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Research Article

Antiplasmodial, Cytotoxic and Acute Toxicity Activities of *Vernonia lasiopus* O. Hoffman

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Background: Malaria continues to cause heavy morbidity and mortality and it is the fifth leading cause of death globally. The disease causes over one million deaths annually and affects many more, particularly due to increasing multi-drug resistant strains of *Plasmodium falciparum*. Sustained investigations in both curative and prophylactic interventions have supported the ethno-pharmacological approach to identify novel compounds as a major channel towards achieving a solution. *Vernonia lasiopus* has been used in traditional medicine for their antimalarial, antiviral and analgesic properties.

Objective: To investigate the antiplasmodial activity and toxicity profile *Vernonia lasiopus* extracts.

Methodology: Extraction of aerial parts and roots was done using dichloromethane:chloroform (1:1) and the resulting crude extracts each fractionated into six fractions by vacuum liquid chromatography using solvents of different polarities. The crude extract and fractions were investigated for antiplasmodial activity using the chloroquine (CQ) sensitive D6 and chloroquine (CQ) resistant W2 laboratory adapted *Plasmodium falciparum* strains. Cytotoxicity was evaluated on Vero 199 cells at starting concentrations of 100µg/ml, whereas acute toxicity (LD_{50}) determined on healthy female Swiss mice (20±2 gm.). Selectivity index was used as an indicator of antiplasmodial viability.

Results: The fractions of *V. lasiopus* roots showed higher activity combined than individually. The crude *V. lasiopus* root extract had an IC_{50} 13.1 µg/ml and selectivity index >7.63. Fraction 1 of the crude root extract (VLR1) was the most viable fraction with an IC_{50} of 16.8 µg/ml and S.I >5.95. Both had CC_{50}>100 µg/ml and LD_{50} >5000mg/kg.

Conclusion: extracts of *V. lasiopus* aerial parts and roots were found to exhibit notable viable antiplasmodial effects, and had minimal acute toxicity in mice.

Key words: *Plasmodium falciparum*, *Vernonia lasiopus*, antiplasmodial activity, toxicity, selectivity index.

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1. Introduction

Malaria is a major disease in many regions particularly in the tropics as it has much socio-economic impact. It is also responsible for 8% of children deaths globally, 16% being in Africa. It is widespread in tropical and subtropical regions, including much of sub-Saharan Africa, Asia, and the Americas. There are approximately 9000-10000 cases reported annually in Europe, and there is estimated to be 1.1% fatality rate among

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Plasmodium falciparum cases. These may also include travellers exposed in other areas (White, 2009). Malaria exerts heavy socio-economic impact on the society; especially due to *Plasmodium falciparum* strains (Sach's, 2002) hence the very important need for solutions. Mortality and morbidity has increased over the last two decades and this is due to development of resistance to the classes of drugs used (Hyde, 2002) , with artemisinin based drugs currently being among the most recently introduced and effective. However, artemisinin resistance has also been reported in murine models of malaria (Arjen et al, 2011).In humans treatment failure of artemisinin based combinations have been described in the Greater Mekong sub region in Asia, and molecular markers for artemisinin resistance have also been identified (WHO, 2014).

Concerted efforts to obtain novel compounds are necessary with the ethno pharmacological approach being able to contribute substantially. The first antimalarial drug, quinine, was isolated from the bark of *Cinchona ledgeriana* (Rubiaceae) in 1820, and is one of the most important and oldest therapeutic options today (Sudhanshu, 2003). Othersemi synthetic and synthetic aminoquinoline based analogues such as chloroquine, amodiaquine, primaquine and mefloquine have been derived from its structure (Hyde, 2002). Artemisinin, an endoperoxysesquiterpene lactone first isolated in 1970, is the parent compound from a Chinese anti-malarial plant *Artemisia annua* (Asteraceae). Several structural analogues having varying pharmacokinetic properties such as, sodium artesunate, dihydroartemisinin, arteether and artemether have been derived from it and constitute the most potent and safe plant-derived anti-malarial drugs in clinical use today. Several higher vascular plants are known to constitute potential anti-protozoa and other therapeutic activities (Sudhanshu, 2003).Medicinal plants have become lucrative in the global market due to popular use. There was a significant rise in the demand for herbal medicines both in the developing and developed countries resulting in global sales of about US$60 billion dollars in the year (WHO, 2003).

V. lasiopus is reputed to have several health benefits traditionally where an infusion of powdered leaves is used to cure indigestion, stomach-ache, malaria and also as a purgative. A root decoction is said to be a very effective treatment for stomach ache (Dharani et al, 2010). Its use in treating other diseases varies among communities in Kenya, for example malaria (Kikuyu), scabies (Kamba), venereal diseases (luo) and sores (Maasai) (Erasto, 2001).The organic fraction extracts of the plant was shown to possess sedative, analgesic (leave and seeds), anti ulcerogenic (leaves and seeds), and membrane stabilizing activity (leaves and roots) as shown by reduced RBC lysis (Erasto, 2001). The chemical compounds isolated from alcoholic extracts of dried aerial parts of *V. lasiopus* are the elemanolides: epivernodalol and lasiopulide. These are carbon-10 epimers of the sesquiterpene lactones vernodalol and demethyl acroylated vernodalol, which have been found present in other species of *Vernonia*, such as *Vernonia amygdalina* (Dharani et al, 2010).

Vernonia lasiopus was selected due to its use traditionally to treat malaria in Kenya and some other African countries.

2. Methodology

2.1 Plant materials

The plants, *V. lasiopus* aerial parts and roots were collected the medicinal garden. Authentication was done at the University of Nairobi Herbarium School of Biological Sciences University of Nairobi and voucher specimen deposited: numbers BN/2011/1 and BN/2011/2.

2.2 Extraction and Fractionation

The plant parts were air-dried for two weeks at room temperature then ground separately into a fine powder using a Willy mill grinder. The yield of *V. lasiopus* aerial parts and roots was 213gm and 594gm respectively.They were then extracted repeatedly with $MeOH/CH_2Cl_2$ (1:1) for 48 hours (x2) at room temperature and the filtrate was dried *in vacuo.* The solvent mixture was used in order to optimize extraction of both polar and non-polar components. The extracts were stored in sterile air-tight vials at 4 °C in readiness for bioassay.

Vacuum liquid Chromatography(VLC) was employed to fractionate the extracts with a mixture of petroleum ether and ethyl acetate as follows; 95% (F1), 4:1 (F2), 3:2 (F3), 3:7 (F4), a mixture of ethyl acetate: methanol 8:2 (F5) and 100% methanol (F6). The fractions were abbreviated respectively such as VLA1 for *V. lasiopus* aerial parts fraction **1**. The crude extracts were adsorbed on silica gel 1:1 ratio, and silica gel equivalent to ×4 the extract weight used as the stationery phase.14gm of the aerial parts and 11gm of root extracts were fractionated. The yield was as in **Table 1**.

2.3 Animals

8 weeks old female Swiss albino mice, weighing 20±2 g, used in this study were sourced from the breeding unit at Kenya Medical Research Institute (KEMRI). They were housed in clearly labeled, standard Macrolon type II cages at 22 °C and 60–70% relative humidity. The cages were placed in a well aerated room with access to natural light during the day. They were fed on commercial rodent feed, water *ad libitum* and allowed acclimation for 7 days. The study was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the US guidelines (NIH publication #85-23, revised in 1985).

These were supplemented by the KEMRI and OECD guidelines on animal care and use. Permission to carry out the study was granted by KEMRI's scientific steering committee and the Ethical Review Committee.

2.4 Parasites

Chloroquine (CQ) sensitive D6 and CQ resistant W2 *Plasmodium falciparum* strains were obtained from cryopreserved banks in KEMRI, revived and used for *in vitro* assay.

2.5 Determination of IC_{50}

Parasitaemia for continuous cultures was determined by microscopy using Giemsa staining. The plasmodia

were cultured at 37 °C in a media containing RPMI with HEPES, 10% human serum and human type O+ erythrocytes (in acid citrate dextrose, ACD, anticoagulant). The atmosphere was maintained at 92% N_2, 5% CO_2, and 3% O_2. Parasitaemia was continuously diluted at 6% with experiments set at levels >4% comprising predominantly (>80%) ring stage plasmodium. They were introduced in serially diluted drug concentrations on a 96 well plate and radio-labelled hypoxanthine added after 48hours. Inhibition was determined via modification of technique described by Desjardins (Desjardins et al, 1979). IC_{50} was obtained by logarithmic transformation of drug concentration and radioactive counts per minute as read by a liquid scintillation counter. Chloroquine was used as the control.

2.6 Determination of CC_{50}

In vitro cytotoxicity assay was done using vero199 cell obtained from mammalian kidney epithelium following a modified rapid colorimetric assay (Mosmann et al, 1983). It measured the activity of cellular enzymes in surviving cells that reduce the tetrazolium salt, MTT, to the purple coloured formazan crystals. These were solubilized by adding dimethyl sulfoxide (DMSO). Absorbance was determined by scanning the plates with a multiwell spectrophotometer and the cytotoxic concentration 50% (CC_{50}) determined. Chloroquine was used as the control.

2.7 Determination of LD_{50}

Five dose levels ranging 500-5000mg/kg of the samples were administered orally to the mice: 500, 889.6, 1581.6, 2812.4 and 5000mg/kg**.** They received 0.2ml of samples with the control group getting the aqueous 0.1 % Tween 80 diluent. The mice were observed individually at least once within the first 30 minutes and periodicallyfor48 hours; with altered behaviour and deaths recorded. For acute oral toxicity testing, those receiving the upper dose were observed for 14 days after which they were also euthanized. The LD_{50} was calculated by probit.

2.8 Statistical analysis

All data was expressed as the mean ± standard deviation. Data was recorded on Microsoft Office Excel (2007) spreadsheet. LD_{50} was determined by probit.

3. Results

The yield of various fractions were as shown in **Table 1**.

Activity was classified into four categories: highly active (IC_{50}< 5 µg/ml), active (IC_{50} 5-10 µg/ml), moderately active (IC_{50} 11- 50 µg/ml) and inactive (IC_{50}> 50 µg/ml), (Charity et al, 2012) and findings were as at **Table 2**.

VLA4 and VLA5 showed the best for the aerial parts whereas VLRT was best for the root extract, as indicated by their IC_{50} values. However due to its high cytotoxic value (19.2µg/ml) VLA4 had low selectivity index. Four fractions VLA4, VLR2, VLR4 and VLA5 were cytotoxic with the rest found to have safe profiles. Crude extracts of both *V. lasiopus* aerial parts and roots were non-toxic *in vivo* with high LD_{50} values (>5000mg/kg). The tested fractions were also found to be non-toxic (**Table 2**).

Table 1: Yield of *V. lasiopus* Fractions on Vacuum Liquid Chromatography

Fraction	Weight of Starting Material (gm.)	Weight of Fraction (gm.)	Yield (%)
VLAT	14.0		
VLA1		0.43	3.07
VLA2		0.64	4.55
VLA3		0.20	1.40
VLA4		0.23	1.65
VLA5		1.20	8.60
VLA6		8.31	59.35
VLRT	11.0		
VLR1		0.04	0.35
VLR2		0.04	0.35
VLR3		0.22	2.01
VLR4		0.67	6.11
VLR5		0.45	4.07
VLR6		7.00	63.64

VLA: *Vernonia lasiopus* aerial parts VLR: *V. lasiopus* root

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Table 2: Crude Extracts and Fractions in Descending Order of Viability

Sample	Activity (IC_{50}) µg/ml	Cytotoxicity (CC_{50}) µg/ml	SI Value	LD_{50} mg/ml
CQ	0.06±0.006	>100	>1667	
VLRT	[c] 13.1±0.96	>100	>7.63	>5000
VLR1	[c] 16.8±0.39	>100	>5.95	ND
VLA2	[c] 18.2±0.50	>100	>5.49	>5000
VLA3	[c] 18.4±0.41	>100	>5.43	>5000
VLR5	[c] 22.2±0.70	>100	>4.5	>5000
VLAT	[c] 25.0±1.47	>100	>4.00	>5000
VLR6	[c] 29.5±0.75	>100	>3.39	>5000
VLA6	[c] 30.6±0.61	>100	>3.27	>5000
VLA5	[c] 21.4±0.59	65.22±1.84	3.05	>5000
VLR3	[c] 33.5±0.91	>100	>2.99	ND
VLR2	[c] 18.2±0.50	41.05±1.24	1.73	ND
VLR4	[c] 37.5±0.78	63.835±9.0	1.70	ND
VLA4	[c] 11.2±0.52	19.2	1.68	>5000
VLA1	[d]68.8±1.19	>100	>1.45	>5000

[c] - Moderately active (IC_{50}: 11- 50 µg/ml), [d] - inactive (IC_{50}: <50 µg/ml), CQ: Chloroquine, ND: Not determined
Selectivity index (SI) = CC_{50}/ IC_{50}

The crude *V. lasiopus* root extract, VLRT (IC_{50}:13.1 µg/ml and S.I >7.63), had higher activity than any individual fraction. Fraction 1 of the crude root extract (VLR1) was the most viable fraction with an IC_{50} of 16.8 µg/ml and S.I >5.95. Both had CC_{50}>100 µg/ml and LD_{50} >5000mg/kg.

4. Discussion

The IC_{50} and CC_{50} for the extracts were determined on *P. falciparum* and their results presented as shown in Table 2. A total of 7 different samples were obtained per plant part, being one crude extract and the six fractions.

For *V. lasiopus* aerial parts, fractions 2 and 3 had the best activity at $IC_{50}$of 18.2 and 18.4µg/ml respectively. They were not cytotoxic at CC_{50}> 100 µg/ml and safe in the animal studies at LD50 >5000mg/kg.

VLA4 showed antiplasmodial activity of 11.2µg/ml, but due to the high cytotoxicity (CC_{50} 19.2µg/ml) the selectivity index was low at 1.71, hence not viable as an antiplasmodial agent. However it was not toxic *in vivo* hence may need to be tested for *in vivo* antiplasmodial activity. An assumption would be that it may be deemed nontoxic by other components before it reaches the cells, such as low pH in the stomach and protective mucous lining.VLA5 was also cytotoxic at 65.22µg/ml although had it had no *in vivo* toxicity at LD_{50}>5000mg/kg.

In *V. lasiopus* Roots, the crude extract (VLRT) is more active than any fraction by itself clearly showing synergy among the various fractions. Synergy has been reported among other plant extracts such as the flavonoids in *Artemisia annua*, which are structurally unrelated to artemisinin but enhance its *in vitro* antiplasmodial activity (Bodekar, 2007). Also synergism has been observed between the alkaloids of *Ancistrocladus peltatum*. The total alkaloid extract of this plant has much greater antiparasitic activity than any of the six alkaloids isolated individually (Bodekar, 2007). VLRT had a kill at day 4, showing evidence of delayed toxicity even though this was past than the 48hr observation period. Fractions 1, 5, 6 and 3 were categorized as moderately active with IC_{50} of 16.8, 22.2, 29.5 and 33.5µg/ml respectively. They all had CC_{50}>100µg/ml. VLR 5 and 6 had LD_{50}>5000mg/kg. Fraction 4 was cytotoxic at 37.5 µg/ml hence had low selectivity index of 1.70.

Presence of antiplasmodial/antimalarial activity in *V. lasiopus* concurs with findings by Muregi (Muregi et al, 2005) and Dharani (Dharani et al, 2010), who reported favourable activity.

5. Conclusion.

The findings showed that *V. lasiopus* aerial parts and roots have favourable antiplasmodial activity in their crude extracts and specific fractions, with good safety margins. This partially supports the favourable use of *V. lasiopus* traditionally as an antimalarial.

Conflict of Interest declaration

The authors declare no conflict of interest.

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