In Vivo Activity of Ethanolic Leaves and Stem Bark Extracts of *Alstonia boonei* Against *Plasmodium berghei* in Infected Mice

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Abstract

Malaria is commonly associated with poverty and also a cause of poverty. It is a major hindrance to economic development of tropical and subtropical regions, including parts of America, Asia and Africa. Recent estimates revealed that more than 3.2 billion people are at risk of malaria, with 200 million infections per year, while approximately 0.6 million deaths per year were recorded according tothe World Health Organization (WHO).This has led to investigations of the potential of natural product-based chemotherapeutic agents to ameliorate the effects of malaria and also in anticipation that new drugs may certainly emerge from the tropical plant sources. The ethanolic extracts of *Alstonia boonei* leaves and stem were used for this study; qualitative phytochemical analysis was carried out alongside acute toxicity and suppressive effect. The mice were administered 200mg/kg, 400mg/kg and 800mg/kg body weight of leaf and stem bark extracts separately, positive control mice were given standard drug chloroquine (5mg/kg) and the negative control were given distilled water. Phytochemical analysis showed that the stem and leaves of *Alstonia boonei* had some compounds in common and also had some differences. After 24 hours of acute toxicity, the test mice neither died, showed any sign of toxicity nor any strange behavior. This result showed that the *Alstonia boonei* was safe and the lethal dose was >5000mg/kg. The suppressive treatment showed that the leaf and stem bark extracts of *Alstonia boonei* were dose independent. The stem extract of *Alstonia boonei* was more effective at the dose of 200mg/kg body weight and the leaf extracts were more effective at the dose of 400mg/kg body weight. The standard drug (chloroquine) used for both test extracts gave 100% suppression. Comparatively, the standard drug at certain dose level was more effective compared to both extracts.

Introduction

Malaria is globally a destructive and dangerous infectious disease in developing countries (Greeenwood *et al*., 2005*;* Winter *et al., 2006*),It is caused by parasitic transmission of anopheles mosquitoes bite and is characterized by energy loss, debilitation, loss of work capacity and economic damage (Sachs and Malaney 2002). WHO(2014) estimated 198 million cases of malaria and 584,000 deaths in children under the age of 5, furthermore among the affected and most studied were children within the age bracket of 2 and 12 years old in Africa with the highest death rate (Crompton *et al.,* 2014).

Plasmodium falciparum and *Plasmodium malariae* are the two most common causes of malaria in Nigeria. The malaria parasites are transmitted all

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year round in the south, east and south-south parts of Nigeria while it is seasonal in the north of Nigeria (Adebayo and Kretti, 2011).The Federal Government of Nigeria spends billions of naira annually for treatment since it is equally the leading cause of death in pregnant women (Government in action, 2005). *Plasmodium berghei* parasites are usually used in predicting treatment outcomes of any suspected antimalarial agent due to its high sensitivity to chloroquine with positive outcome (Iwu *et al*., 1999). Due to limited availability and/or affordability of pharmaceutical medicines in many tropical countries, the majority of the population depends on traditional medical remedies (WHO,2002; Zirihi *et al.,*2005).The rate of death numbers recorded and caused by malaria is increasing due to mosquito resistance to chemical insecticides, thus it is a huge challenge to mankind (Kamalinder *et al.,* 2008). Plants have always been considered to be a possible alternative to and a rich source of new drugs, indeed most of the antimalarial drugs in use today such as quinine and artemisinin were either obtained directly from plants or were developed using chemical structures of plant-derived compounds as templates (Basco *et al.,*1994). *Alstonia boonei* plant has been found to be effective in the treatment of several diseases such as fever, painful micturition, insomnia, chronic diarrhea, rheumatic pains,and have nephrotoxicological effect, anxiolytic and neuroleptic properties (Ojewole, *1984;* Asuzu and Anaga, 1991*;*Olajide *et al.,* 2000; Elizabetsky and Costa-Campos, 2006;Oze *et.al*., 2007; Odeku *et al.,* 2008). Olajide *et al., (*2000) also reported that the stem bark of *A.boonei* has anti-inflammatory, anti-pyretic and analgesic properties. Additionally, it contains minerals such as magnesium, sodium, calcium, phosphorus, and iron (Elijah *et al.,* 2010). Studies have revealed that the bark and leaves of *Alstonia boonei* possesses antibiotic and antimicrobial properties (John-Prosper *et al.,* 2012). Furthermore, the stem barks have been found to possess anti-diabetic properties (Akinloye *et al.,* 2013). *A.boonei* as an antimalarial drug is the focus of this research.

Materials and Methods

Plant materials and extraction

Fresh leaves and barks of *Alstonia boonei* were obtained from the botanical garden of the University of Lagos, Akoka (Lagos, Nigeria). Identification and authentication (LUH 6309) were done at the Herbarium, in the Department of Botany, Faculty of Science at the University of Lagos. The samples were air-dried at room temperature under shade for two weeks and ground into fine powder using an electric mill. The powder was stored in an air-tight vessel at room temperature.

The crude extracts of *Alstonia boonei* leaves (100g) and stem bark (100g) were prepared by cold maceration technique (O'Neill *et al.,* 1985). Ethanol (70%) extract of each specimen was prepared. The extraction was done by weighing 100g of each plant specimen in 1000ml of ethanol and soaked for 48hours. The mixture was filtered using Whatman filter paper (No.3, 15cm size with retention down to 0.1µm in liquid). The filtrates were evaporated to dryness in a water bath at 40^{o}C. The extracts were used for analysis.

Experimental Animals

Thirty female mice with different weights of 16g to 28g were used for the study. The animals were obtained from the animal unit of the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos State, Nigeria. They were housed in stainless steel wire mesh cages up to a maximum of 5 per cage, in a well-ventilated room. They were allowed to acclimatize for two weeks before experimentation. All animals had regular supply of clean drinking water and food.

Animal Grouping

The 30 female mice were grouped into 2 groups, one for the application of stem extract and the other for leaf extract. Each group was further divided into 3 sub groups of 5 mice per cage. Groups A, B and C for each extract were administered at 300mg/kg, 2000mg/kg and 5000mg/kg body weight respectively. Each cage was identified by a tag. This tag stated the weight

of the animals it contained, test substance code and administration dose level.

Screening of Phytochemical Components

Phytochemical components were analyzed through qualitative chemical test (Harborne 1973 and Sofowora 1993).

Acute Toxicity Test

The crude extracts were evaluated for toxicity in *P. berghei* non-infected female albino mice with different weights of 16g to 28g. For the test of the extract,30 mice were randomly divided into six groups of five animals per cage. Before oral administration of a single dose of each extract, the mice were not fed for two hours (WHO 2000; OECD 2001). Then, 0.2ml of *Alstonia boonei* leaves and stem extract were administered separately at 300mg/kg, 2000mg/kg, and 5000mg/kg body weight and monitored for 24 hours (CDER 1996).The mice were observed for gross behavioral changes such as in hair, excretion, lactation, mortality and other manifestations (Pillai and Santhakumari 1984).

The Parasite and Infection

The NK65 strain of *Plasmodium berghei* which is sensitive to Chloroquine used for this study was obtained from the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos State. The parasites were maintained by serial passage of blood from infected mice to non-infected ones. To infect the mice, blood samples were collected from auxiliary vessels of a donor mouse with a rising parasitaemia of 37%, blood sample was then diluted with normal saline. Each mouse was passaged with 0.1ml of the infected blood containing about 1×10^{6} *Plasmodium berghei* parasitized red blood cells via intraperitoneal route (IP).

Antimalarial Activity of Plant Extract

A four (4) day standard suppressive test was adopted(Peter *et al.,* 1975), and used for this study. 50 female albino mice with different weights of 16g to 28g were divided into groups of five (5) with each group having five (5) animals in a cage for each extract. Group A served as a negative control and was given distilled water while groups B, C, and D were administered the extracts at 200mg/kg, 400mg/kg and 800mg/kg respectively and the last group E was given chloroquine, the chloroquine was used as a positive control. The animals were administered with the extract 2 hours after the inoculation of the parasite on day 1 (D1) and everyday till day 3 (D3) using an oral cannula (Plant extracts were administered orally). All animals had regular supply of clean drinking water and food (standard mice food). On the fourth day, a thin blood film was made from the tail of each mouse. The smear was prepared by spreading the blood on a clean slide over an area of 1.5 cm × 2.5 cm, allowed to dry and fixed with methanol, stained with 3ml of 3% Giemsa stain for 45 minutes and examined with microscope under the oil immersion objective to determine the parasite density microscopically (Olympus CX, Japan) at 100x times magnification. This is to monitor the level of parasitaemia. The suppression of parasitaemia in relation to the control was assessed using the recommended formula.

$$\text{Average (Av) \% suppression} = \frac{\text{Av\% parasitaemia in negative control} - \text{Av\% parasitaemia in test group} \times 100}{\text{Av\% parasitaemia in negative control}}$$

Statistical Analysis

Data were expressed as mean ± standard error using SPSS version 15.

Results

Phytochemical screening result revealed that ethanolic extract of *Alstonia boonei* stem bark contains alkaloids, flavonoids, saponins, phlobatannins, anthraquinones, steroids and reducing sugars. Ethanolic extract of *Alstonia boonei* leaves contain tannins, alkaloids, cardiac glycosides, reducing sugar, saponins, anthraquinones and steroid.

Table 1: Phytochemical constituents of *Alstonia boonei* extract

Constituents	Observation	
	Stem extract	Leaves extract
Flavonoid	+	–
Tannin	–	+
Alkaloid	+	+
Cardiac glycosides	–	+
Steroid	+	+
Reducing sugar	+	+
Saponin	+	+
Anthraquinone	+	+

+ = presence of compound; - = absence of compound

Acute toxicity

The acute toxicity study indicated that none of the two extracts caused mortality of mice within 24 hrs. The behavioral and physical observation of the experimental mice showed no sign of acute toxicity, the skin fur, lethargy, salivation and eyes were all normal at 5000mg/kg slight weaknesses was observed for about 30 minutes and were later physically active.

Antimalarial Activity of Plant Extract

The antimalarial experimental results indicated that ethanol extracts of *Alstonia boonei* stem and leaves possess intrinsic anti-plasmodial activity that was evident from the suppression they produced during the 4- day suppressive test. The ethanolic extract of *Alstonia boonei* stem showed the highest suppression of 55.4% at 200mg/kg body weight of mice while the mean parasitaemia count of the test groups were 3.3 ± 0.51 to 6.2 ±2.75 to 6.0 ± 2.75 respectively and the negative control was 7.4 ± 3.16 (Table 2). *Alstonia boonei* leaves extract showed the highest (43.1%) suppression of parasitaemia at 400mg/kg body weight in mice (Table 2). Percentage suppression was observed to indicate the exact increase in concentration, after four days treatment with different doses, the mean parasitaemia count of the test groups ranged from 5.1 ± 3.30 to 3.4 ± 1.62 to 5.2 ± 3.45.

Table 2: Activity of ethanolic extract of *Alstonia boonei* against *P. berghei* in mice

Extract %Parasites Count	Dose (mg/kg/day) % Suppression	Antimalarial activity	
Alstonia boonei stem	NC	7.4 ± 3.16	0.00
200 3.3 ± 0.51	55.4%		
400 6.2 ±2.75	16.2%		
800 6.0 ± 2.75	18.9%		
CQ0.00100%			
Alstonia boonei leaf	NC	6.6 ± 2.35	0.00
200 5.1 ± 3.30	23.1 %		
400 3.4 ± 1.62	43.1%		
800 5.2 ± 3.45	20.0%		
CQ 0.00100%			

Values are Mean ±SE; n=5, NC: Negative control, CQ: chloroquine

Discussion

The ethanolic extracts of *Alstonia boonei* leaves and stem bark were tested for toxicity against albino mice and their antimalarial activity against NK65 strain of *Plasmodium berghei* in mice. After 24 hours, they showed no sign of toxicity or weakness or death at the various doses employed (300mg/kg, 2000mg/kg and 5000 mg/kg). This means that the extracts were safe and the lethal dose (LD50) was>5000mg/kg (CDER 1996). However, there was mild weakness for those treated at 5000mg/kg stem extract and leaves extract for about 30 minutes then they became active. The observed mild weakness of the treated mice showed that the extract possesses central nervous system depressant effect, consistent with previous studies (Awe and Opeke, 1990; Olajide *et al*., 2000; Odugbemi and Akinsulire, 2007; Bello *et al*., 2009; Idowu *etal*., 2010; Gbadamosi *et al*., 2011). Furthermore, the extract showed no toxic manifestation from the acute toxicity study which shows the test extract were non-toxic on the mice.

Ethanolic extracts of *Alstonia boonei* leaves possess moderate antiplasmodial activity. The suppressive effect of the leaf extract may be linked to some phytochemical compounds like tannins, phlobatannins, alkaloids, cardiac glycosides, reducing sugar, saponins, anthraquinones and steroids. *In vivo* suppression of the extract showed average % suppression of 23.1%, 43.1% and 20.0% for leaf extract applied at 200, 400 and 800 mg/kg body weight respectively against *Plasmodium berghei.* Studies have revealed that the leaves are effective in treatment of hypertension and analgesic activity (Irvine *et al.,* 1961; Iyiola *et al*., 2011).

The result obtained from ethanolic extracts of *Alstonia boonei* stem shows that it contains alkaloids, flavonoids, saponins, phlobatannins, anthraquinones, steroids and reducing sugars these could be responsible for the antimalarial efficacy (Oigiangbe *et al*., 2010). The extract manifested a significant reduction in suppression with average % suppression of 55.4%, 16.2% and 18.9% for stem bark respectively as compared with negative control. The stem bark is reported to be a remedy for inflammation and anti-pyretic agent and diarrhea in human infection (Oliver-Bever*et al.,* 1986; Olajide and Awe *et al*., 2000), anti-venom for snake bites and in the treatment of arrow poisoning (Faparusi and Bassir, 1972; Oliver-Bever *et al.,*1986; Asuzu and Anaga, 1991; Kweifo-Okai *et al*., 1995).

In this study, both the leaf and stem extracts of *Alstonia boonei* manifested antiplasmodium properties, the suppression caused by both extracts were significantly greater than the value obtained from the negative control.

Comparatively, the antimalarial efficacy of *Alstonia boonei* stem extract is greater than those of the leaves extract but the standard antimalarial drug (chloroquine, 5mg/kg) at certain dose level was significantly greater than both test extracts. This might indicate that the active compounds for the observed antimalarial activities of the two extracts are different, the better performance observed for chloroquine compared to the extracts maybe as a result of the inability of the parasites to develop as much, it may result from differences in the chemical composition of the extracts compared to chloroquine .

Conclusion

The study established suppressive activity of the leaf and stem bark extracts against *Plasmodium berghei* indicating that this plant contains some antiplasmodial compounds. However, further research should be done in order to consider them as potential sources for antimalarial drug development for human malaria.

References

Akinloye, O.A., Oshilaja, R.T., Okelanfa O.A., Akinloye, D.I and O.M.O Idowu (2013).Hypoglyceamicactivity of *Alstonia boonei* stem bark extract in mice. *Agriculture and Biology Journal of North America* 45: 2151-7517.

Asuzu.I.U and Anaga. A.O.(1991). Pharmacological screening of the aqueous extract of *Alstonia boonei* stem bark. *Fitoter.* 63:411-417.

Awe.S.O, Opeke.O.O. (1990). Effects of *Alstonia congensis* on *Plasmodium berghei* in mice. *Fitoter*.61:225-229.

Basco.L.K, Mitaku.S, Skaltsounis.A.L, Ravelomanaintsoa. N, Tillequin. F, Koch.M and Le Bras. J.(1994). *In vitro* activities of acridone alkaloids against *Plasmodium falciparum. Antimicrobial Agents Chemother.* 38:1169-1171.

Batista.R, Junior.A.J.S.and A.B. de Oliveira, (2009). Plant-derived antimalarial agents: New leads and efficient phytomedicines. Part II. Non-Alkaloidal natural products. *Molecules* 14: 3037-3072.

Bello.I.S, Oduola.T, Adeosun.O.G, Omisore.N.O.A, Raheem. G.O. and Ademosun. A.A. (2009). Evaluation of antimalarial activity of various fractions of *Morindalucida* and *Alstonia boonei* stem bark. *Global J. Pharmacol.*3(3):163-165.

Center for Drug Evaluation and Research (1996).Guidance for industry single dose acute toxicity testing for chemicals.

Crompton PD, Moebius J, Portugal S, Waisberg M, Hart G, Garver LS.(2014) Malaria immunity in man and mosquito: insights into unsolved mysteries of a deadly infectious disease. *Annu Rev Immunol.;* 32:157–87.

Delmas.F, Di Giorgio.C, Elias.R.(2000). Antileishmanial activity of three saponins isolated from ivy, α-hederin, β-hederin and hederacolchiside A1, as compared to their action on mammalian cells cultured *in vitro. Planta Medica.* 66(4): 343-347.

Elisabetsky.E and Costa-Campos.L. (2006). The alkaloid alstonine: A review of its pharmacological properties. *Evidence-Based Complementary and Alternative Medicine.*3(1):39-48.

Elijah, A.I., Ojimelukwe, P.C., Ekong,U.S and Asamudo, N.U (2010). Effect of *Sacoglottisgabonesis* and *Alstonia boonei* on the kinetics of *Saccharomyces cerevisae* isolated from palm wine. *Afr. J. Biotechnol.* 9(35): 5730-5734.

Ene. A.C, Ameh.D.A, Kwanashie.H.O, Agomuo. P.U and Atawodi.S.E. (2008). Preliminary invivo antimalarial screening of petroleum ether, chloroform and methanol extracts of fifteen plants grown in Nigeria. *Journal of Pharmacol Toxicology* 32:254-260.

Faparusi.S.I and Bassir.O.(1972). Triterpines from *Alstonia boonei. Phytochemistry.* 21:3083-3084.

Gbadamosi.I.T, Moody.J.O and Lawal.A.M. (2011). Phytochemical screening and proximate analysis of eight ethnobotanicals used as antimalarial remedies in Ibadan. *Nig. J. Appl. Biosci.* 44: 2967-2971.

Greenwood.B. (2004).The use of anti-malarial drugs to prevent malaria in the population of malaria-endemic areas. *Am J Trop Med Hyg.* 70 (1):1-7.

Harborne, J.B., (1973). Phytochemcial methods London, Chapman and Hall, Ltd., pp: 49-188.

Idowu. O.A, Soniran.O.T, Ajana. O and Aworinde. D.O.(2010). Ethnobotanical survey of antimalarial plants used in Ogun State, Southwest, Nigeria. *African Journal of PharmacolToxicology*. 4:055-060.

Irvine. F.R. (1961).The Woody Plants of Ghana. Oxford University Press, London.p.868.

Iwu. M.W, Duncan.A.R, Okunji.C.O. (1999). New antimalarials of plant origin. In: Janick J, (ed.) Perspective on new crops and new uses. Alexandria: VA. *American Society* of *Horticultural Science*.457-462.

Iyiola. O.A, Tijani. A.Y and Lateef. K.M. (2011). Antimalarial activity of ethanolic stem bark extract of *Alstonia boonei* in mice. *Asian J. Biol. Sci*.4:235-243.

John-Prosper K.A, Adukpo G.E, Boahen Y.O and Armah F.A (2012). A Review of the Ethnobotany and Pharmacological Importance of *Alstonia boonei* De Wild (Apocynaceae) Article ID 587160, pp 9.

Kweifo-Okai.G, Bird.D, Field. B, Ambrose. R, Carroll.A.R, Smith. P, Valdes. R. (1995). Anti-inflammatory activity of a Ghanaian herbal preparation III. *Journal of Ethnopharmacology*. 46:7-15.

Odugbemi.T.O and Akinsulire.O.R.(2007). Medicinal plants useful for malaria therapy in Okeigbo, Ondo State, Southwest, Nigeria. *Africa Journal of Alternative Medicine* 4(2):191-198.

Oigiangbe.O.N, Igbinosa.I.B and Tamo. M. (2010). Insecticidal properties of an alkaloid from *Alstonia boonei* De Wild. J. Biopesticides. 3(1):265-270.

Ojewole, J.A.O. (1984). Studies on the pharmacology of echitamine, an alkaloid from the stem bark of *Alstonia boonei* L. (Apocynaceae). *Int. J. Crude Drug Res*.22:121-143.

Olajide.O.A, Awe.S.O, Makinde.J.M, Ekhelar.A.I, Olusola. A, Morebise. O. and Okpako. D.T. (2000). Studies on the anti-inflammatory, antipyretic analgesic. *Journal of Ethnopharmacology*. 44:7-15.

Okwu. D.E and Okwu. M.E.(2004). Chemical composition of *Spondiasmombin* Linn plant parts.*J. Sustain. Agric. Environ.* 6:140-147.

Organization of Economic Co-operation and Development (2001). The OECD guidelines for testing of chemicals: 420 Acute Oral Toxicity.

Oze, G.O., Nwanjo, H.U. and Onyeze, G.O (2007).Nephrotoxicty caused by the extract of *Alstonia boonei* (De Wild) stem bark in Guinea pigs. *The Internet J. Nutr. and Wellness,* 3(2): 1-9.

Peters. I.T and Antoli. V.K. (1998): The Current global malaria situation. Malaria parasite biology, pathogenesis and protection ASM Press. W.D.C Pp 11-12.

Peter.W, Portus.H, Robinson.L. (1975). The four-day suppressive *in vivo* antimalarial test. *Ann. Trop. Med. Parasitol.* 69: 155 – 171.

Pillai R, Santhakumari G. (1984) Toxicity studies in Nimbidin, a potential antiulcer drug. *Planta Medica*. 54(2): 146-148.

Sachs.J and Malaney. P. (2002). The economic and social burden of malaria. *Nature*, 415:680- 685.

Sofowora, A., (1993). Medicinal plants and Traditional medicine in Africa.Spectrum Books Ltd, Ibadan, Nigeria, pp: 289.

Titanji.V.P.K, Zofou.D and Ngemenya. M.N. (2008). The antimalarial potential of medicinal plants used for the treatment of malaria in Cameroonian folk medicine. *Africa Journal of Traditional Complimentary Alternative Medicine*.,5:302-321.

WHO (2000).General guidelines for methodologies on research and evaluation of traditional medicine. World Health Organization. Geneva.

WHO (2008). World Malaria Report. Geneva: World Health Organization. 2008.

WHO(2014)World Malaria Report. Geneva: World Health Organization; 2014.

Wilson. R.J. M. (2002). Progress with parasite plastids. Journal of Molecular Biology. 319:257-274.

Winter. R.W, Kelly.J.X, Smikstein. M.J, Dodean. R and Bagby. G.C.(2006). Evaluation and lead optimization of antimalarial acridones. *Exp. Parasitol.* 114:47-56.