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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* leave, *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 hydroxyvernolide (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* antiplasmodial 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 hydro-ethanolic (50:50 v/v) extracts using standard extraction procedures (Ouattara *et al*., 2006). The extracts were concentrated using a rotary vacuum evaporator, freezed dried and stored at -20^{o}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 ï 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% (IC_{50}) 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 IC_{50} 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% $NaHCO_3$ at 37^{o}C in an atmosphere of 95% humidity, 5% CO_2. Assay was performed in 96-well microplates using neural red uptake method as described by Borenfreund and Puerner (1985) and Repetto *et al.* (2008). Concentration ranges tested were between 0.3 ï 1000 µg/ml for plant extracts and 0.3 ï 100 µg/ml for doxorubicin. All cultures were performed in triplicates (three assays). Doxorubicin hydrochloride was used as the standard cytotoxic drug. IC_{50} was calculated from dose-response curve as described earlier.

RESULTS AND DISCUSSION

The results from the *in vitro* antiplasmodial 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 IC_{50} < 10µg/ml, moderately active if IC_{50} is between 10µg/ml and 50µg/ml and inactive if IC_{50} > 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 9.82μg/ml when compared with the aqueous (IC_{50} = 41.69 μg/ml) and hydroalcoholic extracts (IC_{50} = 44.03 μg/ml) (Ttable 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 (Mbatchi *et al*., 2006; Kaou *et al*., 2008).

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

Extracts	IC_{50} (μg/ml) Plasmodium	IC_{50} (μg/ml) VeroCells	Selectivity Index (SI)[a]
VA ethanol	9.82 ± 0.43	60.33 ± 0.24	6.14
VA aqueous	41.69 ± 0.21	414 ± 0.16	9.93
VA hydroethanol	44.03 ± 0.32	224.6 ± 0.22	5.1

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 ï 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.

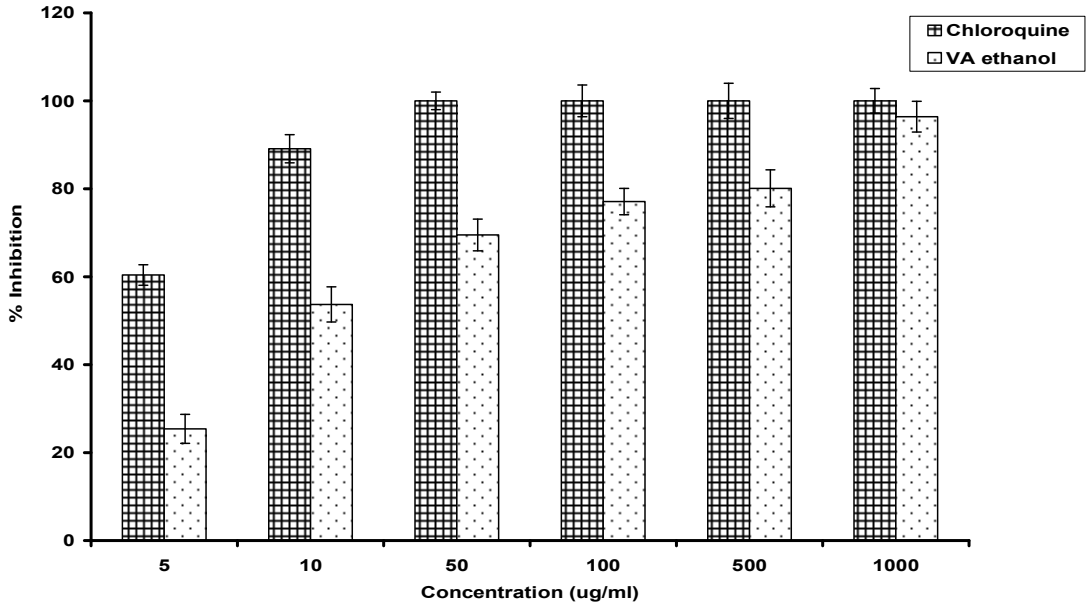

Fig. 1: Showing effect of different concentrations of ethanolic Extract of *V. amygdalina* and chloroquine on *in vitro* growth of *P. falciparum*

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

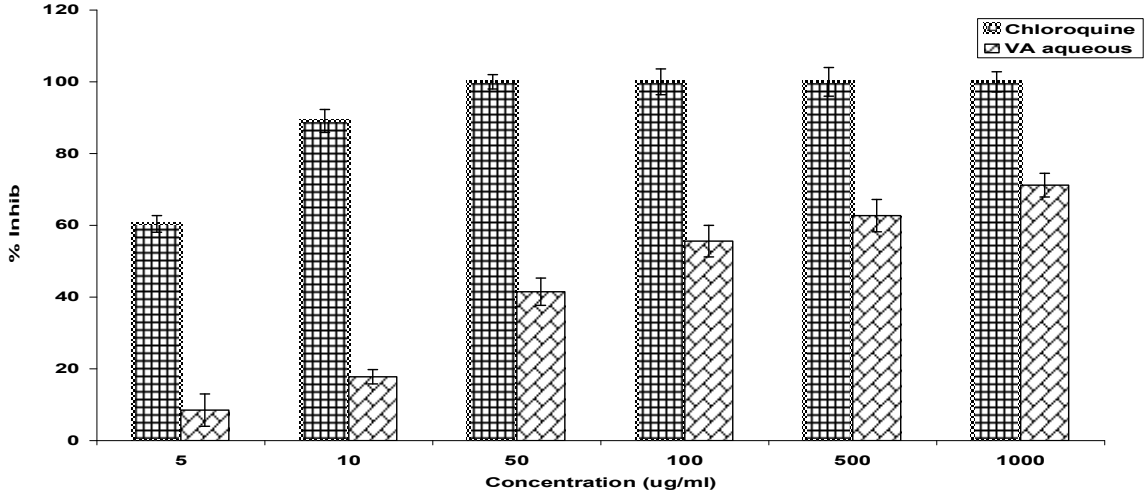

Figure 2: Effect of different concentrations of aqueous extract of *V. amygdalina* and chloroquine on *in vitro* growth of *P. falciparum*

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

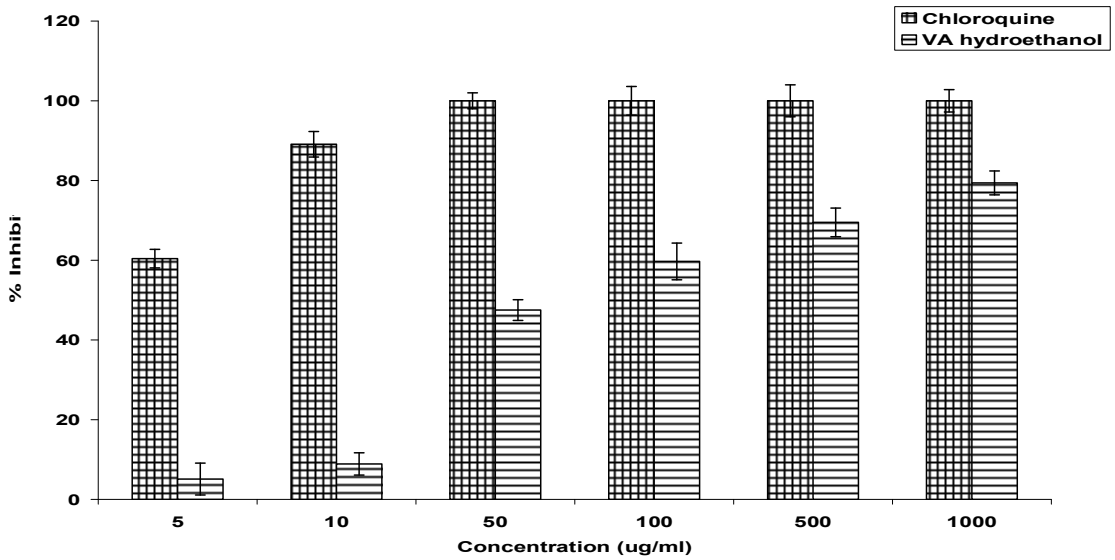

Figure 3: Effect of different concentrations of hydroethanolic (50:50) extract of *V. amygdalina* and chloroquine on *in vitro* growth of *P. falciparum*

Data are expressed as mean ± three separate experiments. VA hydroethanol = Hydroethanolic extract of *Vernonia amygdalina leaves*)

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