



Therapeutic potential of concurrent administration of *Hippocratea africana* and *Eremomastax speciosa* in the treatment of *Plasmodium berghei* infected mice

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Abstract

Context: Despite the commonness of polyherbal therapy among the locals in the treatment of malaria in Nigeria, there are no adequate data on the therapeutic potentials and safety profile of these herbal combinations. The use of these plants in combination in the treatment of suspected and confirmed malaria infection is very common among the Niger Delta dwellers in Nigeria.

Aim: To evaluate the therapeutic potential of co-administration of *Hippocratea Africana*, a medicinal plant with well documented antimalarial properties, and *Eremomastax speciosa*, a tropical plant with well reported antianaemic potential and haematoprotective properties.

Materials and Methods: Thirty albino mice, whose weights ranged between 32 - 37g, were divided into five groups having six mice in each. Clinical features, weight changes and parasite clearance were evaluated to determine therapeutic potential of treatments.

An inoculum which consisted of 5×10^7 *Plasmodium berghei* infested erythrocytes per ml of blood from a donor mouse with 64% parasitaemia was injected into each mouse by intraperitoneal route. The mice were kept at room temperature of $28.0 \pm 20^\circ\text{C}$ for 7 days for the parasite to develop. A non-parasitized mice group served as normal control. After parasitaemia was confirmed using standard procedure, 200mg/kg and 300mg/Kg body weights of *Hippocratea Africana* root bark and *Eremomastax speciosa* leaf extracts respectively, were administered by oral routes to the respective groups of mice for 6 days. A parasitized group was treated with fixed doses of 3mg/kg body weight of Artemether and 18mg/kg body weight of Lumefantrine. Another parasitized group was left untreated.

Results: Mice treated concurrently with the extracts of *H. africana* and *E. speciosa* showed a significant improvement in clinical signs in comparison to the untreated group. The mean body weights of mice administered both extracts was significantly ($P < 0.05$) increased when compared to the parasitized untreated mice and those treated with extracts separately. The mice treated concurrently with the two extracts also showed significant ($P < 0.05$) reduction in percentage parasitaemia and significant ($P < 0.05$) increase in percentage parasite clearance comparable to that of Artemether-lumefantrine. The parasitized untreated group recorded 50% mortality, while the group treated concurrently with the two extracts did not record any mortality.

Conclusion: Concurrent administration of *E. speciosa* crude leaf extract and *H. africana* ethanolic root extract had good therapeutic potential in the treatment of *Plasmodium berghei* infected mice. This justified the use of these extracts by Sub-Saharan African traditional medical practitioners and Nigerian Niger Delta rural dwellers in the treatment of human malaria.

Keywords: *Hippocratea africana*, *Eremomastax speciosa*, malaria, multiple herbal therapy

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Introduction

The burden and challenge of malaria treatment is as heavy and complex as the disease itself. A scale up in antimalarial efforts in sub-Saharan Africa and the tropics has been intensified, and various strategies for combating the new face of malaria are being developed.^{1,2} In recent years, infections by *Plasmodium* species have become more difficult to treat because the parasites have developed resistance to available drugs, and mosquitoes that transmit the disease causing parasites have also become resistant to insecticides.^{1,3} The multi-organ affection of the disease and its complex pathogenesis contribute enormously to the challenge of treatment.^{4,5,6} These have led to intensification of the quest for effective malaria treatment regimen and modality, especially in the face of co-infections and concurrent diseases.^{3,7} The use of artemisinin-based combination therapy (ACT) recommended by the World Health Organization has been limited by such reported factors as high costs, limited production of artemisinin derivatives, toxicity and parasite resistance.^{3,8,9,10} With the emergence of Chloroquine-resistant and multiple drug-resistant malaria parasites, development of new treatment modalities and new antimalarials to surmount these challenges becomes imperative.¹¹

Plants have always been considered to be possible alternative and rich sources of new drugs. Most of the antimalarial drugs in use today, such as quinine and artemisinin, were either obtained directly from plants or developed using chemical structures of plant-derived compounds as templates.¹² It is estimated that up to 80% of the world's population living in the developing world rely on medicinal plants products as a primary source of healthcare.¹³ Moreover, traditional medical practice which primarily involves the use of herbs is an integral part of the culture in those communities.¹³ In Sub-Saharan Africa, there are renewed interests in indigenous medicine, and even worldwide in the last

decade, arising from the realization of the limitations of orthodox drugs.^{11,12,14} An acquaintance with antimalarial plants may be a springboard for new phytotherapies that could be affordable to treat malaria, especially among the less privileged native people living in endemic areas of the tropics, mostly at risk of this devastating disease.^{15,16}

Hippocratea africana (Wild) Loess *Hippocrateaceae*, commonly known as African paddle-pod, inhabits the green forests and is a perennial climber with glabrous hairs and is widely distributed in tropical Africa, reproducing from seeds.^{3,17} The plant is known by various names in West Africa, including *nnoto* (Akan-Asante in Ghana), *onchom* (Mandyak in Guinea-Bissau), *njabo(li)* (Loko in Sierra Leone) *Rdelbi* (Fula-Pulaa in Senegal) and *kesayso* (Manding-Mandinka in Gambia).¹⁸ In Nigeria, the plant is called *godayi or gwad'ayi* in Hausa, *balandibi* in Fulfulde, *ipungwa* in Tiv, *ponju owiwi* in Yoruba and *mba* or *eba enang enang* in Ibibio languages.^{17,19,20} The Ibibios of the Niger Delta region of Nigeria use the root of the plant in the treatment of various ailments such as fever, malaria, body pains, diabetes and diarrhea.^{17,21}

The root is also used traditionally as an antipoison or antidote to treat liver diseases.²⁰ The plant has been shown to contain significant quantities of phytochemicals such as alkaloids, cardiac glycosides and flavonoids, tannins and flavonoids as the major constituents.²² The root of *H. africana* has been reported to possess *in vivo* antiplasmodial activity with LD50 of 2.45g kg⁻¹.¹⁶ The blood schizontocidal activity and chemosuppressive effect, both in early and established infection in mice, were comparable to chloroquine at 5mg/kg.²⁰

Eremomastax speciosa (Hochst) *Acahaceae* is a tropical stout erect multi-branched herb that grows as a weed in the forest.^{19,23} The plant is commonly known as blood tonic plant in southern Nigeria, *Edem iduduot* (purple back) by the Ibibios and *Ikpo ikong* by the Efiks in Akwa Ibom and Cross River States respectively.²⁴ Due to its numerous medicinal values, it is now grown in farmlands around living houses.²⁴ The plant is a perennial plant that orderly distributed in tropical Africa, from West Africa through Central African Republic, North Congo-Kinshasa to South Sudan and South West Ethiopia and Madagascar.²⁵ The crude leaf extract of *E. speciosa* is locally used in southern Nigeria as

enema to treat splenic disorders, fever and internal heat (especially in pregnant women). Leaf extract is also drunk as an anti-anaemic.^{24,26} The Douala people of Cameroon employ *E. speciosa* variously for treatment of malaria, kidney pains, scabies, anaemia, diabetes, and nerves pain.²⁷ Ethanolic crude leaf extract was demonstrated to possess antianaemic property,^{24,28} and improved haematological indices in plasmodium infected mice.¹⁹ Earlier published worked showed that administration of the Artemether-Lumefantrine with *E. speciosa* leaf extract may relieve plasmodium-induced anaemia and malaria-induced immunosuppression.³

Despite the commonness of multiple herbal therapy among the locals in the treatment of malaria In Nigeria, there are no adequate data on the therapeutic potentials and safety profile of these herbal combinations. Our present study evaluated the therapeutic potential of co-administration of *Hippocratea Africana*, a medicinal plant with well documented antimalarial properties, and *Eremomastax speciosa*, a tropical plant with the well-reported antianaemic potential.

Materials and methods

Collection and identification of plant material

The roots of *Hippocratea africana* (Willd) Loes and fresh leaves of *Eremomastax speciosa* were harvested from their natural habitats and were identified and authenticated by a taxonomist in the Department of Botany, University of Uyo, Nigeria. The roots of *H. africana* were washed with clean water and the bark scrapped with a sharp knife, sun dried and crushed with a mortar into pellets. The pellets were blended into powdered form using an electric blender. About 500g of the powdered *H. africana* root bark was blended in 1000ml of 80% ethanol. It was left overnight to achieve a good extraction. The mixture was filtered and the filtrate was concentrated in vacuo at 40°C to obtain a dry crude extract which could dissolve homogeneously in normal saline and distilled water. The leaves of *Eremomastax speciosa* were washed, deveined and blended with an electric blender. About 3kg of the sample was macerated with 4000ml of tap water. The mixture was sieved and filtered to remove the residue. The filtrate was concentrated in a water bath at 40°C to obtain a greenish black crude extract which dissolved

completely in distilled water and normal saline to give a homogeneous solution. The extracts were stored in the refrigerator at 4°C and used in this study.

Inoculation of experimental mice with *Plasmodium Berghei*

Albino mice were obtained from the Animal House of the Faculty of Basic Medical Science, College of Health Sciences, University of Uyo. A donor mouse was obtained from the Faculty of Pharmacy, University of Uyo, Uyo. Ethical clearance was obtained from the Faculty of Basic Medical Science Animal Ethics and Research Committee, College of Health Sciences, University of Uyo, Uyo, Nigeria, with number RP/REC/15/01.

Thirty albino mice that weighed between 32 - 37g, were divided into five groups having six mice in each. About 0.2ml of infected blood obtained from donor mouse was mixed with 20ml of normal saline, from where 0.2ml of the mixture, which contained about 1.0×10^7 *Plasmodium berghei* parasitized erythrocytes, was injected into each mouse by intraperitoneal route. The inoculum consisted of 5×10^7 *P. berghei* infested erythrocytes per ml of blood from the donor mouse with 64% parasitaemia. A non-parasitized mice group served as normal control. The animals were fed *ad libitum* with rat chow and water, and kept at room temperature of 28.0 ± 2 °C for the period of seven days for the parasite to develop.^{3,22} On the eighth day, thick films were prepared from blood collected through tail puncture of the parasitized animals to ascertain parasitaemia using the method earlier described.³

Experimental design and treatment of experimental animals

Based on already established safety dosages of the plants extracts by various scholars.^{17,21,29} 200mg/kg and 300mg/Kg body weights of *H. africana* root bark and *E. speciosa* leaf extracts respectively, were administered by oral routes to the respective groups of mice for 6 days. The extracts were sustained in 0.5ml of solvent and carefully administered with the aid of a cannula to avoid spillage. A group was administered the therapeutic doses of 3mg/kg body weight of Artemether and 18mg/kg body weight of Lumefantrine daily for 6 days. The untreated control groups were administered normal saline.

Clinical observation of mice

All the mice were monitored visually for behavioural changes and signs of illness which included lethargy, piloerection, decreased locomotor activity and diarrhea. Any signs of illness observed were quantified using arbitrary scale and recorded as either absent (-), mild (+), moderate (++) or severe (+++). Pre-treatment and post-treatment weights were measured.

Determination of parasitaemia

A drop of blood was collected from the mice by tail-puncture and transferred onto the edge of a microscope slide (single, 76 × 26 mm thickness) and drawn evenly across a second slide to make a thin

blood film and allowed to dry at room temperature. The smear was stained with Leishman stain and examined under light microscopy with oil immersion (x1000 magnification). Parasitaemia of the mice were determined using standard procedure as earlier described.³

Statistical analysis

Standard computerized statistical tools were used in the analysis of the results obtained. All data were expressed as mean ± standard deviation (SD). Analysis of Variance was used to analyze data, while Student's t-test was used for comparison. Any difference in mean was considered significant at $P < 0.05$

Table 1: Clinical features of *Plasmodium berghei* infected mice treated with *H. Africana*, *E. speciosa* and artemether-lumefantrine

Group	Treatment	Lethargy	Piloerection	Tail/Pinnae pallor	Decreased Locomotor	Diarrhea
I	Normal Control	-	-	-	-	-
II	Parasitized Untreated	+++	+++	+++	+++	-
III	AL	+	+	++	+	-
IV	<i>H. africana</i>	++	+	++	+	-
V	<i>E. speciosa</i>	++	++	++	++	-
VI	<i>H. africana</i> + <i>E. speciosa</i>	+	+	+	+	-

(-) = Absent, (+) = Mild, (++) = Moderate, (+++) = Severe, AL = Artemether-Lumefantrine

Table 2: Mean body weights of *Plasmodium berghei* infected Mice treated with *Eremomastax speciosa* leaf and *Hippocratea africana* root extracts

Group ^e	Treatment	Initial MBW (g)	MBW before treatment (g)	MBW after treatment (g)	MBW change after treatment (g)	% MBW change After treatment
I	Normal Control	31.85±1.85	32.27 ± 1.52	34.40±1.65	2.13 ± 0.41	6.6 0± 1.02
II	Parasitized Untreated	31.50±1.34	30.12 ± 2.12	25.65±1.80	-4.47±0.52 ^a	-14.84 ± 0.74
III	AL	30.80±1.55	28.46 ± 1.35	31.53±1.67	1.07±0.74 ^{a,b}	3.76 ± 0.24 ^{a,b}
IV	<i>E. speciosa</i> Only	32.45±1.58	30.09 ± 1.47	29.11±1.28	-0.98±0.09	-3.26 ± 1.08 ^{a,b}
V	<i>H. africana</i> Only	30.20±1.10	28.52±1.75	29.08±1.48	0.56±0.05 ^{a,b}	1.96 ± 0.09 ^{a,b}
VI	<i>E. speciosa</i> + <i>H. Africana</i>	30.85±1.22	29.30 ± 1.21	30.95 ± 1.67	1.65±0.19 ^b	5.63 ± 1.06 ^b

e = Mean ± Standard Deviation, a = significantly different when compared with normal control (administered normal saline) at $p < 0.05$, b = significantly different when compared with test group II (parasitized untreated) at $p < 0.05$, ACT = Artemether-Lumefantrine, BW = Body weight

Table 3: Levels of parasitaemia, parasite clearance and mortality in *Plasmodium berghei* infected Mice treated with *Eremomastax speciosa* leaf and *Hippocratea africana* root extracts

Group ^c	Treatment	Before Treatment %	After Treatment %	Percentage Parasitaemia	Parasite Clearance %	Mortality	Percentage Mortality
I	Normal Control	0.00	0.00	0.00	0.00	0.00	0.00
II	Parasitized untreated	23.00 ± 3.12	42.00 ± 5.10	182.61 ± 3.54	-86.36 ± 2.63	3.00	50.00
III	AL	29.50 ± 5.02	2.00 ± 0.05 ^a	6.78 ± 0.33 ^a	93.10 ± 3.63 ^a	0.00	0.00
IV	<i>E. speciosa</i> Only	25.10 ± 3.45	16.00 ± 2.27 ^{ab}	63.75 ± 3.11 ^{ab}	30.00 ± 3.72 ^{ab}	2.00	33.33
V	<i>H. africana</i> Only	21.00 ± 2.80	3.75 ± 4.40 ^a	17.56 ± 2.71 ^{ab}	82.15 ± 2.84 ^a	1.00	16.67
VI	<i>E. speciosa</i> + <i>H. Africana</i>	27.15 ± 2.18	2.89 ± 2.45 ^a	10.64 ± 1.31 ^{ab}	89.36 ± 2.11	0.00	0.00

e= Mean ± Standard Deviation, a = significantly different when compared with test group II

(Parasitized untreated compared with test group III (AL) at p < 0.05, AL = Artemether-Lumefantrine

Results

At the end of treatments, clinical examination of the parasitized untreated mice (group II) showed severe lethargy with marked piloerection (Table 1). The mice clustered together at the corner of their cages, with marked decrease in locomotor activity. The tail and pinnae were markedly paler compared to the normal animals (Group I). The remnants of food in the containers were markedly increased compared with that of the normal non-parasitized mice. There was no evidence of passage of watery stool. Mice treated with combination of *E. speciosa* and *H. africana* (Group VI) showed negative lethargy and piloerection. There were increased locomotor activities in comparison with parasitized untreated group. Clinical features of treatment groups were as shown in Table 1.

As shown in Table 2, there was a significant (P < 0.05) reduction in the mean body weight of the parasitized untreated mice when compared with non-parasitized normal control. The parasitized untreated group showed a negative percentage body weight change, which was significantly (P < 0.05) lower than that of *E. speciosa* treated mice. All other treatment groups showed positive percentage weight change at the end of treatment. There was significant (P < 0.05) increase in mean body weight of mice treated separately with *E. speciosa* and *H. africana* extracts. The mean body weight of mice treated with the combination of *E. speciosa* and *H.*

africana was significantly (P < 0.05) increased when compared to the parasitized untreated mice and *E. speciosa* treatment alone. The mean body weight of the mice treated with the combination of the two extracts was slightly higher (P > 0.05) than that treated with Artemether-lumefantrine.

As shown on Table 3, the untreated parasitized mice showed significantly (P < 0.05) increased percentage parasitaemia at the end of treatment compared to that before treatment. Mice treated separately with *E. speciosa* and *H. africana* showed significant (P < 0.05) reduction in percentage parasitaemia and significant (P < 0.05) increase in percentage parasite clearance compared to the parasitized untreated mice. Percentage parasitaemia for *E. speciosa* treatment only was significantly higher than that of Artemether-lumefantrine, with significantly reduced parasite clearance compared to AL. The mice treated concurrently with the two extracts showed significant (P < 0.05) reduction in percentage parasitaemia and significant (P < 0.05) increase in percentage parasite clearance comparable to that of artemether-lumefantrine. The parasitized untreated group recorded 50% mortality, while the group treated concurrently with the two extracts did not record any mortality, just like the group treated with AL.

Discussion

Treatment of malaria remains a major challenge in Sub-Saharan Africa where the disease is endemic.

Search for therapeutic agents that are efficacious, available and affordable intensifies, especially in the face of obvious resistance against the available standard regimen and global economic recession. Resort to medical plants in the treatment of malaria has surged in recent times. In our study, regression of clinical signs, body weight recovery and parasites clearance from blood were used in assessing therapeutic efficacy of concurrent administration of the two commonly used plants extracts.

Effect of treatments on clinical signs

The pretreatment parasitized mice showed clinical signs consistent with infection by *Plasmodium* species. The clumping together of the mice at the corner of the cages, reduced locomotor activities and increases in food remnants were clinical manifestation of hypothermia, malaise and anorexia respectively, usually associated with *Plasmodium* parasitaemia in mice.^{3,30} Paleness of the pinnae and tails of the parasitized untreated mice may be as result of anaemia and reduced haemoglobin, which correlates with earlier reports.²⁴ *Plasmodium berghei* infection in mice is a well-employed animal model used in malaria research, and this includes analyses on the severe pathology associated with malaria infections.^{4,30} All treated parasitized groups showed some improvement of clinical signs of anaemia, evidenced by slight regression of paleness of pinnae and tails. The actions of the two extracts administered separately in relieving pinnae and tail paleness were similar to that of artemether-lumefantrine. However, parasitized mice administered the two extracts concurrently showed significant regression of pinnae and tail paleness. This implies that the combination of the extract was more efficacious in relieving parasite-induced anaemia than AL or the extracts administered separately. This may be attributed to the direct synergistic stimulation on haemopoietic tissues such as the liver and bone marrow by the phytochemicals in the plant extracts.^{3,31,32} Extract of *E. speciosa* has been reported to possess antianaemic potentials attributed to alkaloids in the herbs, which stimulate the phosphorylation of proteins, hence increased haematopoiesis.^{20,24,33} Concurrent administration of the plant extracts also produced significant improvement in piloerection, decreased locomotor and lethargy, and was as

effective as artemether-lumefantrine administration. Administrations of the extracts separately were less effective in making these clinical signs to regress. Piloerection, lethargy and decreased locomotor may have been due to hypothermia, electrolytes imbalance as a result of reduced food intake and dehydration, which are characteristic derangements in *Plasmodium* parasitaemia. Rich anti-inflammatory and antioxidant properties of *E. speciosa* reported are believed to compliment the strong antiplasmodial property of *H. africana* in causing the regression of these clinical signs.^{34,35}

Effect of treatments on body weight

Parasitized untreated mice exhibited significant acute weight loss, which was consistent with reports of other scholars.^{36,37} This has been attributed to diminished food intake due to parasite-induced anorexia. A study also reported gastric mucosal lesions in plasmodium infected mice, which was associated with sudden decrease in food intake, causing weight loss.³⁶ Parasitized mice treated with *E. speciosa* leaf extract only did not exhibit appreciable weight increase probably due to poor activity against the parasites. Treatment with *H. africana* root extract only brought about a significant positive weight changes. *H. africana* has been reported to exhibit excellent antiplasmodial activity.^{17,20} Parasitized mice concurrently administered *H. africana* and *E. speciosa* extracts exhibited excellent weight gain. The parasitized mice administered the two extract gained more weights in comparison with those administered either Artemether-lumefantrine or *H. africana* alone. *Eremomastax speciosa* leaf extract has been demonstrated to possess strong anti-inflammatory, antioxidant and antianaemic activities.^{3,20,24,34,35} Potent phytochemicals reported in the extracts may have protected the gastric mucosa against inflammatory cytokines and edema which are believed to cause anorexia, reduction in food intake and weight loss.³⁶ Onaja et al reported that *E. speciosa* exhibited significant antioxidant activities,³⁵ which relieved plasmodium infected mice of parasite-induced oxidative stress capable of inducing gut mucosal injuries, reduce food intake and weight loss. This probably justifies the folkloric use of *Eremomastax speciosa* leaf in the

management of malaria and febrile illnesses in traditional medicine. *H. africana* may have provided the necessary suppressive and antiplasmodial action which facilitated body weight recovery.¹⁷

Effect of treatment on parasitaemia

Treatment of the parasitized mice with *E. speciosa* leaf extract only did not achieve a satisfactory antiplasmodial action, leaving a residual parasitaemia of 63.75 ± 3.11 per cent, with a minimal parasites clearance of 30.00 ± 3.72 only. This implies that *E. speciosa* extract only is not efficacious enough for parasites elimination. Treatment with *H. africana* root extract only, however, yielded excellent parasite elimination that was comparable to that of AL. Antiplasmodial activity of *H. africana* has been well reported by various researchers.^{3,17,20,36} A study showed that ethanolic root extract of the plant demonstrated blood schizontocidal activity, both in early and established infection at oral doses of 200 to 600 mg/kg/day in mice. The chemosuppressive effect of *H. africana* at 400 and 600 mg/kg were 81.8 and 90.9%, and was comparable to that of chloroquine.²¹ *H. africana* was reported to contain significant quantities of phytochemicals such as alkaloids, cardiac glycosides and flavonoids, tannins and flavonoids as the major constituents.²² Concurrent administration of *H. africana* and *E. speciosa* demonstrated higher parasite clearance than *H. africana* only. The methoxylated flavones artemetin and casticin were reported to demonstrate synergistic action with Artemisinin, and flavanoids present in *Artemisia annua* was considered to probably contribute to the antimalarial action of extracts or herbal teas prepared from this species.³⁷ Hence, the increased antiplasmodial activities of the combined extract may be due to synergistic action of the phytoconstituents in the plants.

Conclusion

Concurrent administration of the two plants extracts demonstrated excellent antiplasmodial activity that was comparable the standard antimalarial drug Artemether-lumefantrine, and better than the extracts administered separately. The higher antiplasmodial action was probably due to the synergistic action of the phytochemicals in the plant

extracts.

Clinically, signs of plasmodium parasitaemia regressed significantly upon concurrent administration of the two plant extracts. The animals showed a significant recovery from acute weight loss, clinical signs of anaemia, hypothermia and weakness, upon concomitant treatment with the two plants extracts. It was concluded that concurrent administration of *E. speciosa* crude leaf extract and *H. africana* ethanolic root extract exhibited good therapeutic potential in treatment of plasmodium berghei infected mice, probably due to synergistic antiplasmodial action of the phytochemicals in both extracts. This justifies the use of these extracts by Sub-Saharan African traditional medical practitioners and Nigerian Niger Delta rural dwellers in treatments of human malaria. However, there is the need for further research to elucidate the active antiplasmodial constituents of these plants and their actual mechanisms of action.

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