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# Antimalarial Activity of the n-Butanol Fraction of *Uapaca togoensis* (Pax) Stem Bark in Mice

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ABSTRACT

The stem bark extract of *Uapaca togoensis* has been used in traditional medicine for the management of fever, epilepsy, fatigue and rheumatism. This study was aimed at evaluating the *in vivo* antimalarial activity of the n-butanol fraction of the methanol stem bark extract of the plant. Antimalarial activity was investigated in *Plasmodium berghei* NK65 infected mice using three experimental animal models including; the Peters 4-day suppressive, Curative and Prophylactic models. Phytochemical screening and acute toxicity tests (using the oral route in mice) were also conducted. The n-butanol fraction at all tested doses (250, 500 and 1000 mg/kg) exhibited significant ($p < 0.01$) and dose-dependent reduction in parasitaemia levels with percentage chemosuppression of 70.4, 80.3 and 90.3% respectively in the Peters 4-day suppressive test. In the curative and prophylactic studies, the fraction exhibited significant ($p < 0.01$) dose-dependent decrease in parasitaemia levels (9.25 ± 1.37, 6.42 ± 1.84, 3.18 ± 1.79) and (6.47 ± 1.39, 3.27 ± 1.16, 3.18 ± 1.02) at doses of 250, 500 and 1000 mg/kg, respectively. The mean survival time of the mice treated with the fraction was significantly ($p < 0.05$) prolonged compared to the distilled water treated group. The oral $LD_{50}$ in mice was estimated to be greater than 5,000 mg/kg. Phytochemical screening of the fraction revealed the presence of alkaloids, tannins, saponins, steroids, triterpenes and cardiac glycosides. These results suggest that the n-butanol fraction of *Uapaca togoensis* possesses antimalarial activity that justifies its use in ethnomedicine to treat malaria infection.

***Keywords***: Antimalarial, n-butanol fraction, Chloroquine, *Plasmodium berghei*, *Uapaca togoensis*

## Introduction

Malaria is an infectious disease that remains a global health problem greatly affecting underdeveloped and poverty-stricken countries.[1] The burden of malaria is heaviest in Sub-Saharan Africa where the population has continued to suffer from a disproportionately high incidence of the disease.[2] Malaria is transmitted to people through the bites of infected female *Anopheles* mosquitoes, blood transfusion, organ transplantation, shared use of needles and syringes that are contaminated with blood, or from a mother to her foetus before or during delivery.[3] Malaria imposes an enormous economic challenge on individuals and nations through high health care cost, missed days at work or school, reduced economic output and productivity.[4] Over the years, numerous strategies including the use of long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS), intermittent preventive treatment in pregnancy (IPTp) and the management of infection with available antimalarials, have been employed in the fight against malaria.[5] However, these strategies are still insufficient to achieve elimination in many countries, especially in the developing countries.[6] This situation including *P. falciparum* resistance to the currently used antimalarial drugs, resistance of mosquitoes to insecticides, adverse effects associated with some of the currently used antimalarials, high costs and logistical problems especially in poor endemic countries as well as a lack of effective vaccines has contributed to the global burden of mortality and morbidity from malaria.[7] Thus, the continious search for newer antimalarial drugs is of great importance.

There has been a re-awakened interest in the use of medicinal plants for the treatment of various diseases, malaria inclusive.[8] In Nigeria, the use of medicinal plants is evidenced by the massive patronage of traditional health-care practitioners by the people.[9] This patronage is probably due to the fact that medicinal plants are believed to be abundant, affordable and accessible especially to the poor in developing countries.[10] Quinine and Artemisinin, the two main drugs used in the treatment of malaria were derived from medicinal plants that have been used over the centuries.[11] Thus medicinal plants represent a virtually unlimited reservoir of molecules, which can be explored chemically to provide lead compounds for the development of newer more effective antimalarial drugs.[12]

The rodent malaria model has been used in identifying several conventional antimalarial agents including chloroquine, halofantrine, mefloquine and more recently artemisinin derivatives.[13] In particular, *Plasmodium berghei* has been used frequently to study the activity of potential antimalarial agents both in mice and rats[14] as it has been reported

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to have a high sensitivity to chloroquine and produces signs and symptoms of disease similar to those exhibited by human *Plasmodium* infection.[15] The rodent malaria parasite using *P. berghe*i was thus employed in the present study.

The plant *Uapaca togoensis* belonging to the family Euphorbiaceae is a tree about 13 m high with a short bole, usually without stilt-roots on dry sites, but may be stilted in damp localities of savanna regions from Nigeria, Senegal to western Cameroon, and in eastern Cameroon to Central African Republic.[16] The stem bark and leaves of the plant have been used folklorically for the treatment of various ailments including pneumonia, cough, fever, rheumatism, vomiting and epilepsy.[17] Phytochemical constituents including glycosides, steroids, triterpenes, saponins, tannins, flavonoids and alkaloids have been found present in the methanol stem bark extract of *U. togoensis*.[18] Methanol extracts from the fruits, bark and leaves of the plant as well as six compounds isolated from the fruit were tested and found to have antimicrobial activities against some Gram-negative bacteria.[19] Isolated compounds from the fruits of *U. togoensis* namely; *β*-amyryl acetate, 11-oxo-*α*-amyryl acetate, lupeol, pomolic acid, futokadsurin B, arborinin, and 3-O-*β*-D-glucopyranosyl sitosterol have demonstrated strong cytotoxic activity on many drug-resistant and sensitive cancer cell lines.[20] The methanol stem bark extract of the plant has been reported to posess antimicrobial activity.[18] The ethyl acetate fraction of the stem bark also exhibited antiplasmodial, analgesic and anti-inflammatory properties.[21-23] The present study aimed to evaluate the antimalarial activity of the n-butanol fraction of *Uapaca togoensis* with the aim of providing data from which possible compounds responsible for the observed pharmacological activity can be isolated.

## Materials and Methods

*Plant Material*

The stem bark of *Uapaca togoensis* was collected in July 2013, at Okpopokwu Local Government Area, Benue, Nigeria. The plant sample was identified and authenticated at the Herbarium Section of the Department of Biological Sciences, Ahmadu Bello University, Zaria. This was compared with the already deposited specimen (voucher number 1279) in the herbarium. The stem bark was air-dried at room temperature under shade to a constant weight. The dried samples were powdered using a wooden mortar and pestle. The powdered sample (2 kg) was macerated with 15 L of 70% v/v methanol in water and the filtrate was concentrated by evaporating to dryness at room temperature.

*Phytochemical Screening*

Preliminary phytochemical screening was carried out using the method of Trease and Evans.[26]

*Fractionation of the Crude Methanol Extract*

The crude methanol extract (200 g) was dissolved in distilled water in a separating funnel and fractionated successively with organic solvents to obtain ethyl acetate, n-butanol and residual aqueous fractions. The weight of the dried fractions was taken to determine the percentage yield. The fractions were kept in airtight containers and stored in a desiccator until needed.

*Animals*

Swiss Albino mice (18–22 g) were obtained from Animal House Facility, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. They were maintained under standard laboratory care, fed with standard animal feeds (Vital Feeds, Jos, Nigeria), and allowed access to water *ad libitum.* All experimental protocols were in accordance with Ahmadu Bello University, Zaria Research Policy; and ethics and regulations governing the care and use of experimental animals as contained in "Principles of Laboratory Animal Care".[24]

*Drugs and Reagents*

Chloroquine Phosphate (Sigma-Aldrich), Pyrimethamine (GSK Pharmaceuticals), Geimsa stain, Trisodium citrate.

*Acute Toxicity Test*

The oral median lethal dose ($LD_{50}$) of the fraction was estimated in mice using the method as described by Lorke.[27] This involved two phases. In the first phase, three doses of the fraction (10, 100 and 1,000 mg/kg) were administered to three groups of three mice each. The animals were then observed for manifestation of physical signs of toxicity such as writhing, decreased motor activity, decreased body/limb tone, decreased respiration and death for the first four hours. The number of deaths in each group within 24 hours was recorded and the second phase initiated in which 4 mice were divided into 4 groups each and administered with the fraction at doses of 1,200, 1,600, 2,900 and 5,000 mg/kg, respectively. The animals were again observed for another 24 hours after which the $LD_{50}$ was calculated as geometrical mean of the maximum dose producing no mortality and the minimum dose producing mortality.

$LD_{50} = \sqrt{(\text{Highest non-lethal dose} \times \text{Lowest lethal dose})}$ [27]

*Malaria Parasite*

*Plasmodium berghei* NK65 (Chloroquine sensitive strain) was obtained from the Department of Microbiology, Nigerian Institute of Medical Research, Yaba, Lagos. The parasite was maintained by continuous re-infecttion intraperitoneally in mice every 4 days by injecting 0.2 mL of infected erythrocytes containing approximately 1 x $10^7$ *P. berghei* parasitized eryhtrocytes.[25]

*Parasite inoculation*

Blood from a donor mouse infected with *P. berghei* (with rising parasitaemia level of about 20-30% infected erythrocytes) was used to infect a clean mouse. Each mouse used in the experiment was inoculated intraperitoneally with 0.2 mL of infected blood containing about $1 \times 10^7$ *P. berghei* parasitized erythrocytes. This was prepared by determining both the percentage parasitaemia and the erythrocytes count of the donor mouse and diluting the blood with isotonic saline in proportions indicated by both determinations.[12]

*Grouping and dosing of animals*

For each model (suppressive, curative and prophylactic), thirty mice were grouped into five groups of six mice. Group I mice were treated with the vehicle (distilled water, 10 mL/kg, which served as the negative control), groups II, III and IV mice were treated with 250, 500, and 1,000 mg/kg of fraction, respectively and group V mice were treated with the standard drug chloroquine phosphate, 5 mg/kg (for suppressive and curative studies) and Pyrimethamine, 1.2 mg/kg (for prophylactic study).

*Drug administration*

The standard drugs (chloroquine phosphate and pyrimethamine) and fraction used in the *in vivo* antiplasmodial study were orally administered with the aid of a cannula.

*Evaluation of antiplasmodial activity of the n-butanol fraction of Uapaca togoensis*

***Suppressive Test (4-day early infection)***

The Peters 4-day Suppressive test was used to evaluate the schizontocidal activity of the n-butanol fraction and chloroquine against early *P. berghei* infection in mice. The method as described by Peters and Robinson[28] was employed. Thirty (30) mice were weighed and randomly divided into five groups of six mice each. Each mouse received standard inoculum of 1 x $10^7$ *Plasmodium berghei* infected erythrocytes through the intraperitoneal route at the commencement of the experiment (day 1). Two hours after parasite inoculation, graded doses of the extract 250, 500, and 1, 000 mg/kg per day orally were given to the test mice in groups II, III and IV, respectively. While groups I (negative control) and V (positive control) received distilled water (10 mL/kg) and Chloroquine (5 mg/kg), respectively. Treatment was through the oral route and lasted for four days starting from the day of infection (day 1). On the fifth day, drops of blood samples were taken from the tail of each mouse. Thin blood films were made on a slide, air-dried, fixed in absolute methanol and stained with 3% Giemsa solution at pH 7.2. The slides were examined under the microscope and parasitaemia was determined by counting the number of parasitized erythrocytes in 4 random fields using $\times 100$ objective lens. Percentage parasite suppression relative to the negative control group was calculated for each dose using the formula below.[29]

$$\% \text{ Suppression} = \frac{\text{Av. parasiatemia in control} - \text{Av. parasitaemia in each treated group}}{\text{Av. parasitaemia in control}} \times 100$$

Av = Average

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*Curative Test*

The method described by Ryley and Peters[30] was used to evaluate the schizontocidal activity of the fraction and chloroquine against established *P. berghei* infection in mice. Thirty mice were weighed and randomly divided into five groups of six mice each. Each mouse was inoculated intraperitoneally with 0.2 mL of blood containing approximately 1 x $10^7$ *Plasmodium berghei* infected erythrocytes on the first day of the experiment. Seventy-two hours (72 h) after the animal was infected (parasitaemia established microscopically), groups II, III and IV animals received 250, 500 and 1,000 mg/kg, respectively of the extract orally for 4 days. Group I received 10 mL/kg of distilled water while group V received 5 mg/kg per day of chloroquine for four days. On day 7 of the experiment, thin blood films from the animals' tails stained with 3% Giemsa stain were prepared. Percentage chemosuppression relative to the negative control was determined for each dose as previously described.[29] After the seventh day, the animals were fed *ad libitum* and observed for 28 days. Any death that occurred during this period was noted to determine the mean survival time as shown in the formula below.[31]

$$\text{MST} = \frac{\text{Sum of survival time of all mice in a group (days)}}{\text{Total number of mice in that group}}$$

*Prophylactic test*

The repository activity of the fraction was assessed by using the method described by Peters.[32] Thirty mice were weighed and randomly divided into five groups of six mice each. Groups II - IV were administered with 250, 500 and 1,000 mg/kg/day of the fraction respectively, Groups I and V were respectively administered with 1.2 mg/kg/day of pyrimethamine (positive control) and 10 mL/kg of distilled water (negative control). Administration of the fraction/drug continued for three consecutive days. On the fourth day, the mice were inoculated with 0.2 mL of infected erythrocytes containing approximately 1 x $10^7$ *Plasmodium berghei* infected erythrocytes. Parasitaemia levels were assessed by blood smears seventy-two hours later. Percentage chemosuppression relative to the negative control was determined for each dose as previously described.[29]

*Statistical Analysis*

Data were analysed using one-way analysis of variance (ANOVA) followed by Dunnett's Post-hoc test. Results were expressed as mean ± standard error of mean (SEM) and presented as tables. Results considered as statistically significant at $p < 0.05$.

## Results and Discussion

In the present study, the antimalarial activity of the n-butanol fraction of *Uapaca togoensis* against *Plasmodium berghei* infection in mice is reported. The percentage yield of the n-butanol fraction was 42.8 %. Preliminary phytochemical screening of the n-butanol fraction of *Uapaca togoensis* revealed the presence of flavonoids, saponins, alkaloids, cardiac glycosides, carbohydrates, steroids and triterpenes; however anthraquinones were absent (Table 1). There was no mortality recorded on the administration of the n-butanol fraction of *Uapaca togoensis* at doses up to 5,000 mg/kg. The oral median lethal dose value of the n-butanol fraction in mice was thus estimated to be greater than 5, 000 mg/kg.[27] According to Lorkes's toxicity scale, the n-butanol fraction of *Uapaca togoensis* can thus be classified as relatively non-toxic.

The rodent malaria parasite model was used to investigate the antimalarial activity of the n-butanol fraction as it is a reliable and highly reproducible *in vivo* model for evaluating new antimalarial agents.[33] The n-butanol fraction in the Peters 4-day suppressive test showed a significant ($p < 0.001$) dose-dependent chemosuppressive effect on the parasitaemia levels relative to the control (Table 2). The percentage chemosupression obtained were 70.4, 80.3 and 90.3% at the doses of 250, 500 and 1,000 mg/kg respectively. For the curative study, the n-butanol fraction significantly ($p < 0.05$) reduced the level of parasitaemia at all tested doses compared to the negative control group (Table 3). The standard drug (Chloroquine, 5 mg/kg) produced a chemosuppression of 92.1% which was however not significantly different from that of the n-butanol fraction at 1,000 mg/kg (86.3%). Also in the prophylactic test, administration of the n-butanol fraction to the mice produced a significant ($p < 0.05$) dose-dependent parasitaemia suppression. The percentage chemosuppression produced at the 500 mg/kg dose was comparable to that produced at the 1,000 mg/kg dose (Table 4).

These results obtained from the *in vivo* antimalarial study shows that the n-butanol fraction of *U. togoensis* possesses significant chemosuppressive effect against early infection, curative effect against established infection and prophylactic effect against residual infection in mice infected with *P. berghei* in a dose-dependent manner. Studies have shown that a compound is considered as active when percentage chemosuppression is 30 % or more.[34-35] According to another study, [36] *in vivo* antimalarial activities of plant extracts can be categorized as: moderate, good or very good if the extract shows 50 % or more chemosuppression at 500, 250 and 100 mg/kg/day extract dose, respectively. Based on these classifications,

**Table 1:** Phytochemical Constituents Present in the n-butanol Fraction of *Uapaca togoensis*

| Phytochemical Constituents | Inference |
|---|---|
| Anthraquinones | – |
| Steroids/Triterpenes | + |
| Cardiac glycosides | + |
| Saponins | + |
| Tannins | + |
| Flavonoids | + |
| Alkaloids | + |

Key: + = present, - = absent

**Table 2:** Effect of n-butanol Fraction of *Uacapa togoensis* on Early *P. berghei* Infection in Mice.

| Treatment groups | Dose (mg/kg) | Average Parasitemia | Percentage chemosuppression (%) |
|---|---|---|---|
| DW | 10 mL/kg | 36.08 ± 0.93 | - |
| NBUT | 250 | 9.13 ± 2.43* | 70.4 |
| NBUT | 500 | 6.06 ± 1.00* | 80.3 |
| NBUT | 1,000 | 3.00 ± 0.98* | 90.3 |
| CQ | 5 | 0.69 ± 0.36** | 92.5 |

Values are presented as Mean ± SEM; Data analysed by one-way ANOVA followed by Dunnett's Post-hoc test; n = 6; * = $p < 0.01$ and ** = $p < 0.001$ versus control; DW = Distilled Water; NBUT = n-butanol Fraction of *Uapaca togoensis*; CQ = Chloroquine; Route of administration = oral.

**Table 3:** Effect of n-butanol Fraction of *Uacapa togoensis* on Established *P. berghei* Infection in Mice

| Treatment groups | Dose (mg/kg) | Average Parasitemia | Percentage chemosuppression (%) |
|---|---|---|---|
| DW | 10 mL/kg | 22.22 ± 0.83 | - |
| NBUT | 250 | 9.25 ± 1.37* | 60.2 |
| NBUT | 500 | 6.42 ± 1.84* | 72.4 |
| NBUT | 1,000 | 3.18 ± 1.79** | 86.3 |
| CQ | 5 | 1.82 ± 0.42** | 91.8 |

Values are presented as Mean ± SEM; Data analysed by one-way ANOVA followed by Dunnett's Post-hoc test; n = 6; *= $p < 0.01$ and ** = $p < 0.001$ versus control; DW = Distilled Water; NBUT = n-butanol Fraction of *Uapaca togoensis*; CQ = Chloroquine; Route of administration = oral

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**Table 4:** Prophylactic Effect of the n-butanol Fraction of *Uacapa togoensis* in *P. berghei* Infected Mice

| Treatment groups | Dose (mg/kg) | Average Parasitemia | Percentage chemosuppression (%) |
|---|---|---|---|
| DW | 10 mL/kg | 21.37 ± 1.36 | - |
| NBUT | 250 | 6.47 ± 1.39* | 69.7 |
| NBUT | 500 | 3.27 ± 1.16* | 84.7 |
| NBUT | 1,000 | 3.18 ± 1.02* | 85.1 |
| PY | 1.2 | 2.79 ± 0.96* | 90.3 |

Values are presented as Mean ± SEM; n = 6; Data analysed by One-way ANOVA followed by Dunnett's Post-hoc test; Values were significantly different between test and control group at *p = < 0.001 versus control; NBUT = n-butanol Fraction of *Uapaca togoensis*; DW = Distilled water; PY = Pyrimethamine; Route of administration = oral

**Table 5:** Effect of n-butanol Fraction of *Uacapa togoensis* on Mean Survival Time in *P. berghei* Infected Mice

| Treatment groups | Dose (mg/kg) | MST (days) |
|---|---|---|
| DW | 10 mL/kg | 10.5 ± 2.2 |
| NBUT | 250 | 28.00 ± 0.00* |
| NBUT | 500 | 28.00 ± 0.00* |
| NBUT | 1,000 | 24.17 ± 3.83* |
| CQ | 5 | 27.8 ± 0.40* |

Values are presented as Mean ± SEM; Data analysed by one- way ANOVA followed by Dunnett's Post-hoc test; n=6; * = $p < 0.05$ versus control; DW = Distilled Water; NBUT = N-butanol Fraction of *Uapaca togoensis*; CQ = Chloroquine; MST = Mean Survival Time; Route of administration = oral

the n-butanol fraction of *U. togoensis* can be classified as having good antimalarial activity. There was a significant ($p < 0.05$) increase in the mean survival time of the mice treated with the n-butanol at all tested doses, however, death was observed to occur earlier in the distilled water treated group (Table 5). In antimalarial studies, a test compound that has the ability to extend mean survival time beyond 12 days is regarded as having good parasite suppressing activity.[15] Animals that received the n-butanol fraction survived for a longer period of time (beyond 12 days) compared to the animals that received distilled water. The longer mean survival time observed in this study may thus be a reflection of the parasite reducing ability (antimalarial activity) of the fraction. The current findings were in agreement with studies done on medicinal plants used for malaria such as *Nigella sativa* [37] and *Dodonaea angustifolia*.[38]
Secondary metabolites such as alkaloids, saponins, flavonoids, tannins and phenols which were found present in the n-butanol fraction have been reported to possess antimalarial actions.[39-40] Alkaloids have been reported to produce antimalarial properties by blocking protein synthesis in *Plasmodium* parasites.[41] Saponins, flavonoids and tannins have been suggested to act as primary antioxidant or free radicals scavengers that can counteract the oxidative damage induced by the malaria parasite.[42] The antimalarial activities of the n-butanol fraction observed in this study may therefore have been due to the presence of these phytochemicals exerting their activity through one or both of these mechanisms, or may be even through a yet to be identified mechanism. The chemosuppression produced by the n-butanol fraction of *Uapaca togoensis* in the current study was in agreement with other studies which reported that the n-butanol fraction of medicinal plants like *Asparagus africanus*[43] and *Dodonaea angustifolia* [38] possesses significant antimalarial activity compared to the other fractions studied. It is noteworthy that the chemosuppression produced by the n-butanol fraction of the plant was better than that of the ethyl acetate fraction reported in a previous study.[21] This difference in antimalarial activity may be linked to the fact that the ethyl acetate fraction was devoid of saponins and tannins and studies have reported these phytochemicals to possess antimalarial activity.[42] Research on medicinal plants contributed to the isolation of compounds with potent antimalarial activity including artemisinin and quinine. Thus the isolation of compounds from the n-butanol fraction of *U. togoensis* is desirable as this may lead to the discovery of lead compounds which could be further adjusted by semi-synthetic approaches to obtain effective antimalarial agents.

## Conclusion

The results obtained from this study suggest that the n-butanol fraction of *U. togoensis* possesses significant antimalarial activity providing the scientific basis for the use of the plant in traditional medicine for the management of malaria. Further research on the fraction should be carried out in order to isolate, identify and characterize the active principles from the plant that may be responsible for the antimalarial activity observed.

## Conflict of interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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