivo* animal models. In addition, bioactivity-guided fractionation as well as metabolomic studies are recommended to identify other antiplasmodial compounds present in the roots and leaves of *V. guineensis*.

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References

Alonso PL, Brown G, Arevalo-Herrera M, Binka F, Chitnis C, Collins F, Doumbo OK, Greenwood B, Fenton Hall B, Levine MM, Mendis K, Newman RD, Plowe CV, Rodríguez MH, Sinden R, Slutsker L, Tanner M. A research agenda to underpin malaria eradication. Plos Medicine. 2011; 8:1–8.

Buskuhl H, Oliveira FL, Blind LZ, Freitas RA, Barison A, Campos FR, Corilo YE, Eberlin MN, Caramori GF, Biavatti MW. Sesquiterpene lactones from *Vernonia scorpioides* and their *in vitro* cytotoxicity. Phytochemistry. 2010; 71:1539–1544. [PubMed: 20599237]

Chea A, Hout S, Long C, Marcourt L, Faure R, Azas N, Elias R. Antimalarial Activity of Sesquiterpene Lactones from *Vernonia cinerea*. Chemical and Pharmaceutical Bulletin. 2006; 54:1437–1439.

Chukwujekwu JC, Lategan CA, Smith PJ, Van Heerden FR, Van Staden J. Antiplasmodial and cytotoxic activity of isolated sesquiterpene lactones from the acetone leaf extract of *Vernonia colorata*. South African Journal of Botany. 2009; 75:176–179.

de Ridder S, van der Kooy F, Verpoorte R. *Artemisia annua* as a self-reliant treatment for malaria in developing countries. Journal of Ethnopharmacology. 2008; 120:302– 314. [PubMed: 18977424]

Donfack ARN, Toyang NJ, Wabo HK, Tane P, Awoufack MD, Kikuchi H, Tamokou JDD, Kuiate JR, Oshima Y. Stigmatane derivatives from the root extract of *Vernonia guineensis* and their antimicrobial activity. Phytochemistry Letters. 2012; 5:596–599.

Fairhurst RM, Nayyar GML, Breman JG, Hallett R, Vennerstrom JL, Duong S, Ringwald P, Wellems TE, Plowe CV, Dondorp AM. Artemisinin-Resistant Malaria: Research challenges, opportunities, and public health implications. American Journal of Tropical Medicine and Hygiene. 2012; 87:231–241. [PubMed: 22855752]

Iwu, MM. Handbook of African Medicinal Plants. CRC Press; London: 1993. p. 415p

Kraft C, Jenett-Siems K, Siems K, Jakupovic J, Mavi S, Bienzle U, Eich E. Herbal remedies traditionally used against malaria. In vitro antiplasmodial evaluation of medicinal plants from Zimbabwe. Phytotherapy Research. 2003; 17:123–128. [PubMed: 12601673]

Meshnick, SR.; Dobson, M. The history of antimalarial drugs. In: Rosenthal, P., editor. Antimalarial Chemotherapy. Mechanisms of Action, Resistance, and New Directions in Drug Discovery. Humana, Totowa; New Jersey: 2001. p. 15-16.

Moll, K.; Ljungström, I.; Perlmann, H.; Scherf, A.; Wahlgren, M. MR4/ATCC. Manassas, Virginia. Paris, France: BioMalPar; 2008. Methods in malaria research.

Smilkstein M, Sriwilaijaroen N, Kelly JX, Wilairat P, Riscoe M. Simple and inexpensive fluorescence-based technique for high-throughput antimalarial drug screening. Antimicrobial Agents and Chemotherapy. 2004; 48:1803–1806. [PubMed: 15105138]

Noumi E. Ethno medicines used for treatment of prostatic disease in Foumban, Cameroon. African Journal of Pharmacy and Pharmacology. 2010; 4:793–805.

Nyunt MM, Plowe CV. Pharmacological advances in the global control and treatment of malaria: combination therapy and resistance. Clinical Pharmacology and Therapeutics. 2007; 82:601–605. [PubMed: 17898709]

Pillay P, Vleggaar R, Maharaj VJ, Smith PJ, Lategan CA, Chouteau F, Chibale K. Antiplasmodial hirsutinolides from *Vernonia staehelinoides* and their utilization towards a simplified pharmacophore. Phytochemistry. 2007; 68:1200–1205. [PubMed: 17408709]

Schmidt TJ, Nour AMM, Khalid SA, Kaiser M, Brun R. Quantitative structure – Antiprotozoal activity relationships of sesquiterpenes lactones. Molecules. 2009; 14:2062–2076. [PubMed: 19513006]

Smilkstein M, Sriwilaijaroen N, Kelly JX, Wilairat P, Riscoe M. Simple and inexpensive fluorescence-based technique for high-throughput antimalarial drug screening. Antimicrobial Agents Chemotherapy. 2004; 48:1803–1806.

Snow RW, Guerra CA, Noor AM, Myint HY, Hay SI. The global distribution of clinical episodes of *Plasmodium falciparum* malaria. Nature. 2005; 434:214–217. [PubMed: 15759000]

Tchinda AT, Tsopmo A, Tane P, Ayafor JF, Connolly JD, Sterner O. Vernoguinosterol and vernoguinoside, trypanocidal stigmastane derivatives from *Vernonia guineensis* (Asteraceae). Phytochemistry. 2002; 59:371–374. [PubMed: 11830150]

Tchinda AT, Tsopmo A, Tane P, Ayafor JF, Connolly JD. Stigmatane derivatives and isovaleryl sucrose esters from *Vernonia guineensis* (Asteraceace). Phytochemistry. 2003; 63:841–846. [PubMed: 12877926]

Toyang NJ, Wabo HK, Ateh EN, Davis H, Tane P, Kimbu SF, Sondengam LB, Bryant J. In-vitro anti-prostate cancer and ex vivo antiangiogenic activity of *Vernonia guineensis* Benth. (Asteraceae) tuber extracts. Journal of Ethnopharmacology. 2012a; 141:866–871. [PubMed: 22465590]

Toyang NJ, Ateh EN, Keiser J, Vargas M, Bach H, Tane P, Sondengam LB, Davis H, Bryant J, Verpoorte R. Toxicity, antimicrobial and anthelmintic activities of *Vernonia guineensis* Benth. (Asteraceae) crude extracts. Journal of Ethnopharmacology. 2012b In Press.

Toyang NJ, Wabo HK, Ateh EN, Davis H, Tane P, Sondengam LB, Bryant J, Verpoorte R. Cytotoxic and antiangiogenic sesquiterpene lactones from the leaves of *Vernonia guineensis* Benth. (Asteraceae). Journal of Ethnopharmacology. 2012c In Press.

Waako PJ, Katuura E, Smith P, Folb P. East African medicinal plants as a source of lead compounds for the development of new antimalarial drugs. African Journal of Ecology. 2007; 45:102–106.

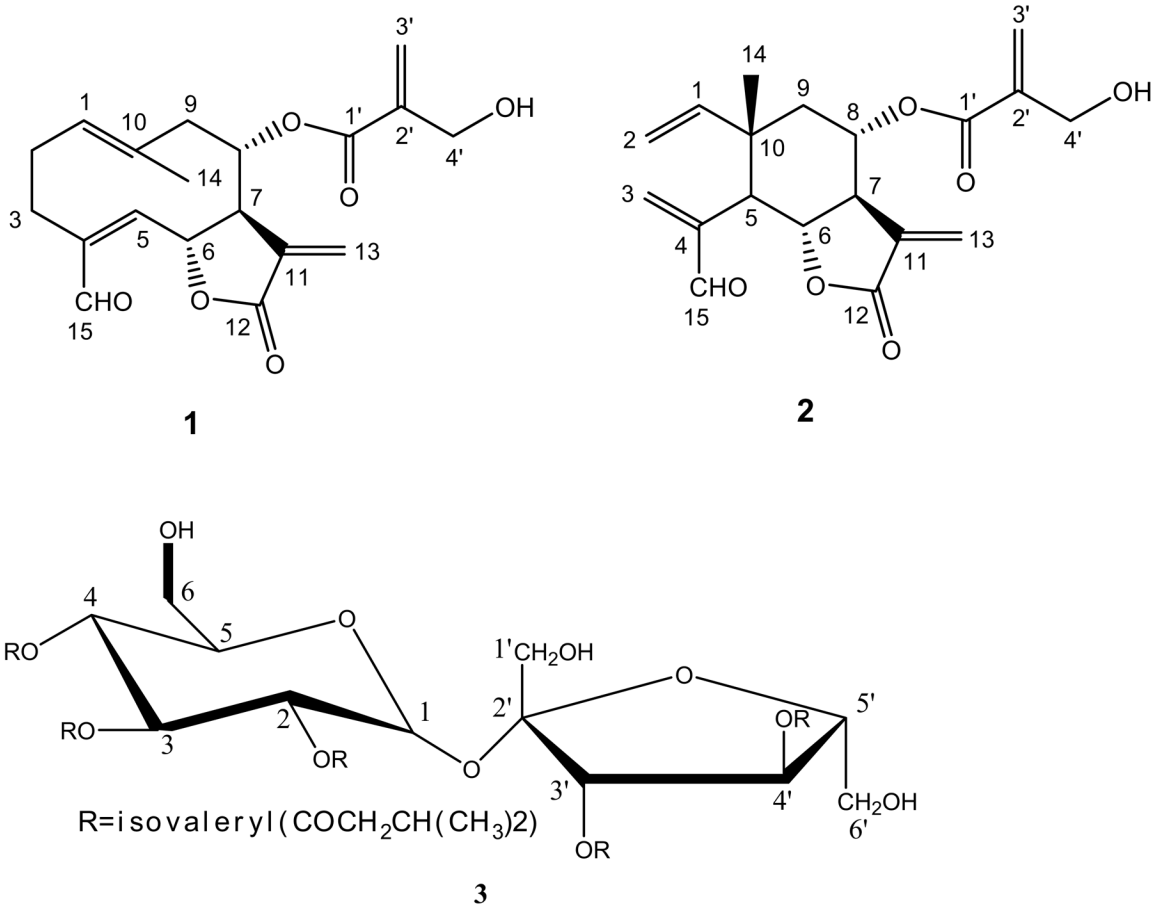

Figure 1.
Chemical structures of vernopicrin (**1**), vernomelitensin (**2**) and pentaisovalerylsucrose (**3**).

Table 1

Antiplasmodial activity *Vernonia guineensis* crude extracts and isolated compounds

	Antiplasmodial activity IC50 (μg/ml)		
	Chloroquine resistant (Dd2)	**Chloroquine sensitive (Hb3)**	**P-value (Dd2 *vs.* Hb3)**
Antimalarials			
Chloroquine	0.070 ± 0.017 (219 ± 52.2 nM)	0.009 ± 0.0009 (28±2.75 nM)	0.005
Artesunate	0.002 ± 0.0001 (5 ± 0.31 nM)	0.0015 ± 0.0004 (4 ± 0.91 nM)	0.112
Pure compounds			
Vernopicrin (**1**)	0.807 ± 0.197 (2332 ± 569 nM)	0.614 ± 0.110 (1774 ± 317 nM)	0.138
Vernomelitensin (**2**)	0.569 ± 0.076 (1644 ± 219 nM)	0.472 ± 0.0861 (1364 ± 248 nM)	0.142
Sucrose ester (**3**)	1.495 ± 0.174 (1962 ± 228 nM)	1.623 ± 0.167 (2130 ± 219 nM)	0.329
Crude extracts			
VGRD	3.162 ± 0.295	3.218 ± 0.310	0.802
VGRM	29.977 ± 1.212	27.084 ± 1.514	0.024
VGRA	26.115 ± 4.027	27.238 ± 3.316	0.682
VGLD	1.823 ± 0.503	1.635 ± 0.429	0.590
VGLM	3.980 ± 1.246	2.055 ± 0.894	0.046
VGLA	11.354 ± 1.148	9.546 ± 1.229	0.075

Root extracts: VGRD (dichloromethane); VGRM (methanol); VGRA (aqueous)

Leaf extracts: VGLD (dichloromethane); VGLM (methanol); VGLA (aqueous)

Results of four replicate experiments are presented as mean ± standard deviation.