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# Evaluation of Metabolite Compositions and Antimalarial Efficacy of *Alstonia boonei* (Cheese wood) in Plasmodiun-infected white albino mice (*BALB/c)*

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## Abstract

**Background of the study:** The recent increase in resistance of the malaria parasite has led to the urgent need for new and novel antimalarial molecules with different modes of action to combat the resistance. Plants have in the past proven to be sources of new molecules for drug development against diseases including malaria.

**Statement of problem:** The increase in antimalarial drug resistance has greatly affected efforts towards the control and prevention of malaria in recent years. Hence, there is an urgent need for the discovery of new molecules for the development of new antimalarial drugs. Research on plants used traditionally for malaria treatment, such as *Alstonia boonei* in this study, may yield new antimalarial molecules.

**Aim of the study:** The aim of this study was to evaluate the metabolite compositions and antimalarial efficacy of *Alstonia boonei* (cheese wood) in plasmodium-infected white albino mice (*BALB/c* mice*).*

**Methodology**: Standard chemical tests were carried out on the extract to determine the presence of phytochemical metabolite compounds in the extract by using the modified methods described in an earlier study [10]. Each test was qualitatively expresses as negative (-) or positive (+). The antimalaria efficacy of the extract was evaluated in *Plasmodium berghei*-infected white albino (*BALAB/c*) mice. The levels of parasitaemia inhibition by the extract was determined by the examination of thin and thick blood smear preparations from the treated mice under the microscope.

**Results:** Metabolite compounds obtained from the phytochemical evaluation of the extract include tannins, flavonoids, steroids, phenols, alkaloids, saponins, glycosides and terpenoids. The antimalarial tests of the ethanolic root extract of *A.* boonei showed significant dose- dependent antimalarial activity against *Plasmodium berghei* infection in white albino mice (*BALB/c* mice) as indicated by the, suppressive (41.64%, 53.92% and 62.81% for 100, 200 and 400 $mgkg^{-1}$ body weights), prophylactic (45.79%, 58.54% and 66.73% for 100, 200 and 400 $mgkg^{-1}$ body weights), and curative (49.46%, 65.97% and 69.58% for 100, 200 and 400 $mgkg^{-1}$ body weights) effects.

**Conclusion:** It was concluded that the extract contains important metabolite compounds and also has significant antimalarial efficacy against malaria infection.

**Recommendation:** It is recommended that *Alstonia boonei* plant extracts should be further investigated to determine their bioactive compounds. The bioactive compounds should then be isolated and then tested to determine their antimalarial potential and position them for new antimalarial drug development.

**Keywords:** Antimalarial, Bio active compounds, Efficacy, Metabolites, *Plasmodium berghei*

## 1.0 Introduction

Malaria is a tropical parasitic disease caused by five species of the *Plasmodium* parasite. These include *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae* and *Plasmodium knowlesi*. *P. falciparum* is the most prevalent in Africa and also causes the most severe type of malaria infection [1]. Malaria is still a disease of great public health importance and leads to huge morbidity and mortality annually especially in Africa where it is endemic [2]. In recent years the efforts geared towards the control of malaria have been greatly affected by the appearance of resistance of the malaria parasite to the currently used antimalaria drugs and this has been reported to be on the increase [3]. This situation has led to urgent need for new and novel antimalarial molecules for new antimalarial drugs to combat the resistance [4]. Research for new antimalarial molecules have been intensified and plants may produce important leads for new antimalarial drugs development [5].

Some of the world's population especially in developing countries still depends on traditional system of medicine for treatment of variety of diseases [1, 6]. The use and efficacy of plants in the treatment of diseases is attributed to the metabolite compounds contained in them. Many modern medicines are derived from plants that have been used by traditional medical practitioners. *Alstonia boonei* is one of the many medicinal plants found in Nigeria and other African countries [3, 7]. The plant parts have been traditionally used to treat various ailments including malaria [5, 6]. Several metabolite compounds have been found to be present in *A. boonei,* and some of them have been found to possess a range of pharmacological activities including antimalarial activities [6, 8].

The recent increase in antimalaria drug resistance has led to an urgent need for new antimalarial molecules. This research is necessary and very important because it could provide a very valuable source of new and novel molecules antimalarial drugs development.

The aim of this study was to evaluate the metabolite compositions and antimalarial efficacy of *Alstonia boonei* (commonly known as cheese wood) in plasmodium-infected white albino mice (*BALB/c* mice*).*

## 2.0 Materials and Methods

### 2.1 Source of the Plant Materials

The root parts of *Alstonia boonei* were collected from Obukpa village in Nsukka Local Government Area in Enugu State, Nigeria. It was identified by a botanist at the Department of Plant Science and Biotechnology, University of Nigeria Nsukka, Enugu State, Nigeria. The sample of the authenticated materials was deposited in the herbarium section with voucher number UNN/PSB/7244.

### 2.2 Sources of Animal and Management

White albino mice (*BALB/*c) of both sexes weighing between 20 and 22g were sourced from the animal house, Department of Animal and Environmental Biology, University of Nigeria, Enugu State, Nigeria. Animal tests were carried out according to the National Institute of health (NIH) guide for the care and use of laboratory animals. Approval was obtained for all animal experiments from the University of Nigeria Ethical Committee on the use of laboratory animals for research.

### 2.3 *Plasmodium berghei* Parasite for the Study

Artesunate sensitive *Plasmodium berghei* NK65 strain was used for this study. It was obtained from the Biology Laboratory of the department of Zoology and Environmental Biology, University of Nigeria Nsukka.

### 2.4 Preparation of Plant Materials

The root parts of *A. boonei* earlier collected were cut into small pieces, washed and air dried for two weeks under room temperature. The dry samples were then ground into powder with a well cleaned grinding machine (model: NM-8300, manufactured by Nina Ltd., China).

### 2.5 Procedures of Extraction from the Root Parts

The extraction was carried out using the modified procedures described in an earlier study [9]. A quantity of 500g 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 72h after which it was vacuum filtered. The resultant extract was then concentrated using a rotary evaporator at a temperature not

exceeding 400ºC. The concentrate was then heated over a temperature-regulated water bath pre-set at 40ºC to obtain a solvent free extract. The extract thus obtained was stored in the refrigerator at 4ºC until use.

### 2.6 Phytochemical Test of the Extract

Standard chemical tests were carried out on the extract to determine the presence of phytochemical metabolite compounds in the extract by using the modified methods described in an earlier study [10]. Each test was qualitatively expresses as negative (-) or positive (+): The intensity of the characteristic colour was expressed as (+), (++) or (+++).

### 2.7 Antimalarial Tests of the Extract

#### 2.7.1 Test for suppressive activity

The malaria suppressive efficacy of the extract was ascertained by using the methods described in an earlier study [11]. 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 $mgkg^{-1}$body weight of the extract or 10 $mgkg^{-1}$ body weight of artesunate. Group 1, the negative control, was given $5mlkg^{-1}$ normal saline. Group 2, the positive control, was treated with 10 $mgkg^{-1}$ artesunate. 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).

During 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.

#### 2.7.2 Test for prophylactic activity

The malaria prophylactic efficacy of the extract was ascertained using the methods described in another study [12]. Fifteen mice were randomly divided into five groups of three mice each. Group 1, the negative control, was given $5mlkg^{-1}$ normal saline. Group 2, the positive control, was treated with $10mgkg^{-1}$ artesunate. 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.

#### 2.7.3 Test for curative activity

The malaria curative efficacy of the extract was ascertained using the method described in a previous study [13]. 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 $5mlkg^{-1}$ normal saline while the positive control group (Group 5) was treated with $10mgkg^{-1}$ artesunate.

The treatment 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).

### 2.8 Data Analysis

The values obtained was analyzed using Statistical Package for the Social Science (SPSS) version 20. The mean values of the parameters studied were compared using one-way Analysis of Variance (ANOVA) at 95% confidence interval. Probability values of $p < 0.05$ were considered significant.

## 3.0 Results

The qualitative phytochemical analysis showed that tannin and phenol showed highest intensity of the characteristic colour. Thus, tannin and phenol were the most abundant in the extract. The next in abundance were flavonoids, steroids, alkaloid, saponin, glycoside and terpenoid. They showed the same colour intensities. The results of the qualitative phytochemical analysis of the extract is

shown in Table 1 below. In the quantitative analysis, phenol was the highest followed by tannin, flavonoid, terpenoid, glycoside, alkaloid, steroid and saponin. The result of the quantitative phytochemical analysis is shown in Table 2 below.

**Table 1:** Qualitative phytochemical analysis of *A. boonei* ethanolic root extract.

| Compounds | Tannin | Flavonoid | Steroid | Phenol | Alkaloid | Saponin | Glycoside | Terpenoid |
|---|---|---|---|---|---|---|---|---|
| Composition | + | + | ++ | +++ | + | + | + | + |

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

**Table 2:** Quantitative phytochemical analysis of *A. boonei* ethanolic root extract

| Compounds | Tannin | Flavonoid | Steroid | Phenol | Alkaloid | Saponin | Glycoside | Terpenoid |
|---|---|---|---|---|---|---|---|---|
| Composition (mg/100g). | 266.988 | 211.059 | 4.151 | 885.917 | 50.248 | 0.664 | 108.689 | 175.879 |

**Antimalarial tests**

The malaria suppressive test of ethanolic root extract showed a significant suppression, at $p < 0.05$, by the extract. The suppressive activity was dose dependent with a suppression of 41.64%, 53.92% and 62.81% respectively, as compared to the positive control, artesunate, with a chemo suppression of 95.75%. The results of the suppressive effect of the extract are shown in Table 3 below.

**Table 3:** Suppressive effect of ethanolic root extract of *A. boonei* and artesunate in mice infected with *Plasmodium berghei*

| Treatments | Suppression (%) |
|---|---|
| Distilled water 5mlkg$^{-1}$ | 0.00 |
| Compound 100mgkg$^{-1}$ | 41.64 |
| Compound 200mgkg$^{-1}$ | 53.92 |
| Compound 400mgkg$^{-1}$ | 62.81 |
| Artesunate 5mgkg$^{-1}$ | 95.75 |

Significantly different from the control at $p < 0.05$

The malaria prophylactic test of the ethanolic root extract also showed a significant dose dependent reduction, at $p < 0.05$, in parasitaemia levels of 45.79%, 58.54% and 66.73% while 5mgkg$^{-1}$ body weight of artesunate produced 92.69% reduction in levels of parasitaemia. The significant reduction in parasitaemia by the extract indicates that the extracts possess schizonticidal activity in blood. The results of the prophylactic effect of the extract are shown in Table 4 below.

**Table 4:** Prophylactic effect of ethanolic root extract of *A. boonei* and artesunain mice infected with *Plasmodium berghei*

| Treatments | Suppression (%) |
|---|---|
| Distilled water 5mlkg$^{-1}$ | 0.00 |
| Compound 100mgkg$^{-1}$ | 45.79 |
| Compound 200mgkg$^{-1}$ | 58.54 |
| Compound 400mgkg$^{-1}$ | 66.73 |
| Artesunate 5mgkg$^{-1}$ | 92.69 |

Significantly different from the control at $p < 0.05$

The curative test of the ethanolic root extract also showed a dose dependent reduction in the levels of parasitaemia in the groups treated with the extracts as indicated an average percentage suppression of parasitaemia of 49.46%, 65.97% and 69.58% respectively while 5mgkg$^{-1}$ body weight of artesunate produced a reduction in parasitaemia of 93.41%. The results of the curative effect of the extract are shown in Table 5 below.

**Table 5:** Curative effect of ethanolic root extract of *A. boonei* and artesunate in mice infected with *Plasmodium berghei*

| Treatments | Suppression (%) |
|---|---|
| Distilled water 5mlkg$^{-1}$ | 0.00 |
| Compound 100mgkg$^{-1}$ | 49.46 |
| Compound 200mgkg$^{-1}$ | 65.97 |
| Compound 400mgkg$^{-1}$ | 69.58 |
| Artesunate 5mgkg$^{-1}$ | 93.41 |

Significantly different from the control at $p < 0.05$

**4.0 Discussion**

The discovery of new and novel antimalarial molecules with modes of action that are different from that of the currently used antimalarial drugs will help in a great measure in the tackling of resistance malaria [10]. Plants as they have done in the past may provide such new and novel molecules. The scientific study of plants and their extracts which are traditional used to treat malaria such as the plant extract in this present study is thus very important in the search for new antimalarial molecules for potential drug development [11].

The results of the phytochemical analysis of the root ethanolic extract of *A. boonei* showed that the extract contains medicinally crucial metabolite compounds such as tannins, flavonoids, steroids, phenols, alkaloids, saponins, glycosides and terpenoids.

The implication of the presence of these medicinal metabolite compounds in the extract is that *Alstonia boonei* is a plant with medicinal property with the potential for producing new molecules for new drugs development. The extract exhibited significant antimalarial efficacy against malaria infection. The implication of this is that the extract has great potential for the development of new antimalarial drugs from it.

The presence of metabolite compounds of medicinal importance in plants have been reported by earlier studies [14, 15, 16].

The malaria suppressive efficacy test of the ethanolic root extract revealed a significant dose dependent suppression of parasitaemia of the malaria parasite infection, which agrees with the findings in earlier studies by Ayoola et al [17], Edoga et al [18] and Elisabetsky et al [19], who recorded significant suppression of parasitaemia in their studies. In the malaria prophylactic efficacy test of the extract, a significant dose dependent inhibition of the establishment of infection of the malaria parasite was recorded in the treated mice, which is similar with the results of earlier studies by Gbadamosi et al [20], Gosse et al [21] and Tarkang et al [22] who obtained significant prophylactic property in their antimalarial tests. The malaria curative efficacy test of the ethanolic root extract also exhibited a dose dependent reduction in the levels of parasitaemia in the infected treated white albino mice. The findings of the malaria curative efficacy test obtained in this present study agree with the results of earlier studies by Obdoni et al [23], Bankole et al [24] and Bekono et al [25], who reported significant curative activity in their studies.

**5.0 Conclusion and Recommendation**

The results from this study revealed that the ethanolic root extract of *Alstonia boonei* contains important metabolite compounds which exhibited significant antimalarial efficacy in the infected treated mice. Having recorded a significant antimalarial efficacy in this study, it is recommended that the extract and other extracts from parts of *Alstonia boonei* should be further investigated to determine their bioactive compounds, and further analysis and characterization in order to find out the molecular weights and chemical structures of each of them. The antimalarial efficacy of the bioactive compounds should also be evaluated and then position them for potential antimalarial drug development.

**6.0 Competing Interests**

The authors declare that no competing interests exist among them.

**7.0 Funding:** None

**8.0 References**

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