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Antimalarial Activities of Some Selected Traditional Herbs from South Eastern Nigeria Against *Plasmodium* Species

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Abstract: A study of three plants traditionally used in the treatment of malaria in the Southeastern part of Nigeria was investigated to determine their efficacies as antimalarial compounds. The three herbs were collected through a traditional herbalist who uses them in his practice. Ethanolic extracts from the roots of *Enyim ocha* (*Salacia nitida*), *Ovoro ilu* (*Nauclea latifolia*) and stem bark of *Erumeru* (*Enantia chlorantha* Oliv.) were assessed for antimalarial activity against chloroquine sensitive *Plasmodium berghei* in mice using the 4 day suppressive test procedure. The extracts had intrinsic antimalarial properties that were dose dependent. The comparison analysis indicated that 250 mg kg⁻¹ body weight of the root of *S. nitida* produced 71.15% suppression of parasitaemia and the 500 mg kg⁻¹ body weight of the stem bark of *E. chlorantha*, roots of *S. nitida*, *N. latifolia* and the three herbs combined, produced 75.23, 73.28, 71.15 and 77.46%, respectively, compared with chloroquine with 71.15% suppression. The results were significant at p<0.05 when compared to a placebo and support the traditional use of these plants for the treatment of malaria.

Key words: Antimalarial, traditional herbs, Southeastern Nigeria, *Plasmodium* species

INTRODUCTION

Malaria represents a major cause of childhood mortality and adult morbidity in many parts of the world. Recent estimates indicate that 300 to 500 million clinical episodes of malaria occur worldwide every year and are responsible for one in five deaths in Africa (Breeman, 2001; WHO, 1997). Antimalaria drug resistance, particularly *Plasmodium falciparum* resistance, has been a major set back in the fight against malaria and its attendant complications (Wongsrichanalai *et al.*, 2002). The application of herbal drugs to different human and animal disease conditions dates back to human history. Plants have been the basic source of sophisticated traditional medicine systems for thousands of years and were instrumental to early pharmaceutical drug discovery and industry (Elujoba *et al.*, 2005). Traditional medicine practitioners, natural herbal sellers and the local indigenous people have been the source of basic information as a lead to scientific probing of medicinal plants in Africa (Baba *et al.*, 1992). An appreciable level of studies has been done on African traditional medicinal plants. These range from ethno botanical surveys, to the actual extraction of the active ingredients in the plants (Ibeh and Nwifo, 2005; Ajaiyeoba *et al.*, 2006; Dikaso *et al.*, 2006). However, in Nigeria, particularly in the South Eastern part, studies on the extraction and perhaps, testing of the effects of these herbal extracts on malaria parasites have been minimal. The enormity of the malaria burden (WHO, 1997), particularly antimalaria drug resistance (Peters, 1998; Wongsrichanalai *et al.*, 2002),

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demands a commensurate level of studies towards the discovery of novel but potent antimalaria drugs to combat this burden. This has become increasingly exigent, particularly in Africa, with widespread poverty and illiteracy, as neither of the antimalaria drugs in use today is indigenous to her (Milijaona *et al.*, 2003). Therefore, the aim of the study was to investigate the antimalarial activities of some herbs used in the traditional treatment of malaria in the South Eastern part of Nigeria. This exercise would offer a scientific backing in the use of traditional herbs either separately or in combination against malarial diseases.

MATERIALS AND METHODS

Sample Collection

Three medicinal plants namely, *Enantia chlorantha* (stem bark), *Nauclea latifolia* (root) and *Salacia nitida* (root), were collected from Mbutu in Aboh Mbaise Local Government, Imo State, Nigeria. Five herbalists from randomly selected communities in the area were interviewed on their methods of treatment of malaria. After the interviews, 25 herbal plants were mentioned as antimalaria herbs used in their practice. All of them were unanimous in their use of the herbs in combination for enhanced efficacy and their mode of preparation of the herbs (boiling or maceration in alcohol). Six herbs were discovered to be common among the herbalists. Three of them were randomly selected for the study and were obtained by the help of the herbalist, from the village forest. The stem of *E. chlorantha* and the roots of *S. nitida* and *N. latifolia* were collected. In addition their respective leaves were collected for botanical identification. The identification and authentication was done at the Herbarium of University of Port-Harcourt, Choba, Nigeria, where a voucher specimen was deposited.

Extraction Procedure

The roots of *N. latifolia* and *Salacia nitida* and the stem bark of *Enantia chlorantha* were sun-dried at ambient temperature ($30 \pm 0.5^\circ\text{C}$). After drying, 1 kg each of the samples was pulverized to coarse powder using sterile mortar and pestle to avoid contamination. Two hundred and eighty-two grams each of the pulverized herbs was measured using Mettler PN 163 electric weighing machine and transferred into a conical flask. Ninety-four grams each of the three herbs was weighed and mixed together to give Two hundred and eighty-two grams of the combined herbs and transferred into the fourth flask. The respective herbs and their combination were extracted for 72 h using 1 L of 98% ethanol. The herb-ethanol mixtures were shaken daily to ensure proper extraction (Abosi and Raseroke 2003). After 72 h, the extracts were filtered using clean, white cotton handkerchief. The filtrates, were concentrated under vacuum using a vacuum rotary evaporator, to yield, 3.885, 14.035, 5.517 and 7.537 g of gummy oily residues of *E. chlorantha*, *S. nitida*, *N. latifolia* and their combination, respectively and were stored in screw capped vials at 4°C until tested (Abosi and Raseroke, 2003). Prior to use, 1 g mL^{-1} of each extract was prepared in ethanol to ensure adequate dissolution and then 4 mL of sterile water was added to give a stock of 1000 mg in 5 mL. Subsequently, 125, 250 and 500 mg kg^{-1} doses were prepared using distilled water. The extraction was carried out in the Microbiology Research Laboratory, Rivers State University of Science and Technology, Port Harcourt.

Mouse Strain

Healthy albino mice, 5 weeks old and weighing 18-24 kg, were obtained from the animal house of the Department of Pharmacology, University of Nigeria Nsukka. They were kept in plastic cages and adequately fed with livestock feed and water for optimal health. Before the experiment, the animals were bred for one week at the Malaria Research Laboratory, University of Port Harcourt where the research took place, for proper acclimatization.

Malaria Parasite

Chloroquine-sensitive *P. berghei* (NK-65), used in the experiment was obtained from the Malaria Research Laboratories, Institute for Advanced Medical Research and Training, (IAMRAT), College of Medicine, University of Ibadan. Parasites were maintained through weekly passage in mice, by inoculation of known amount of parasite into healthy mice every week.

Inoculation and Treatment

Peter's 4 day test (Peter and Anatoli, 1998; David *et al.*, 2004) was followed to evaluate the blood schizontocidal action against *Plasmodium berghei*. Donor albino mice previously infected with chloroquine-sensitive *P. berghei* and with a rising parasitaemia of 30% as determined using thin blood film, were sacrificed and the blood collected using EDTA bottle. The blood sample was diluted using phosphate buffered saline (concentration of 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, pH 7.4) such that 0.1 mL contained eleven million parasites. To avoid variability in parasitaemia, all the animals used were infected from the same source. Forty-two mice were used to assess the effect of the herbal extracts. On day 0, experimental as well as control groups of animals were inoculated with eleven million *P. berghei*-infected red blood cells. The mice were then randomly divided into groups of three per cage and groups of experimental animals were given different doses of test materials consecutively from day 0 to day 3. Three of the groups were given 500, 250 and 125 mg kg⁻¹ of the plant extract per day, orally. The initial treatment started about two hours after infection. The other two groups received either 5 mg kg⁻¹ chloroquine per day (positive control), or 0.2 mL of phosphate buffered saline (concentration of 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, pH 7.4) (negative control). All experiments were carried out in triplicate. The animals were adequately fed and no death was recorded throughout the duration of the experiment.

On day four of the test, thin blood smears were prepared using well-labeled and properly cleaned slides (Devi *et al.*, 2000). Blood was collected from the tail vein of each animal using heparinized capillary tube. The dry blood films were fixed with methanol and subsequently, stained with Giemsa for 25 min. They were then washed with phosphate buffer, pH 7.2 (Dikaso *et al.*, 2006) and allowed to dry. To ensure optimal film quality, a total of eighty-four slides were made, each animal in duplicate. The slides were then microscopically examined using x100 magnification in oil immersion (Model Olympus microscope) (Dikaso *et al.*, 2006). The percentage suppression of parasitaemia was calculated for each dose level by comparing the parasitaemia in infected controls with those of treated mice. Average percentage suppression of parasitaemia was calculated using the formula:

$$A = \frac{[(B-C)]}{B} \times 100$$

Where:

A = Average percentage suppression

B = Average percentage parasitaemia in the placebo group

C = Average percentage parasitaemia in the test group (Abosi and Raseroke 2003)

Student's t-test was used to compare the differences in the results between the groups.

The mice after four days of treatment looked very healthy and active. They were then monitored for 40 days. All of the treated mice were alive and active. On the 31st day of observation, one of the extract treated mice had obstructed labor and died two days later. Two other extract treated ones had normal delivery of 4 and 5 young ones on the 35th and 38th days, respectively. The chloroquine treated mice did not die within 40 days but were less active than the extract treated animals. However, all of the untreated mice died within 14 days after the experiment.

RESULTS AND DISCUSSION

The results of the study indicated that *in vivo*, ethanolic extracts of the roots of *Salacia nitida*, *Nauclea latifolia* and stem bark of *Enantia chlorantha* Oliv. displayed a very good activity against *P. berghei* malaria parasite when given at doses of 125, 250 and 500 mg kg⁻¹, respectively. The comparison analysis indicated that 250 mg kg⁻¹ of the root of *S. nitida* and the herbal mixture, as well as the 500 mg kg⁻¹ of the stem bark of *E. chlorantha*, root of *S. nitida* and the herbal mixture, showed statistically significant difference on day four parasitaemia level compared to the negative control (p<0.05). A high level of inhibition was observed with 500 mg kg⁻¹ of *E. chlorantha*, *N. latifolia*, *S. nitida* and the herbal mixture (75.23, 71.15, 73.28 and 77.46%), as well as 250 mg kg⁻¹ of *S. nitida* and herbal mixture (71.15 and 69.11%). The result indicated that, the highest level of inhibition (77.46%) was obtained in 500 mg kg⁻¹ of the herbal mixture (Table 1). The overall result showed a higher reduction in mean percentage parasitaemia with increased herbal concentration for each herb and their combination. The positive control, chloroquine induced the same level of chemosuppression (71.15%) with 500 and 250 mg kg⁻¹ of *N. latifolia* and *S. nitida*, respectively

Plants have undoubtedly been a rich source of new drugs in use today. It is estimated that over 1200 plant species from 160 families are used to treat malaria and fever (Wilcox and Gerald, 2004). A number of studies have been done to evaluate the inhibitory effect of various plant extracts on *P. falciparum* (Milijaona *et al.*, 2003; Sanon *et al.*, 2003). Similarly, the *in vivo* antimalarial properties of several plant extracts have been studied in mice (Devi *et al.*, 2000; Abosi and Raseroke, 2003; Ajaiyeoba *et al.*, 2006). Following the trend, this study presents the results got from the evaluation of the *in vivo* antimalarial activity of three herbs namely: *Enantia chlorantha*, *Nauclea latifolia* and *Salacia nitida*, commonly used in the Eastern part of Nigeria against malaria.

N. latifolia (Pin cushion tree) is a staggering shrub or small tree, native to tropical Africa and Asia. It is commonly used in the Eastern part of Nigeria to treat malaria. The root extract of this herb, in the present study was observed to show some intrinsic antimalarial activity. This is evident from its percentage chemosuppression when compared with the positive control, chloroquine in the 4 day suppression test (Peter and Anatoli, 1998; David *et al.*, 2004). Treatment of mice infected with *P. berghei* with ethanolic extracts of *N. latifolia* showed a dose dependent chemosuppression with the 500 mg kg⁻¹ treated group of mice showing the highest percentage suppression. Similar findings were reported by Benoit-Vical *et al.* (1998) that showed that aqueous extracts obtained from the root and stem of *N. latifolia* when tested on two strains of *P. falciparum* (febi strain), inhibited the parasites. Other medicinal ingredients have also been found in this herb. Gidado *et al.* (2005) discovered that the aqueous extracts of *N. latifolia* at 200 mg kg⁻¹ body weight of mice, significantly lowered glucose levels (p<0.05) of diabetic rats by 45% within four hours, but showed no similar effect in normoglycaemic rats.

E. chlorantha (*Annonaceae*) is an ornamental tree of up to 30 m high with dense foliage and spreading crown. It is a component of fever preparations by traditional medicine practitioners in the Eastern part of Nigeria. The ethanolic extract of the stem bark of *E. chlorantha* displayed high level

Table 1: *In vivo* anti-malarial effect of three medicinal plants and their combination

Medicinal plants	Control		Drug dosage in mg kg ⁻¹ body weight		
	Positive	Negative	125 mg	250 mg	500 mg
<i>Enantia chlorantha</i> (stem bark) n = 9	(CQ)	(PBS)	5.00±1.63 (53.62)	3.67±0.94 (65.98)	2.67±0.94 (75.23)*
<i>Nauclea latifolia</i> (Root) n = 9			5.56±3.34 (48.42)	4.11±0.99 (61.87)	3.11±2.74 (71.15)
<i>Salacia nitida</i> (Root) n = 9			4.00±1.50 (62.89)	3.11±0.80 (71.15)*	2.88±0.99 (73.28)*
Herbal mixture n = 9	3.11±1.79 (71.15)	10.78±5.51	3.56±1.16 (66.98)	3.33±0.94 (69.11)*	2.43±1.56 (77.46)*

n: No. of animals in each group; *: Significance at p<0.05; Values indicate mean percentage parasitaemia; Values in parenthesis indicate percentage reduction in parasitaemia compared to control; CQ: Chloroquine; PBS: Phosphate Buffered Saline

of suppression of parasitaemia compared with the positive control. The level of activity was discovered to be dose dependent, with the dose of 500 mg kg⁻¹ having the highest activity (75.23%). Agbaje and Onabanjo (1991) reported that aqueous extract of the plant *E. chlorantha* when tested *in vivo* against *Plasmodium youllei* parasite in mice, was found to be effective in suppressing *Plasmodium youllei* infection in mice if given orally in drinking fluid at 0.2-150 mg mL⁻¹. The animals given the extracts in their drinking fluid survived for over 60 days. They also observed that the ethanolic extract was effective in eliminating the parasites when administered subcutaneously in doses of 0.05-0.5 mg mL⁻¹. They noted that the aqueous and ethanolic extracts had ED50 of 6.9 and 0.34 mg g⁻¹, respectively and were schizontocidal in action. Its antimicrobial activity was also confirmed by Atata *et al.* (2003).

Compared with chloroquine, *S. nitida* was discovered to have a high suppressive ability that was equally dose dependent with the 250 and the 500 mg kg⁻¹ having significant difference at (p<0.05) when compared with the placebo. The overall result shows that the crude extracts of the three herbal plants have intrinsic antimalarial components, which are dose dependent. The extract dose of 500 mg kg⁻¹ displayed the highest level of inhibition of parasitaemia with that of the drug mixture maintaining the lead (77.46%). This drug mixture demonstrated synergistic inhibitory effect on the *in vivo* plasmodial development. This result justifies their use in combination in traditional medicine (Hilda *et al.*, 1994; Azas *et al.*, 2001). Chloroquine at a dose of 5 mg kg⁻¹ gave a percentage suppression of 71.15%, a value equal to that of 250 mg kg⁻¹ of *S. nitida* and lower than the 500 mg kg⁻¹ of the individual herbs and their combination (Table 1). Since chloroquine sensitive parasites were used, it could be possible that the potency of the respective herbs and their combination at 500 mg kg⁻¹ were greater than that of chloroquine at a dose of 5 mg kg⁻¹. Devi *et al.* (2000) and Ajaiyeoba *et al.* (2006) got a clearance rate of 100% parasitaemia with chloroquine sensitive *P. berghei* parasites in their respective studies. However, Ajaiyeoba *et al.* (2006) used a total dose of 25 mg kg⁻¹ of chloroquine in their study while Devi *et al.* (2000) used a total dose of 20 mg kg⁻¹. Both used 1.0×10⁷ parasites whereas, this present study was done with a total dose of 20 mg kg⁻¹ and 1.1×10⁷ parasites. In untreated mice, the parasite count increased as observed in previous studies (Ajaiyeoba *et al.*, 2006; Devi *et al.*, 2000). The high levels of chemosuppression produced at high doses of extracts, is in line with previous studies (Ajaiyeoba *et al.*, 2006; Devi *et al.*, 2000). At 125 mg kg⁻¹ per day, the stem bark extract of *E. chlorantha* and the root extract of *N. latifolia* decreased parasitaemia by 53.62 and 48.42%, respectively. This indicated that the minimum effective dose of these extracts is approximately 125 mg kg⁻¹ day⁻¹. However, under the experimental conditions employed, the extracts failed to eliminate *P. berghei* parasites completely.

CONCLUSIONS

The development of antimalarials from indigenous plants depends to a large extent on the screening of appreciable number of these herbs, particularly those that have been in use by the indigenous people from different geographical areas, especially, from the different tribes where they are endowed by nature. Once the preliminary anti plasmodial effect has been established, further studies to ascertain the active ingredients that exert these effects could then be carried out. This will ultimately help in discovering potent and novel antimalarials to counter the threat posed by malaria in recent times.

There is increasing concern by Medical practitioners as the resistance of malaria parasites to available drugs continues to grow, increasingly limiting our ability to control this serious disease. However, it is reassuring that many new approaches to antimalarial drug discovery are now under evaluation as carried out in this research. Recent increases in the pace of progress in the search especially in the area of herbal medicine, suggest that, if support for antimalarial drug discovery is adequate, the development of novel, but potent antimalaria is underway.

From the present study, it can be concluded that the ethanolic extracts of the roots and stem bark of *N. latifolia*, *S. nitida* and *E. chlorantha* have shown parasite suppressive effects on *P. berghei*-infected albino mice in a dose-related fashion. This result therefore, offers a scientific basis for the traditional use of these herbs separately and in combination against malaria parasite. Further studies on the herbs, especially on the refined extracts, to among other things, assess their pharmacokinetic properties, is recommended.

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REFERENCES

- Abosi, A.O. and B.H. Raseroke, 2003. *In vivo* antimalarial activity of *Vernonia amygdalina*. B. J. Biomed. Sci., 60 (2): 89-91.
- Agbaje, E.O. and A.O. Onabanjo, 1991. The effects of extracts of *Enantia chlorantha* in malaria. Ann. Trop. Med. Parasitol., 85 (6): 585-590.
- Ajaiyeoba, E., M. Falade, O. Ogbole, L. Okpako and D. Akinboye, 2006. *In vivo* antimalarial and cytotoxic properties of *Ammonia senegalensis* extract. Afr. J. Trad. CAM., 3 (1): 137-141.
- Atata, R.F., A. Sani and S.M. Ajawole, 2003. Effect of stem bark extracts of *Enantia chlorantha* on some clinical isolates. Biokemistry, 15 (2): 84-92.
- Azas, N., N. Laurencin, F. Delmas, C. Di Gogio, M. Gasquet, M. Laget and T.P. David, 2001. Synergistic *in vitro* antimalarial activity of plant extracts used as traditional herbal remedies in Mali. Parasitol. Res., 88 (2): 165-171.
- Baba, S., O. Akerele and Y. Kawguchi, 1992. Natural Resources and Human Health. Elsevier Tokyo.
- Benoit-Vical, F., A. Valentine, V. Cournac, M. Malli and J.M. Bastide, 1998. *In vitro* antiplasmodial activity of stem and root extracts of *Nauclea latifolia*. J. Ethnopharmacol., 7 (613): 173-178.
- Breeman, J.G., 2001. The ears of the hippopotamus: Manifestations, determinants and estimates of the malaria burden. Am. J. Trop. Med. Hyg., 64 (Suppl 1): 1-11.
- David, A.F., J.R. Philip, L.C. Simon, B. Reto and N. Solomon, 2004. Antimalaria drug discovery: Efficiency models for compound screening. Nat. Rev., 3: 509-520.
- Devi, C.U., N. Valecha, P.K. Atul and C.R. Pillai, 2000. Antiplasmodial effect of three medicinal plants: A preliminary study. Curr. Sci., 80 (8): 917-919.
- Dikaso, D., E. Makonnen, A. Debella, D. Abebe, K. Urga, W. Makonnen, D. Melaku, A. Assefa and Y. Makonnen, 2006. *In vivo* anti malarial activity of hydro alcoholic extracts from *Asparagus africanus* Lam in mice infected with *P. berghei*. Ethiop. J. Health Dev., 20 (2): 117-121.
- Elujoba, A.A., O.M. Odeleye and C.M. Ogunyemi, 2005. Traditional medicine development for medical and dental primary health care delivery systems in Africa. Afr. J. Trad. CAM., 21: 46-61.
- Gidado, A., D.A. Ameh and S.E. Atawodi, 2005. Effects of *Nauclea latifolia* leaves aqueous extracts on blood glucose levels of normal and alloxan-induced diabetic rats. Afr. J. Biotech., 4 (1): 91-93.
- Hilda, A.P., M. De La Rosa and M. Apitz, 1994. *In vivo* activity of Ajaene against Rodent malaria. Antimicrob. Agents Chemoth., 2 (38): 337-339.

- Ibeh, A.E. and I.M. Nwifo, 2005. Identification, collection and domestication of medicinal plants in South East Nigeria. *Afr. Dev.*, 3: 66-77.
- Milijaona, R., T.R. Valerie, R. Harrison, K.C. Peter, R. Michel, A.M. Dulcie and M. Phillippe, 2003. Plants traditionally prescribed to treat tazo (Malaria) in the Eastern region of Madagascar. *Malaria J.*, 2 (25): 239-241
- Peter, I.T. and V.K. Anatoli, 1998. The Current Global Malaria Situation. *Malaria Parasite Biology, Pathogenesis and Protection*. ASM Press. W.D.C., pp: 11-22.
- Peters, W., 1998. Drug resistance in malaria parasites of animals and man. *Adv. Parasitol.*, 41: 1-62.
- Sanon, S., N. Azas, M. Gasquet, E. Ollivier, V. Mahiou, N. Barro, N. Cuzin Quattara, A.S. Traore, F. Esposito, G. Balansard and P. Timon-David, 2003. Antiplasmodial activity of alkaloid extracts from *Pavetta crassipes* and *Achantospermum hispidum*, two plants used in traditional medicine in Burkina Faso. *Parasitol. Res.*, 90: 314-317.
- WHO, 1997. World malaria situation in 1994. Part 1-Population at risk. *Weekly Epidemiol. Rec.*, 72: 269-274.
- Wilcox, M.L. and B. Gerald, 2004. Traditional herbal medicine for malaria. *BMJ.*, 329: 1156-1159.
- Wongsrichanalai, C., A.L. Pickard, W.H. Wernsdorfer and S.R. Meshnick, 2002. Epidemiology of drug-resistant malaria. *Lancet Infect. Dis.*, 2: 209-218.