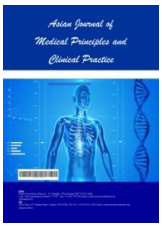

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Antimalarial Potential of Methanolic and Aqueous Extracts of Leaves, Root and Stem-Bark of *Vitex doniana*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was aimed at evaluating the antimalarial potential of methanolic and aqueous extracts of leaves, root and stem bark of *Vitex doniana*.
Materials and Methods: Apparently healthy parts of *V. doniana* (leaves, root and stem bark) were obtained from a farm in Abakaliki, Nigeria. They were air dried and milled into powder and were extracted using methanol and water as solvent respectively. Thirty (30) Swiss male albino mice weighing 15-20 g were used for this study. They were acclimatized for 14 days and randomly divided into 10 groups of 3 mice each. The chloroquine resistant *Plasmodium berghei* (NK 65) used was obtained from the Department of Veterinary Pathology, University of Ibadan. The parasite was maintained by sub-passaging into healthy mice via an intraperitoneal route. Treatment of the animals began 24 hours after infection with the parasite and parasitaemia confirmed. Groups A and B were treated with aqueous and methanol leaf extract of *V. doniana* respectively. Groups C and D were treated with aqueous and methanol root extract of *V. doniana* respectively. Groups E and F were treated with aqueous and methanol stem-bark extracts of *V. doniana* respectively. Groups G, H and I were treated with the standard drugs Artemether, Artesunate and Chloroquine respectively and Group J were administered the vehicle (normal saline) and this group served as the negative

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control group. The administrations were done once a day for 14 days via the intraperitoneal route. Parasitaemia was monitored in all the groups starting from day 0 to day 14 using thick and thin blood films made from blood obtained from the tail vein of mice.

Results: The infected animals treated with methanol stem bark extract of *V. doniana* was compared on day 0 and 10, there was a great reduction in parasitaemia level from 5.33±0.58 to 3.33±0.58 as compared to 6.67±1.15 to 43.0 ±2.65 in untreated group and 5.33±0.58 to 5.67±1.15 in Chloroquine treated group.

Conclusion: From the result of this study, it can be said that methanolic extract of *V. doniana* stem bark is more potent as an antimalarial agent.

Keywords: Antimalarial potential; Vitex doniana; chloroquine resistant; Plasmodium berghei.

1. INTRODUCTION

Malaria is a life-threatening disease prevalent in Nigeria, with a high morbidity and mortality rate, especially among pregnant women and children below 5 years of age. The endemicity of malaria parasite *plasmodium* species, the high morbidity and mortality rate, and its increasing resistance to most antimalarial drugs, has led to diversification of research into potential antimalarial natural medicinal plants. Natural products are important sources for biologically active drugs and wild herbs have been investigated for their antioxidant properties [1]. Medicinal plants containing active chemical constituents with high antioxidant property play an important role in the prevention of various degenerative diseases and have potential benefit to the society [2]. Natural antioxidants from plant sources are potent and safe due to their harmless nature. A free radical in each molecule is determined as an unpaired electron that occupies an atomic or molecular orbital on its own. This reactive molecule is bond to another electron to pair, this in turn leads to an uncontrolled chain reaction that can damage the natural function of the living cell, resulting in different diseases [3]. Many fruits and vegetables, herbs, cereals, seeds that contain natural antioxidants that can extract the lone electron from free-radical molecules and help humans to keep control on these harmful species. Most of these antioxidants in plants are highly colored anthocyanins, proanthocyaninidins, flavans, flavonoids, and their glycosides, carotenoids, like β-carotene and lycopene [4]. Isolation of anti-oxidants from plants depends on the polarity of these compounds.

Vitex doniana, (family *Verbanaceae*) is a perennial shrub widely distributed in tropical West Africa, and some East African countries including Uganda, Kenya and Tanzania and high rainfall areas. It is found in the middle belt of Nigeria particularly Kogi, Benue, and parts of the savannah regions of Kaduna, Sokoto and Kano states [5]. It is variously called *vitex* (English), *dinya* (Hausa), *dinchi* (Gbagyi), *uchakoro* (Igbo), *oriri* (Yoruba) *ejiji* (Igala) and *olih* (Etsako) [6]. *V. doniana* is employed in the treatment of a variety of diseases. Hot aqueous extracts of the leaves are used in the treatment of stomach and rheumatic pains, inflammatory disorders, diarrhoea, dysentery and diabetes. Njoku *et al*. [7] reported the antidiabetic properties of the leaves. The roots and leaves are used for nausea, colic and epilepsy [8]. In North-Central and eastern parts of Nigeria, the young leaves are used as vegetables or sauces and porridge for meals, especially for diabetic patients. The anti-hypertensive effect of extracts of the stem bark of *V. doniana* has been reported [9]. The extract exhibited a marked dose-related hypertensive effect in both normotensive and hypertensive rats [9]. Extract of stem bark of *V. doniana* have also demonstrated some level of *in vitro* trypanocidal activity against *Trypanosome brucei* [10]. The aqueous methanol extract has also exhibited anti-diarrhea activity [11]. Extracts of leaves of *V. doniana* has demonstrated anti-inflammatory and analgesic activities by suppressing paw edema induced by agar in rats and prolonging reaction latency to thermally induced pain in mice by hot plate [12]. The present study was designed to investigate the antimalarial effects of *V. doniana* aqueous and methanol leaf extracts on mice infected with malarial parasites.

2. MATERIALS AND METHODS

2.1 Plant Collection and Extraction

Healthy parts of *V. doniana* (leaves, root and stem bark) were obtained from a farm in Abakaliki, Ebonyi State, Nigeria and were identified by Mr. Alfred Ozioko of International

Center for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Enugu State. It was assigned Voucher Number InterCedd\207 and deposited at the herbarium of the Centre. Plant parts air-dried for about 3 weeks to a constant weight in the laboratory. The dried samples were milled into powdered using an electric grinder and stored separately.100 g of the various plant parts were then soaked in 600 mL of methanol and distilled water respectively for 72 hours and then filtered with a filter paper and evaporated in a water bath at 45°C. All the extracts were weighed and stored in well stoppered containers and refrigerated at 4°C for further analysis.

Fig. 1. Leaves of *Vitex doniana*

2.2 Phytochemical Analyses of *V. doniana* Extracts

The phytochemical analyses of the various extracts were conducted according to the standard methods of AOAC [13].

2.3 Induction of Chloroquine Resistant *Plasmodium berghei* Parasite

Thirty Swiss male albino mice weighing 15-20 g used for this study were obtained from the animal unit of the faculty of Pharmaceutical Science, University of Nigeria, Nsukka. The mice were acclimatized to laboratory conditions for a period of 14 days. The animals were fed with standard animal feed (Vital growers, Nigeria) and clean drinking water *ad libitum*. The chloroquine resistant *Plasmodium berghei* (NK 65) used was obtained from Department of Veterinary Pathology, University of Ibadan. The parasite was maintained by sub-passaging into healthy mice via an intraperitoneal route as earlier described by Ene et al. [14]. Briefly, one millilitre of *P. berghei* infected blood was diluted with 10 mL of phosphate buffer saline (PBS) pH 7.2. The dilution was such that each 0.2 mL had approximately $10x10^7$ infected red cells/parasite per kg of body weight. Infection of each mouse was effected with a single intraperitoneal inoculum of 0.1 mL of diluted infected blood.

2.4 *In vivo* Treatment of the Infected Albino Mice

24 hours after infection with the parasite and parasitaemia confirmed, the mice were weighed and divided into 10 groups of three (3) mice each. Groups A and B were treated with aqueous and methanol leaf extract of *V. doniana* respectively. Groups C and D were treated with aqueous and methanol root extract of *V. doniana* respectively. Groups E and F were treated with aqueous and methanol stem-bark extracts of *V. doniana* respectively. Groups G, H and I were treated with the standard drugs Artemether (1.6 mg/kg body weight), Artesunate (1.6 mg/kg body weight) and Chloroquine (10 mg/kg body weight) respectively and Group J were administered the vehicle (normal saline) and this group served as the negative control group. The dosage of 100 mg/kg b.w.t of the extract was adopted based on a preliminary study carried out in mice. The administrations were done once a day for 14 days via the intraperitoneal route [14].

2.5 Estimation of Parasitaemia

Parasitaemia was monitored in all the groups starting from day 0 to day 14 using thick and thin blood films made from blood obtained from the tail vein of mice [14]. The smear was air-dried, fixed with methanol, stained with 10% Giemsa at pH 7.2 for 15 mins, washed under running tap and allowed to dry. It was then examined under the microscope with x100 objective lens to access the level of parasitaemia.

Percentage parasitaemia was calculated as

$$\text{Percentage parasitaemia} = \frac{\text{No of parasitemia in treated Mice}}{\text{No of parasitemia in control}} \times 100$$

This is normally estimated as:

$$\frac{\text{No of parasitemia in treated Mice}}{500} \times 100.$$

2.6 Statistical Analysis

Data were statistically analyzed using Analysis of Variance (ANOVA). Subsequently, the Tukey post hoc tests with multiple comparisons were used to determine the source of the significant differences. $P < 0.05$ was considered to be statistically significant.

3. RESULTS

The results of the phytochemicals composition of various extracts of *V. doniana* are presented in Table 1 while their respective antimalarial potency are presented in Table 2.

4. DISCUSSION

Plant materials contain a variety of natural products with different polarities and therefore different solubility properties. This study on *V. doniana* has become necessary both to meet the challenges of malaria eradication and to circumvent resistance to most anti-malarial drugs. Several medicinal plants have also been used locally to treat malaria infection [15,16].

The qualitative phytochemical screening of the methanolic extract of *V. doniana* stem bark indicated the abundance of many phytochemicals including alkaloids, tannins, flavonoids, saponins, anthraquinone (Table 1). The phytochemical characteristics possessed by *V. doniana* may be attributed to their antimalarial properties. This finding agrees with that conducted by Emmanuel et al. [17] on phytochemical and antimicrobial screening of the stem bark extracts of *V. doniana*. These are responsible for the medicinal values of the plant. Alkaloids have been reported to be powerful pain relieve [18,19]. Saponins could be considered as natural antibiotic which helps in fighting infections and microbial invasion [20,21]. Flavonoids are antioxidants [22], protections against allergies, inflammation, free radicals, platelet aggregation of microbes and ulcer [23,24,25]. Glycosides are known to lower blood pressure [26]. Steroids and terpenoids show analgesic properties [27]. The effects of methanol and aqueous extracts of different parts of *V. doniana* on Chloroquine resistant *P. berghe*i NK65 is shown in Table 2.

For the negative untreated group, there was a significant increase in the parasitaemia level, which caused the animals to become weak. For the infected mice treated with Chloroquine, there was a reduction in parasite level in day 5, which started increasing again from day 10. For the infected groups treated with Artemether and Artesunate, the parasite almost cleared on day 6, which just a trace of the parasite in the blood, and the animals survived after 14 days. However, when the infected animals treated with methanol stem bark extract of *V. doniana* was

Table 1. Phytochemicals composition of various extracts of *V. doniana*

Phytochemical	Specific test	Results					
		Aqueous leaves	Methanol leaves	Aqueous stem	Methanol stem	Aqueous root	Methanol root
Tannins	Test with ferric chloride solution	+	+	+	+	+	+
Saponins	Frothing test	+	+	+	+	+	+
Flavonoids	Schinoda's test	+	+	+	+	+	+
Alkaloids	Test with Dragendroff's reagent	+	+	+	+	-	+
Cardiac glycosides	Salkowskii test	+	+	+	+	+	+
Anthraquinone	Borntrager's test	-		-		-	
Steroids	Test with Liebermann-Burchard reaction	_	-	_	-	_	-

+ means Present while – means Absent

Table 2. Effect of various extracts of *V. doniana*, Artemether, Artesunate, Chloroquine on parasite count in albino mice

Plant parts	Extract concentration	Number of days of treatment				
		0	4	7	10	14
Leaf	Aqueous Leaf (100 mg/kg b.wt)	6.33 ± 0.58a	3.67 ± 0.58^{a,b}	4.67 ± 0.58^{b}	7.33 ± 0.58^{c}	12.00 ± 1.00^{d}
Leaf	Methanolic Leaf (100 mg/kgb.wt)	6.33 ± 4.04a	6.67± 0.58^{a,c}	6.00 ± 0.00^{b}	11.67 ± 0.58^{d}	23.00 ± 1.41^{e}
Root	Aqueous Root (100 mg/kgb.wt)	5.67 ± 2.08^{a}	9.67± 2.52^{c}	ND	ND	ND
Root	Methanolic Root (100 mg/kgb.wt)	5.33 ± 1.53^{a}	30.67 ± 0.58^{e}	14.33 ± 2.08^{c}	25.33 ± 0.58^{e}	ND
Bark	Aqueous Stem Bark (100 mg/kgb.wt)	5.00 ± 1.00^{a}	6.33 ± 0.58^{b,c}	5.33 ± 0.58^{b}	22.67 ± 1.15^{e}	49.33 ± 0.58^{f}
Bark	Methanolic Stem Bark (100 mg/kgb.wt)	5.33 ± 0.58^{a}	5.00± 1.73^{ab}	3.33 ± 0.58^{ab}	3.33 ± 0.58^{a,b}	3.00 ± 0.00^{b}
Artemether Standard	Artemether (100 mg/kgb.wt)	6.33 ± 1.15^{a}	2.00± 0.00^{a}	1.00 ± 0.00^{a}	1.00 ± 0.00^{a}	1.00 ± 0.00^{a}
Artesunate Standard	Artesunate (100 mg/kgb.wt)	6.00 ± 1.00^{a}	1.67 ± 0.58^{a}	1.00 ± 0.00^{a}	1.00 ± 0.00^{a}	1.00 ± 0.58^{a}
Chloroquine Standard	Chloroquine (100 mg/kgb.wt)	5.33 ± 0.58^{a}	5.33 ± 1.53^{ab}	4.33 ± 1.53^{b}	5.67 ± 1.15^{b,c}	5.00 ± 1.00^{c}
Untreated Control	Untreated	6.67 ± 1.15^{a}	13.67 ± 2.08^{d}	22.00 ± 2.00^{d}	43.00 ± 2.65^{f}	52.00 ± 0.00^{f}

Values are mean ± standard deviation. Values with different superscript per column are statistically significant (p<0.05). ND= Non determinable

compared on day 0 and 10, there was a great reduction in parasitaemia level from 5.33±0.58 to 3.33±0.58 as compared to 6.67±1.15 to 43.0 ±2.65 in untreated group and 5.33±0.58 to 5.67±1.15 in Chloroquine treated group.

From the Table 2, the comparisons were made on days 0, 4, 7 and 14. It was shown that the level of parasitaemia of infected animals treated with methanol stem bark extract of *V. doniana* showed a significant difference (p<0.05) when compared with the other treatment groups and untreated infected group. It was also observed that the other of *V. doniana* showed a reduction in parasitaemia level after day 3, but the parasitaemia level increased rapidly from day 8, which led to the death of some of the animals by day 14. The parasitaemia levels were considerably reduced but not completely cleared in the group treated with methanol stem bark extract as opposed to the parasitaemia levels of the group treated with Artemether and Artesunate which was completely cleared.

In this study, methanol was used to extract the bioactive components of *V. doniana* because the methanol extract of the stem bark demonstrated higher anti-malarial activity than the crude aqueous extract. Methanol is less dense than water and hence possesses greater diffusibility and solubility in the same medium than water.

In this study, the result of the effect of methanol extract of *V. doniana* stem bark on the mean parasitaemia in mice showed significant (p<0.05) reduction in the treated groups when compared to the untreated and Chloroquine treated groups (Table 2). This might be due to the phytochemical content of the plant. This showed that the extract might be effective against malaria parasitaemia due to the reduction of the percentage parasitaemia level in the treatment groups.

5. CONCLUSION

From the result of this study, methanolic extract of *V. doniana* stem bark is more potent as an antimalarial agent.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal ethic Committee approval has been collected and preserved by the author.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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