**In vitro** Antimalarial and Cytotoxic Activities of Leaf Extracts of *Vernonia amygdalina* (Del.)

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**ABSTRACT:** The antiplasmodial and cytotoxic activity of leaf extracts of *Vernonia amygdalina* was studied. The plant leaves were prepared into three extract forms: ethanolic, aqueous, and hydroethanolic (50:50) using standard procedures. The extracts were evaluated **in vitro** for antiplasmodial activity using a 3D7 chloroquine sensitive clone of NF-54 isolate of *Plasmodium falciparum*. The parasite growth inhibition was estimated based on the 48 hours microassay technique. Cytotoxicity of the extracts was evaluated **in vitro** using non-cancerous vero cell line (C-1008 kidney fibroblasts from African green monkey) by the neural red uptake method. The results showed that the ethanolic extract of the plant had the highest antiplasmodial activity with IC$_{50}$ = 9.83 µg/ml, cytotoxicity (IC$_{50}$ = 60.33) and selectivity index (S.I.) of 6.14 when compared with the other extracts. The results suggest that the ethanolic extract of *V. amygdalina* possesses considerable antiplasmodial activity. The study justifies local claims on the efficacy of the plant leaves for treatment of malaria.

**Key Words:** Antiplasmodial, cytotoxicity, *Vernonia amygdalina* leaf, **in vitro**, *Plasmodium falciparum*, vero cell line

**INTRODUCTION**

Malaria constitutes one of the major public health problems in the world, especially in tropical Africa, Asia and Latin America. The World Health Organization (WHO) estimated that over (350-500) million people are infected by malaria parasite (Kumar and Srivastava, 2005; Hilou *et al.*, 2006; Mbatchi *et al.*, 2006). The problem is further compounded by the upsurge in the spread of resistance of the mosquito vector to currently available insecticides as well as the growing resistance of the parasites to treatment. Thus, the search for novel and more effective antimalarial drugs especially from medicinal plants is of utmost importance in the treatment of malaria (Trager and Jensen, 1997; Mbatchi *et al.*, 2006; Hofer *et al.*, 2008).

Traditional methods of malaria treatment still remain promising source of new antimalaria compounds (Ouattara *et al.*, 2006; Chukwujekwe *et al.*, 2009). In Africa, the use of indigenous plants plays an important role in the treatment of malaria by serving as good sources of novel antiplasmodial compounds (Mbatchi *et al.*, 2006; Hilou *et al.*, 2006; Challand and Wilcox, 2009).

The therapeutic properties ascribed to most medicinal plants have been linked to the presence of phytochemical compounds contained in them. Phytochemicals such as alkaloids, terpenes, saponins, flavonoids, etc, have been reported to exhibit anti-plasmodial activity (Martin and Appel, 2010; Erasto *et al.*, 2006).

*Vernonia amygdalina* is an edible rainforest plant. It is a small tree that can grow up to 3 metres high throughout the African tropics (Erasto *et al.*, 2006). The common names are bitter leaf, *ewuro*, *shuwaka*. The leaves, root and twig of the plant are used for treating stomach-ache and gastrointestinal disorders, schistosomiasis, amoebic dysentery, wounds, veneral diseases, hepatitis, diabetes mellitus, cancer and malaria infection (Nwanjo, 2005; Erasto *et al.*, 2006; Challand and Wilcox, 2009).

*V. amygdalina* and its relatives are good sources of sesquiterpenes lactones (Kraft *et al.*, 2003; Tona *et al.*, 2004; Challand and Wilcox, 2009; Chukwujekwu *et al.*, 2009). Several such
compounds have been isolated and identified from the plant leaves including vernolide, vernodalin and hydroxyvernonolide (Kraft et al., 2003; Tona et al., 2004; Challand and Wilcox, 2009; Chukwujekwu et al., 2009). The occurrences of steroidal saponins, terpenes, coumarins, tannins, alkaloids and flavonoids have also been reported (Ighile et al., 1995; Akinpelu 1999; Tona et al., 2004; Erasto et al., 2006). The phytochemicals present in the plant have been suggested to play a role in its biological activity (Kumari et al., 2003; Erasto et al., 2006).

This study reports scientifically based evaluation of in vitro antimalarial activity of leaf extracts of V. amygdalina in chloroquine sensitive P. falciparum 3D7 (NF-54 clone) culture and cytotoxicity of the extracts against non-cancerous vero cell lines.

MATERIALS AND METHODS
Collection of Plant Material: The V. amygdalina leaves were selected for this study based on local claims of its efficacy in the treatment of malaria infection in southern region of Nigeria. The plant leaves were collected during the rainy season between April and June from a private farm at Ugbowo Quarters, Egor Local Government Area, Benin City, Nigeria. The leaves were authenticated by a Botanist, and voucher specimen (ESO/VA/08) of the plant deposited in the herbarium of the University of Benin, Benin City, Nigeria.

Extract Preparation: The leaves were air-dried, crushed and extracted into 3 extract types which consisted of ethanolic, aqueous, and hydroethanolic (50:50 v/v) extracts using standard extraction procedures (Uoattara et al., 2006). The extracts were concentrated using a rotary vacuum evaporator, freeze-dried and stored at -20°C.

Determination of in vitro Antiplasmodial Activity of Extracts: Chloroquine sensitive P. falciparum 3D7 (NF-54 clone) were maintained at 5% haematocrit (human type o-positive red blood cells) in complete RPMI 1640 medium by the candle jar method (Trager and Jensen, 1976). The culture was routinely monitored using Giemsa staining of thin smears. Antimalarial activity was determined in vitro by parasite growth inhibition assay with some modifications. Different concentrations of the standard drug chloroquine (0.05 ï½ 10 μg/ml) and extracts (5, 10, 50, 100, 500, and 1000 μg/ml) were dissolved in sterilized water and DMSO, respectively.

The synchronized culture with parasitaemia of between 1.5% and 3% haematocrit was incubated in 96-well microtitre plates predisposed with multiple concentrations of compounds/extracts for 48 hrs at 37°C in candle jar. Blood smears from each well were prepared and stained with Giemsa stain and the number of infected RBCs per 5000 cells was counted. All cultures were performed in triplicates and the results were expressed as percentage of growth inhibition. The concentration at which parasite growth was inhibited by 50% (IC₅₀) was determined from dose-response curves by a non-linear dose-response curve fitting analysis via Graph Pad Prism (version 4 software). Crude extracts with IC₅₀ values > 50 μg/ml were considered to be inactive (Kraft et al., 2003).

In vitro Cytotoxicity Test: Cytotoxicity of the plant extracts was assessed against Vero cell line (kidney cells from the African green monkey) cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum; 0.2% NaHCO₃ at 37°C in an atmosphere of 95% humidity, 5% CO₂. All cultures were performed in triplicates (three assays). Dose-response curves were determined from near dose–response curve fitting analysis via Graph Pad Prism (version 4 software). Crude extracts with IC₅₀ values > 50 μg/ml were considered to be inactive (Kraft et al., 2003).

RESULTS AND DISCUSSION
The results from the in vitro antimalarial study showed that extracts of V. amygdalina possess parasite suppressive effect against chloroquine sensitive 3D7 strain of P. falciparum (Figures 1 to 3). From literature, an extract is said to be highly active if IC₅₀ < 10μg/ml, moderately active if IC₅₀ is between 10μg/ml and 50μg/ml and inactive if IC₅₀ > 50μg/ml (Mbatchi et al., 2006). Using this
classification, the ethanolic extract of the plant leaf was found to be highly active against the *P. falciparum* parasites with IC$_{50}$ of 9.82 μg/ml when compared with the aqueous (IC$_{50}$ = 41.69 μg/ml) and hydroalcoholic extracts (IC$_{50}$ = 44.03 μg/ml) (Table 1) which showed moderate antiplasmodial activities. The parasite suppressive ability of the extracts was however low when compared with that of the standard drug, chloroquine (C$_{50}$ of < 0.090 μg/ml).

The results is consistent with previous studies on antiplasmodial activity of *V. amygdalina* and its closely related species including *V. colorata* and *V. brazzavillensis* (Masaba, 2000; Madureira et al., 2002; Tona et al., 2004; Kaou et al., 2008; Challand and Willcox, 2009; Chukwujekwu et al., 2009). The presence of some bitter sesquiterpenes lactones compounds, such as, vernolide, vernodalin, hydroxyvernolide and the steroid-related constituents, vernonioside B1 and vernonoid B1 in the plant leaves have been suggested to be responsible for its antiplasmodial activity (Al Magboul et al., 1997; Kraft et al., 2003; Tona et al., 2004; Kaou et al., 2008; Chukwujekwu et al., 2009). These compounds were earlier found to be present in the leaves and the pith of young shoots of the plants which may be responsible for its bitter taste and significant bioactivity (Masaba, 2000).

Chimpanzees have been reported to chew the pith of the young shoots for treatment of parasitic infections (Tona et al., 2004; Challand and Wilcox, 2009). Antimicrobial and cathartic activities have also been previously reported, and in some cases, the isolation of the active constituents had been described (Koshimizu et al., 1994; Akinpelu, 1999; Awe and Makinde, 1999; Tawo et al., 1999; Kambizi and Afolayan, 2001; Tona et al., 2004). In addition to the sesquiterpenes lactones, there may be other constituents present in the plant leaves that can provide therapeutic activities other than antiparasitic effect, such as antipyretic, antioxidant, anti-inflammatory, analgesic, immunomodulatory and cytotoxic properties (Mbathie et al., 2006; Kaou et al., 2008).

**Table 1:** *In vitro* antiplasmodial activity, cytotoxicity and selectivity index of various leaf extracts of *V. amygdalina*

<table>
<thead>
<tr>
<th>Extracts</th>
<th>IC$_{50}$ (μg/ml) <em>Plasmodium</em></th>
<th>IC$_{50}$ (μg/ml) <em>VeroCells</em></th>
<th>Selectivity Index (SI)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>VA ethanol</td>
<td>9.82 ± 0.43</td>
<td>60.33 ± 0.24</td>
<td>6.14</td>
</tr>
<tr>
<td>VA aqueous</td>
<td>41.69 ± 0.21</td>
<td>414 ± 0.16</td>
<td>9.93</td>
</tr>
<tr>
<td>VA hydroethanol</td>
<td>44.03 ± 0.32</td>
<td>224.6 ± 0.22</td>
<td>5.1</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± three separate experiments

$^a$ Selectivity Index = IC$_{50}$ *Vero Cells*/ IC$_{50}$ *Plasmodium*

VA ethanol (*Vernonia amygdalina* ethanolic extract), VA aqueous (*Vernonia amygdalina* aqueous extract), VA hydroethanol (*Vernonia amygdalina* hydroethanolic extract). IC$_{50}$ chloroquine 0.090 μg/ml.

All the extracts showed moderate cytotoxicity towards the normal vero cell lines; ethanolic extract had the highest cytotoxicity. The selectivity index (S.I.) was highest for the aqueous extract group, which probably indicates its high selectivity when compared with the other extracts (Table 1). Our results seem to agree with previous report that the vernodalin and vernolide compounds isolated from *V. colorata*, a closely related species of *V. amygdalina*, have cytotoxic effects on human KB cells (Kraft et al., 2003; Chukwujekwu et al., 2009). In the ethnomedicinal use of the leaf, the preparation of the plant decoction however involves some preliminary processing procedures which may reduce the cytotoxicity; and the decoction is usually given in small doses or in combination with other herbal preparation for the treatment of malaria.

The high antiplasmodial and moderate cytotoxic activity of the crude ethanolic extract in this study makes it an ideal candidate for investigating the active compounds in terms of structure–activity relationship with various modifications of the compounds to reduce toxicity while retaining antiplasmodial activity. The study justifies local claims on the efficacy of the plant leaves and provides effective intervention for malaria.
infection. Further work is desirable to ascertain the optimum dosage regimen, possibly in combination with other antimalarial plants.

**Fig. 1:** Showing effect of different concentrations of ethanolic Extract of *V. amygdalina* and chloroquine on *in vitro* growth of *P. falciparum*

Data are expressed as mean ± three separate experiments. VA ethanol = Ethanolic extract of *Vernonia amygdalina* leaves

**Figure 2:** Effect of different concentrations of aqueous extract of *V. amygdalina* and chloroquine on *in vitro* growth of *P. falciparum*

Data are expressed as mean ± three separate experiments. VA aqueous = Aqueous extract of *Vernonia amygdalina* leaves
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REFERENCES


