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Antimalarial Activity, Phytochemical Composition and Acute Toxicity Tests of Ethanolic Stem Bark Extract of Alstonia boonei De Wild

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Authors' contributions

This work was carried out in collaboration among all authors. Authors CAO, FCO, RNNO and CIO designed the study and managed the analyses of the study. Authors ICJO, AQAO, SSE and EOU performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Authors ICE and HUY managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Many modern medicines are derived from the chemicals available in plants. The utilization of plants against diseases by traditional medical practitioners is common in many parts of the world and several researches have been carried out to determine the scientific basis for the use of such

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plants. Alstonia boonei is one of the many medicinal plants found in Nigeria. The plant parts have been traditionally used to treat various ailments including malaria. This study was carried out to evaluate the antimalarial activity, phytochemical composition and toxicity of ethanolic stem bark extract of Alstonia boonei. The extract showed substantial dose dependent antimalarial activity as indicated by the recorded suppressive (45.67%, 58.53% and 74.68% for 100, 200 and 400 $mgkg^{-1}$ body weights) prophylactic (33.57%, 45.64% and 61.23% for 100, 200 and 400 $mgkg^{-1}$ body weights) and curative effects (62.35%, 68.57% and 79.63% for 100, 200 and 400 $mgkg^{-1}$ body weights) on Plasmodium berghei infected white albino mice. The results of the antimalarial tests were significantly different compared to the negative control at $P < 0.05$. The phytochemical evaluation showed that the plant contained important chemical compounds including tannins, flavonoids, steroids, phenols, alkaloids, saponins, glycosides and terpenoids. The acute toxicity test showed that the extract is safe as observed on the tested mice. It was concluded that the extract contains important active antimalarial compounds that are safe and should be further investigated for antimalarial drug development.

Keywords: Antimalarial; phytochemical; toxicity; plasmodium berghei; suppressive; prophylactic; curative.

1. INTRODUCTION

Products from nature play important roles and leads to the discovery and development of new drugs [1]. Plants have proved to be sources of important new drugs. Drugs for treating malaria such as quinine and artemisinin came from plants [2]. With increasing reports of resistance of malaria parasite to currently used antimalarial drugs and no approved malaria vaccine yet, malaria continues to cause high morbidity and mortality rates especially in areas where it is endemic [3]. There is an urgent need for the scientific investigation of new and safe medicinal plants for treating malaria [4]. The development of novel and new antimalarial drugs will play key roles in malaria control and prevention. The use of traditional medical products has increased globally due to their relatively low cost and the urgent need to reduce the overuse of chemicals which posing a serious public health threat [5]. These traditional medical products are obtained from local herbal plants. One of such plants is Alstonia. Alstonia is a plant which comprises about 40 species and has a pan tropical distribution. There are about twelve species of genus 'Alstonia'. Alstonia boonei belongs to the family Apocynaceae and is an herbal medicinal plant of West African origin [6,7]. The plant's parts has been used for the treatment of malaria and other forms of diseases in Nigeria and other West African Countries. The parts have been traditionally used for its antimalarial, aphrodisiac, anti-diabetic, antimicrobial, and antipyretic activities. [8-11].

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant Material

Alstonia boonei stem bark was collected from Obollo Afor town in Enugu State, Nigeria and identified and authenticated by a botanist expert at the Department of Plant Science and Biotechnology, University of Nigeria Nsukka, Enugu State, Nigeria. The specimen was documented and assigned voucher number 7601.

2.2 Chemicals

All chemicals used for this research were of analytical grade.

2.3 Experimental Animals

Animal tests were carried out according to the National Institute of health (NIH) guide for the care and use of laboratory animals, NIH publication (volume 25, number 28), revised 1996. Approval for all animal experiments was obtained from the University of Nigeria Ethical Committee on the use of laboratory animals for research with approval number UNN-ERC/Z/9875 - 7/5/18. Inbred white albino mice of both sexes weighing between 20 and 22g were used for this study. The animals were obtained from the animal house of the Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria. The white albino mice were housed in well-ventilated wooden wire gauzed cages with saw dust as bedding and were acclimatized for

seven days and fed mice feed and tap water ad libitum.

2.4 Parasite Strain for the Study

Chloroquine sensitive Plasmodium berghei NK 65 strain was used for this study. It was obtained from the Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria and maintained in mice by serial passage.

2.5 Preparation of Plant Materials

The stem bark of Alstonia boonei were collected and cut into small pieces, washed and air dried for two weeks under room temperature. The dry samples were then ground into powder with a mechanical grinder.

2.6 Extraction Method

500 g of the ground plant material powder was measured and dispersed in 2.5 L of ethanol. The mixture was shaken with a mechanical shaking machine (GFL shaker No. 3017 MBH, Germany) for 72 h after which it was vacuum filtered. The resultant extract was then concentrated using a rotary evaporator at a temperature not exceeding 400°C. The concentrate was then heated over a temperature-regulated water bath pre-set at 40°C to obtain a solvent free extract. The extract thus obtained was stored in the refrigerator at 4°C until use.

2.7 Phytochemical Test of the Extract

Various phytochemical tests were carried out on the extract in order to determine the presence of phytochemical compound following standard techniques [12]. Each test was qualitatively expressed as negative (-) or positive (+) with the intensity of the characteristic colour expressed as (+), (++) or (+++).

2.8 Acute Toxicity Test of the Extract

The acute toxicity test of the ethanolic stem bark of A. boonei was evaluated using the methods described by Lorke 1983 [13]. The vehicle for the extract administration to experimental mice was corn oil. A 4 hour test period was done after which mice were divided into groups of three. The extract doses were calculated in reference to the body weight of the mice. Each mouse was then treated with a single oral dose of the extract. The administered doses were 5, 50, 300, 1200 and 1500 $mgkg^{-1}$ body weight. The animals were observed for three hours after dosing for signs of toxicity. A single high oral dose of 5000 $mgkg^{-1}$ body weight was then administered to a group of three male and three female mice while the control groups were administered with the vehicle. The animals were given food one hour after the administration of the extract. The animals were observed 30 minutes after dosing followed by hourly observation for a period of 8 hours and then once a day for the next 13 days. Daily observations including physical change, signs of illness and mortality were recorded and surviving mice were weighed.

2.9 Antimalarial Tests of the Extract

2.9.1 Test for suppressive activity

The suppressive activity of the extract was evaluated in early Plasmodium berghei infection in white albino mice using the methods described by Peters 1967 [14]. Fifteen mice were randomly divided into five groups of three mice each. On the first day (D0), the mice were each infected with 10^7 Plasmodium berghei. Three hours later the infected mice were each treated orally with 10 $mLkg^{-1}$ body weight of the extract or 10 $mgkg^{-1}$ body weight of chloroquine. Group 1, the negative control, was given 5 $mLkg^{-1}$ normal saline. Group 2, the positive control, was treated with 10 $mgkg^{-1}$ chloroquine. Groups 3 to 5 were treated with the extract.

The extract was administered orally at a dose of 100, 200 and 400 mg extract kg^{-1}. Treatment was carried out for four consecutive days (D0 – D3). The body weight of each mouse was measured on the first day (D0) and on the fifth day (D4). The body temperature was also taken before infection and three hours after infection (on D0) and then monitored daily to the fifth day (D4).

On the fifth day (D4), thin blood film was prepared from the tail blood of the mice. The thin blood film was fixed in methanol and stained with Giemsa to reveal parasitized erythrocytes. Parasitaemia was determined using light microscopy with 100X objective lens.

2.9.2 Test for prophylactic activity

The prophylactic activity of the extracts was determined using the methods of Peters 1965 [15]. Another set of fifteen mice were randomly divided into five groups of three mice each. Group 1, the negative control, was given 5 $mLkg^{-1}$ normal saline. Group 2, the positive

control, was treated with 10mgkg^{-1} chloroquine. Groups 3 to 5 were treated with the extract. The extract was administered orally at a dose of 100, 200 and 400 mg extract kg^{-1}. Treatment was carried out for three consecutive days (D0 – D2). On the fourth day (D3) the mice were inoculated with 10^7 P. berghei infected red blood cells. After 72 hours the level of parasitaemia was then determined using microscopy.

2.9.3 Test for curative activity

The curative activity of the extract on established infections of Plasmodium berghei on mice was assessed using the method earlier described by Ryley and Peters [16]. Another set of fifteen mice were infected with 10^7 P. berghei by intra peritoneal injection on the first day (D0). 72 hours later the mice were randomly divided into five groups of three mice each. Three groups of the mice (Groups 1 to 3) were treated orally with a dose of 100, 200 and 400 mg kg^{-1} body weight of the extract. The negative control group (Group 4) was given 5 mLkg^{-1} normal saline while the positive control group (Group 5) was treated with 10 mgkg^{-1} chloroquine.

The treatments with the extract and drug was done once daily for five days. Parasitaemia levels was checked each day by preparing Giemsa-stained thin smears from blood samples collected from the tail of the mice and examined under the microscope. The body weight and temperature were taken before infection (D0) and from the fourth day (D3) to the eight day (D7). The mean survival time (MST) of the mice in each treatment group was determined over a period of 29 days (D0 – D28) by dividing the number of days each mice survived with the total number of days and multiplying by 100 as follows:

$$MST = \frac{\text{Numberofdayssurvived}}{\text{Total number of days}} \times 100$$

3. RESULTS

3.1 Phytochemical Analysis

The result of the phytochemical analysis revealed that the extract contained important compounds including tannins, flavonoids, steroids, phenols, alkaloids, saponins, glycosides and terpenoids. Phenol showed the highest intensity followed by flavonoids, steroids, terpenoids, tannins, alkaloids, saponins and glycosides. The result of the qualitative analysis of the extract is shown in Table 1 while the result of the quantitative analysis is shown in Table 2 and Fig. 1.

3.2 Acute Toxicity Studies

No mortality was recorded in all the doses used for the toxicity test which was 5, 50, 300, 1200 and 1500 mgkg^{-1} body weight during the four days the treated mice were observed. This was an indication that the extract was not toxic. For the acute toxicity test of the extract, at the doses of 1500 and 5000 mgkg^{-1}body weight, signs observed in the tested mice included licking of the paws, stretching, salivation and a reduction in activity. The oral median lethal dose (LD50) was determined to be greater than 5000 mgkg^{-1}.

3.3 Antimalarial Tests

The suppressive test of ethanolic stem bark extract of A. boonei revealed a significant suppression, at $P < 0.05$, on the fourth day of the test by the extract. The suppressive activity was dose dependent with a suppression of 45.67% for 100 mgkg^{-1} body weight, 58.53% for 200 mgkg^{-1} body weight and 74.68% for 400 mgkg^{-1} body weight respectively, as compared to the control, 5 mgkg^{-1} body weight chloroquine, with a chemo suppression of 96.82%. The results were significantly different from the negative control at $P < 0.05$. Table 3 and Fig. 2 show the results of the suppressive effect of ethanolic stem bark extract of A. boonei and chloroquine in mice infected with Plasmodium berghei.

The prophylactic test of the ethanolic stem bark extract produced a dose dependent reduction in parasitaemia levels of 33.57% for 100 mgkg^{-1} body weight, 45.64% for 200 mgkg^{-1} body weight and 61.23% for 400 mgkg^{-1} body weight while 5 mgkg^{-1} body weight chloroquine produced 90.25% reduction in levels of parasitaemia. The results were significantly different from the negative control at $P< 0.05$. The reduction in parasitaemia by the extract indicates that the extract possesses schizonticidal activity in blood. Table 4 and Fig. 3 show the results of the prophylactic effect of ethanolic stem bark extract of A. boonei and chloroquine in mice infected with Plasmodium berghei.

In the curative test of the stem bark ethanolic extract, it was observed that the extract produced a significant dose dependent reduction ($P < 0.05$) in the levels of parasitaemia in the groups treated with the extract. On the seventh day of the curative test the extract showed an average

percentage parasitaemia suppression of of 62.35%, for 100 mgkg^{-1} body weight, 68.57% for 200 mgkg^{-1} body weight and 79.63% for 400 mgkg^{-1} body weight while mgkg^{-1} body weight chloroquine produced a reduction in parasitaemia of 99.42%.The results were significantly different from the control at $P < 0.05$. Table 5 and Fig. 4 show the results of the curative effect of the ethanolic stem bark extract of A. boonei and chloroquine in mice infected with Plasmodium berghei.

Table 1. Results of the qualitative phytochemical analysis of A. boonei ethanolic stem bark extract

Compound	Composition
Tannin	+
Flavonoid	++
Steroid	++
Phenol	+++
Alkaloid	+
Saponin	+
Glycoside	+
Tepernoid	++

Legend: + = Low; ++ = Moderate; +++ = High

Table 2. Results of the quantitative phytochemical analysis of A. boonei ethanolic stem bark extract

Compound	Tannin	Flavonoid	Steroid	Phenol	Alkaloid	Saponin	Glycoside	Terpenoid
Composition (Mg/100 g)	274.368	216.918	29.734	790.381	4.988	121.225	0.724	264.452

Table 3. Suppressive effect of ethanolic stem bark extract of A. boonei and chloroquine in mice infected with Plasmodium berghei

Treatments	Suppression (%)
Distilled water 5 mlkg^{-1}	0.00
Extract 100 mgkg^{-1}	45.67
Extract 200 mgkg^{-1}	58.53
Extract 400 mgkg^{-1}	74.68
Chloroquine 5 mgkg^{-1}	96.82

Significantly different from the negative control at $P < 0.05$

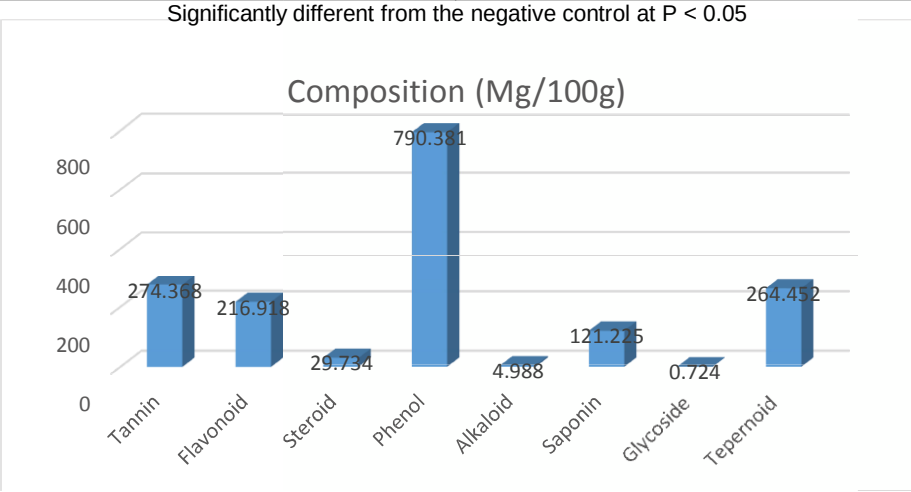

Fig. 1. Results of the quantitative phytochemical analysis of A. boonei ethanolic stem bark extract

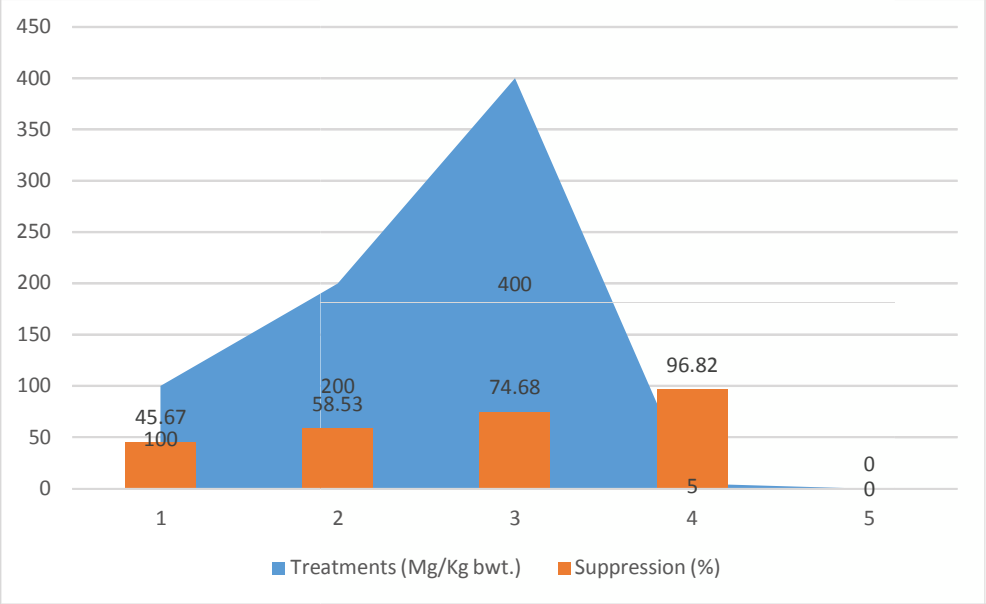

Fig. 2. Suppressive effect of ethanolic stem bark extract of A. boonei and chloroquine in mice infected with Plasmodium berghei

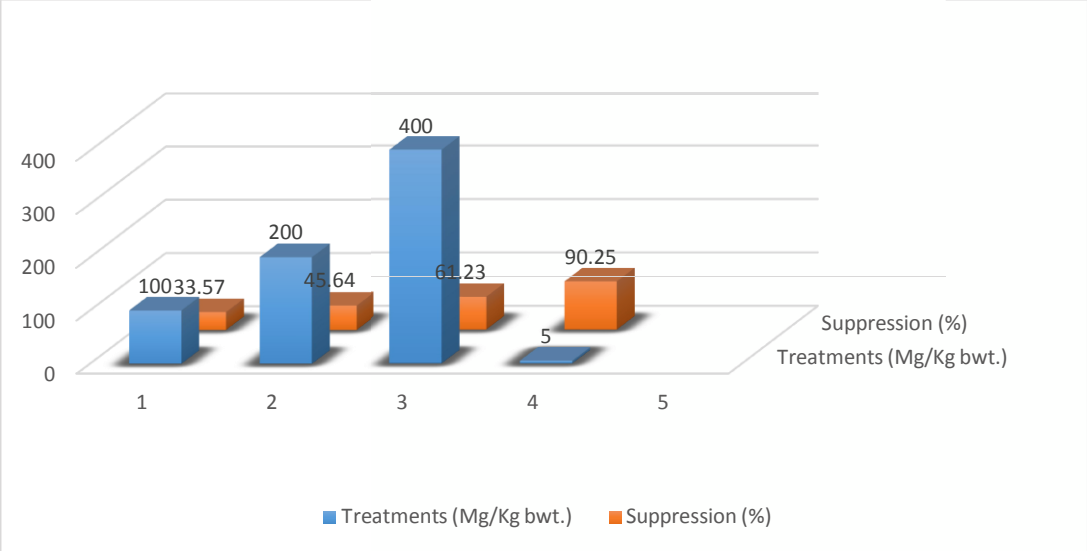

Fig. 3. Prophylactic effect of ethanolic stem bark extract of A. boonei and chloroquine in mice infected with Plasmodium berghei

Table 4. Prophylactic effect of ethanolic stem bark extract of A. boonei and chloroquine in mice infected with Plasmodium berghei

Treatments	Suppression (%)
Distilled water 5 $mlkg^{-1}$	0.00
Extract 100 $mgkg^{-1}$	33.57
Extract 200 $mgkg^{-1}$	45.64
Extract 400 $mgkg^{-1}$	61.23
Chloroquine 5 $mgkg^{-1}$	90.25

Significantly different from the negative control at $P < 0.05$

Table 5. Curative effect of ethanolic stem bark extract of A. boonei and chloroquine in mice infected with Plasmodium berghei

Treatments	Suppression (%)
Distilled water 5 mlkg^{-1}	0.00
Extract 100 mgkg^{-1}	62.35
Extract 200 mgkg^{-1}	68.57
Extract 400 mgkg^{-1}	79.63
Chloroquine 5 mgkg^{-1}	99.42

Significantly different from the negative control at P < 0.05

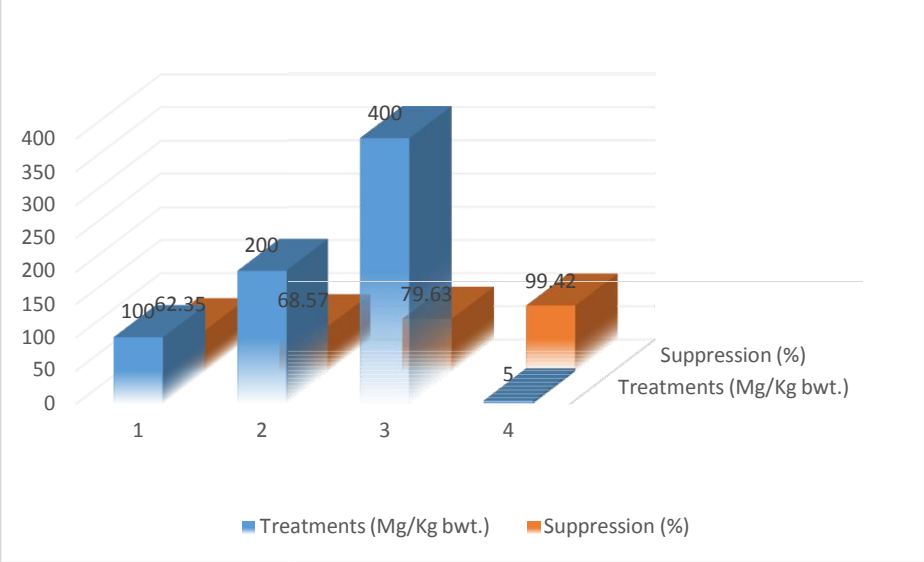

Fig. 4. Curative effect of ethanolic stem bark extract of A. boonei and chloroquine in mice infected with Plasmodium berghei

4. DISCUSSION AND CONCLUSION

From the results of the phytochemical analysis, the ethanolic stem bark extract of Alstonia boonei was found to contain important compounds including tannin, flavonoid, steroid, phenols, alkaloid, saponin, glycoside and terpenoid. The presence of phytoconstituents in plants, including alkaloids, have been reported by other studies [17-20]. Nature is a major resource of medicinal plants which are used for treating many diseases [21-26]. The presence of alkaloid in the extract may contribute to its antimalarial activity. Alkaloids present in plants have been described to contribute to the antimalarial activities of such plants [27,28]. These active bio active compounds present in the plant have been reported by other studies to be antimalarial compounds [29-34].

The result of the toxicity test is similar to that reported by Iyiola et al. 2011 [3] who also observed no mortalities at the doses treated with the oral median lethal dose also at more than 5000 mgkg^{-1}. Several other stud es also follow this trend including the result of studies carried out by Akinmoladun et al. 2007 [35], Adotey et al. 2012 [6], and Obiagwu et al. 2014 [36].

The result of the antimalarial tests of this study, comprising the suppressive, prophylactic and curative tests, agrees with the result from other studies carried out by Olanlokun t al. 2012 [37] who studied the therapeutic effects of various solvents of Alstonia Boonei (apocynaecia) stem bark on Plasmodium berghei-induced malaria and also the study carried out y Afolabi and Abejide 2020 [38], who evaluated the in vivo antiplasmodial properties of Morinda lucida and Alstonia boonei.

The results from this study indicate that the ethanolic stem bark extract of Alstonia boonei has a good safety profile and substantial antimalarial activity and thus should be further investigated for antimalarial drug development.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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