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# Antimalarial Efficacy and Chemopreventive Capacity of Bamboo Leaf (*Bambusa vulgaris)* in Malaria Parasitized Mice

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**ABSTRACT:** The exploration of natural products for the treatment of malarial has been the main focus of scientists in the past decades. In this research, the phytochemical constituents, antimalarial effect, and chemopreventive capacity of aqueous leaf extract of *Bambusa vulgaris* in malaria parasitized mice was investigated. A total of 30 male mice, grouped into six (n=5), was used. The results obtained showed that *B. vulgaris* is rich in flavonoid (262.08 µg CE/g) and phenol (0.91 g AAE/ 100 g). There was significant reduction on the activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and Gamma glutarmyl-transferase (GGT) upon treatment as compared with the control groups (P<0.05). Concentration of total bilirubin (TB), direct bilirubin (DB) and serum electrolytes (sodium, calcium, phosphorus and chloride) decreased in treated groups; serum urea, creatinine and uric acid also reduced significantly as against the control groups (P<0.05). The hepatoprotection and renal function restoration observed upon the administration of the plant extract indicate to a far reaching end that *B. vulgaris* leaf extract would be a promising natural antimalarial product devoid of side effects upon use, especially when administered within the dose range of 100 – 200 mg/Kg body weight investigated in this study.

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Malarial, a condition that depletes hepatocytes remains the prevailing infectious diseases in Sub-Saharan Africa (WHO, 2016). Plasmodium falciparum, the deadliest form of the malarial parasite, is responsible for the enormous majority of the mortality and morbidity associated with malarial infection. Artemisinin Combination Therapies (ACTs) are presently the major drugs in the management of malarial caused by P. falciparum. Although these treatments continue to be effective in many parts of the world, the occurrence of the malarial parasite resistance to ACTs, a combatant is an urgent public health concern (WHO, 2016; Wele *et al*., 2017). Medicinal plants are being used for the treatment of diseases since inception worldwide (Wele *et al*., 2017). Since most of the developing nations have no access to modern therapeutics such as ACT to treat malarial because of financial, geographical and/or cultural impediments, they fall back to the use of medicinal plant for the management of diseases. About 80% population of the world relies on traditional medicinal products for some aspects of primary healthcare (WHO, 2016). Improved knowledge of plants from traditional pharmacopoeias and validated traditional medications in Improved Traditional Medicine (ITM) can result to access to effective, standardized, ever present with moderate prize therapeutics for management of malarial by local populations (WHO, 2012). In Africa and other countries where malarial exists, traditional medicinal plants are habitually used as preventive or curative for malarial (Jenett-Siems *et al*., 1999). It is true that orthodox antimalarials such as quinine and artemisinin derivatives originated from plants. It is therefore reasonable to investigate the antimalarial activity of medicinal plants in order to define their potential as sources of new antimalarial agents (Mustofa *et al*., 2007). Bamboo is a large perennial grass dispersed widely from tropical to subarctic zones (Ambika and Rajagopal, 2017). In Asian countries, different parts of bamboo have been used for medicinal purposes to treat hypertension, arteriosclerosis, cardiovascular disease and certain forms of cancer, antioxidant activities and are non-toxic (Tanaka *et al*., 2013). However, this study focuses on the use of aqueous leaf extracts of *Bambusa vulgaris* for the treatment of *Plasmodium berghei*- infected mice.

## MATERIALS AND METHODS

*Chemicals and Reagents: Plasmodium berghei*, Lonart (antimalarial drug), and all other reagents used for this study were obtained from Alpha Chimika, Mumbia, China.

*Experimental Animals:* Adult male Wistar albino mice of three to four months weighing between 30-35g were obtained from the animal house, Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria. They were fed with grower marsh obtained

from Dutch Farm Limited, Abraka and water *ad libitum*. They were acclimatized for one week.

*Collection and Identification of Plant Material:* The leaves of *Bambusa vulgaris* (Bamboo) were collected from Abraka, Ethiope East Local Government Area, Delta State and the taxonomical identification of the plant was done by the Taxonomist in the Department of Botany, Delta State University, Abraka.

*Preparation of Extract:* The leaves were washed to remove contaminants and air-dried for about three weeks. Thereafter, they were grinded to fine powder using Waren blender. 700g of the powdered form was soaked in 2800ml (2.8L) of distilled water in a ratio 1:4. It was macerated after 48hrs to obtain the crude extract (Anigboro *et al.*, 2018). This was followed by filtration using Whatman No110 filter paper. The purified crude was then concentrated using a vacuum rotary evaporator at reduced temperature (50$^{o}$C) and bathe at 40$^{o}$C. This yielded a dark brown concentrated extract of 29g (4.14%w/w). The obtained crude extract was packaged in an airtight plastic container and stored at 4$^{o}$C until when required for use.

*Inoculation:* The mice were inoculated intraperitoneally with *Plasmodium berghei* and baseline was established as described by Ambika and Rajagopal (2017).

*Experimental design:* A total of thirty (30) mice were used for this experiment. The mice were divided into six (6) groups of five (5) mice each as follows:
Group 1 (NC): Negative control (Healthy mice + no treatment)
Group 2 (PC): Positive control (Malaria-induced mice + no treatment)
Group 3 (D1): Malaria-induced mice + 100 mg/kg of extract of B. vulgaris
Group 4 (D2): Malaria-induced mice + 200 mg/kg of extract of B. vulgaris
Group 5 (D3): Malaria-induced mice + 300 mg/kg of extract of B. vulgaris
Group 6 (STD): Malaria-induced mice + 100 mg/kg of antimalarial drug (Lonart)

The experiment was lasted for two weeks; thereafter, determination of malarial load and biochemical parameters was undertaken.

*Collection of Sample:* The rats were sacrificed by cervical dislocation or decapitation and blood sample was collected using 5ml syringe from each rat. Samples were emptied into anticoagulant containers to prevent clotting (plasma enzymes). Some of the samples were equally placed in universal containers (serum enzymes) assayed biochemically.

*Determination of Biochemical Parameters:* Determination/Estimation of liver enzymes, lipid profile and kidney function were carried out using the *Prietest easylab Biochemistry analyzer*. It measures theoretical densities of samples and it uses algorithm to calculate results, which are used for biochemical investigations. It has direct access to stored programs. The analyzer can analyze the following parameters. Serum Glucose, Urea, Creatinine, Hemoglobin, Cholesterol, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Albumin, Total Protein, Total Bilirubin, Direct Bilirubin, Alkaline Phosphatase, Uric Acid, Triglycerides, Gamma glutamyltransferase (GGT), Phosphorus, Calcium, and Chloride were assayed using *Prietest easylab Biochemical analyzer.*

*Phytochemical Screening:* Plant materials were subjected to qualitative phytochemical screening to determine the presence of the major phytochemical constituents: alkaloids, glycosides, flavonoids, phlabatannins, phenols, resin, saponins, tannins, triterpenes, steroids, proteins, carbohydrates, amino acids, gums, mucilage, non-reducing polysaccharides and non-reducing simple sugar according to standard methods outlined by Petchi *et al*. (2013). Quantitative analysis was carried out to determine the percentage of phenol and flavonoids present in the extract as described by Bharathi *et al*. (2013).

*Statistical Analysis:* The data was analyzed using a computer software (SPSS version 21) and compared using Bonferroni test. The results are represented as Mean ± SD. Mean values compared were considered significantly different at $p<0.05$.

## RESULTS AND DISCUSSION

Malarial, a life threatening disease is known to deplete the levels of red blood cell and eventually cause anemia and loss of life in most cases (WHO, 2016). The secondary metabolites; alkaloids, glycosides, flavonoids, phlabatannins, phenols, resin, saponins, tannins, triterpenes, steroids, proteins, carbohydrates, amino acids, gums, mucilage, non-reducing polysaccharides and non-reducing simple sugar present were screened qualitatively and the result showed the presence of some highly active phytochemicals in the leaf extract of *Bambusa vulgaris* such as phenols, flavonoids, saponin etc (Table 1a). Quantitative analysis also showed that *B. vulgaris* leaf is rich in total phenol (0.91 g ascorbic acid equivalent / 100g dry weight) and total flavonoids (262.08 µg catechin equivalent/ g) (Table 1b). These findings are consistent with the reports of Wele *et al*., (2017), Abdillah *et al*., (2015) and Wang *et al*., (2016). Flavonoids are one of the major antimalarial natural products and have been published to exhibit promising results. The malarial parasite (MP) load and MP percent (%) of the treated groups reduced upon the administration of the extract in a dose-dependent manner (Table 2).

**Table 1a.** Qualitative analysis of phytochemical constituents of *Bambusa vulgaris.*

| Phytochemical | *Bambusa vulgaris* (Bamboo) |
|---|---|
| Saponin | + |
| Tannin | - |
| Terpenes | - |
| Flavonoid | +++ |
| Phlobatannins | - |
| Alkaloid | - |
| Gycosides | + |
| Resin | + |
| Phenol | + |
| Micronutrients | |
| Steriods | - |
| Proteins | + |
| Carbohydrates | + |
| Amino acids | + |
| Gums & Mucilage | - |
| Non reducing polysaccharides | - |
| Non reducing simple sugar | + |

*+ = mildly present; ++ = highly present; +++ = more highly present; - = absent or non-detectable.*

**Table 1b.** Total phenol and flavonoid contents of *Bambusa vulgaris*.

| Phytoconstituent | *Bambusa vulgaris* |
|---|---|
| Total phenol (g AAE / 100g dry weight) | 0.91 |
| Total flavonoid (µg CE/ g of leaf) | 262.08 |

*AAE, Ascorbic acid equivalent; CE, catechin equivalent. The quantitative analysis showed that mast tree leaf is rich in total phenol (0.91 g AAE / 100g dry weight) and total flavonoids (262.08 µg CE/ g).*

**Table 2.** Antimalarial effect of *B. vulgaris* aqueous extract treatment on *Plasmodium berghei*- infected mice.

| Group | MP Load | MP (%) |
|---|---|---|
| NC | 0 | 0 |
| PC | $15.5 \pm 1.44^{a}$ | $62 \pm 5.77^{a}$ |
| D1 | $3.00 \pm 0.00^{c}$ | $12 \pm 0.00^{c}$ |
| D2 | $2.00 \pm 0.81^{c}$ | $8.00 \pm 3.27^{d}$ |
| D3 | $1.50 \pm 0.20^{d}$ | $6.00 \pm 0.80^{e}$ |
| STD | $6.00 \pm 1.63^{b}$ | $24 \pm 6.53^{b}$ |

*MP, Malaria parasites. Values are expressed in Mean ± Standard error of mean. Values in the same column with different superscript letters show significant difference (P<0.05) while those in the same column with same superscript letters show no significant difference (P>0.05).*

The antiplasmodial activity of the plant extract reported in this study may be due to interactions between one or more group of the phytochemicals present in the extract. Several class of phytochemicals have been described in the field of antiplasmodials such as terpenoids, alkaloids, phenolic compounds including flavonoids and quinones (Oliveira *et al*., 2009; Adeleke *et al*., 2014; Tonukari *et al.*, 2015; Aganbi *et al.*, 2017; Vats *et al*., 2011; Tanaka *et al*., 2011).

**Table 3a.** Effect of *B. vulgaris* aqueous extract treatment on liver function biomarkers in malaria-induced mice.

| Group | ALT (U/L) | AST (U/L) | ALP (U/L) | GGT (U/L) |
|---|---|---|---|---|
| NC | $11.80 \pm 0.34^{a}$ | $46.66 \pm 3.58^{a}$ | $40.92 \pm 6.05^{a}$ | $80.00 \pm 0.20^{a}$ |
| PC | $20.33 \pm 1.71^{b}$ | $48.10 \pm 2.82^{b}$ | $60.88 \pm 17.9^{b}$ | $87.50 \pm 0.08^{b}$ |
| D1 | $16.48 \pm 0.41^{c}$ | $42.83 \pm 9.42^{c}$ | $56.47 \pm 9.29^{c}$ | $83.25 \pm 0.34^{c}$ |
| D2 | $15.90 \pm 1.89^{c}$ | $45.48 \pm 6.31^{d}$ | $48.30 \pm 1.54^{d}$ | $81.25 \pm 0.21^{a}$ |
| D3 | $13.30 \pm 0.27^{d}$ | $39.05 \pm 1.48^{a}$ | $47.93 \pm 2.03^{d}$ | $79.50 \pm 0.64^{a}$ |
| STD | $11.91 \pm 0.42^{a}$ | $35.18 \pm 3.50^{ac}$ | $44.66 \pm 4.15^{d}$ | $71.00 \pm 0.68^{d}$ |

*ALT, Alanine aminotransferase activity; AST, Aspartate aminotransferase activity; ALP, Alkaline phosphatase activity; GGT; Gamma glutarmyltransferase activity.*

**Table 3b:** Effect of *B. vulgaris* aqueous extract treatment on liver function biomarkers in malaria-induced mice.

| Group | TB (mg/dl) | DB (mg/dl) |
|---|---|---|
| NC | $0.72 \pm 0.08^{a}$ | $0.72 \pm 0.15^{a}$ |
| PC | $1.95 \pm 2.76^{b}$ | $1.45 \pm 0.92^{b}$ |
| D1 | $1.83 \pm 1.59^{c}$ | $1.40 \pm 0.50^{b}$ |
| D2 | $2.20 \pm 1.43^{d}$ | $1.23 \pm 0.37^{c}$ |
| D3 | $2.85 \pm 0.49^{d}$ | $0.90 \pm 0.42^{d}$ |
| STD | $1.08 \pm 0.71^{c}$ | $0.98 \pm 0.17^{d}$ |

*TB, Total bilirubin level; DB, Direct bilirubin level.*

**Table 4.** Effect of *B. vulgaris* aqueous extract treatment on serum electrolytes levels in *Plasmodium berghei*- infected mice.

| Group | Sodium (mg/dl) | Calcium (mg/dl) | Phosphorus (mg/dl) | Chloride (mg/dl) |
|---|---|---|---|---|
| NC | $108.0 \pm 6.78^{a}$ | $5.720 \pm 1.70a$ | $4.800 \pm 0.84^{a}$ | $114.1 \pm 2.67^{a}$ |
| PC | $137.5 \pm 3.54^{b}$ | $8.500 \pm 0.14^{b}$ | $3.750 \pm 0.35^{b}$ | $79.00 \pm 1.41^{b}$ |
| D1 | $126.5 \pm 4.73^{c}$ | $6.750 \pm 0.17^{a}$ | $5.250 \pm 0.26^{c}$ | $83.70 \pm 7.40^{c}$ |
| D2 | $120.0 \pm 0.00^{d}$ | $6.800 \pm 0.67^{a}$ | $5.050 \pm 0.10^{c}$ | $85.53 \pm 6.88^{c}$ |
| D3 | $123.0 \pm 4.24^{d}$ | $8.650 \pm 0.49c$ | $5.500 \pm 0.71^{c}$ | $91.00 \pm 9.90^{d}$ |
| STD | $110.0 \pm 7.12^{a}$ | $7.850 \pm 0.44^{d}$ | $5.350 \pm 0.60^{c}$ | $84.00 \pm 3.37^{c}$ |

**Table 5.** Effect of *B. vulgaris* aqueous extract treatment on serum creatinine, urea and uric acid levels in malaria-induced mice.

| Group | Creatinine (mg/dl) | Urea ( mg/dl) | Uric acid (mg/dl) |
|---|---|---|---|
| NC | $1.14 \pm 0.40^{a}$ | $24.33 \pm 2.41^{a}$ | $2.40 \pm 1.74^{a}$ |
| PC | $2.20 \pm 0.71^{b}$ | $50.18 \pm 22.1^{b}$ | $4.35 \pm 0.49^{b}$ |
| D1 | $1.88 \pm 0.38^{c}$ | $44.77 \pm 14.5^{c}$ | $3.10 \pm 0.14^{c}$ |
| D2 | $1.79 \pm 0.33^{c}$ | $45.49 \pm 9.10^{c}$ | $3.20 \pm 0.34^{c}$ |
| D3 | $1.60 \pm 0.14^{d}$ | $39.22 \pm 8.77^{d}$ | $3.15 \pm 0.21^{d}$ |
| STD | $1.53 \pm 0.28^{d}$ | $26.55 \pm 8.26^{a}$ | $2.30 \pm 0.29^{a}$ |

The decrease in malarial parasite load and MP% in parasitized mice groups treated with the *B. vulgaris* leaf extract is also in agreement with those of De Donno *et al*. (2012) who reported on the antimalarial activity of Artemisia annua herbal tea and artemisinin. This reduction may be due the interaction of flavonoids and other secondary metabolites present in the extract. Prior to treatment of the infected mice, there was elevated levels of serum electrolytes (sodium, calcium, phosphorus and chloride), liver markers (ALT, AST, ALP and GGT), and renal indices (urea, creatinine and uric acids) of the mice when compared with the normal control.

This was used as a baseline upon treatment. However, upon treatment with the B. vulgaris aqueous extract, the activities of serum ALT, AST, ALP and GGT of the treated groups were significantly reduced in the treated groups (Tables 3a and 3b) when compared with the untreated positive control group ($P<0.05$) and it followed a dose-dependent manner. This indicates a restoration of liver integrity. Liver organic molecules (TB and DB) reduced upon the administration of the extract indicating its hepatoprotective effect (Table 4a and 4b) (Somsak *et al*., 2015; Thiengsusuk *et al*., 2013; Zulkefli *et al*., 2013; Viriyavejakul *et al*., 2014). Similarly, the levels of serum electrolytes (sodium, calcium, phosphorus and chloride) of the treated groups reduced significantly when compared with the positive control (untreated malarial group) ($P<0.05$) (Table 4). This report is consistent with the work of Ajayi *et al.* (2017). The marked reduction is an indication of the restoration of renal activities and follows a dose dependent manner.Serum urea, creatinine and uric acids which are biomarkers of renal damage of the respective treated groups were significantly reduced when correlated with the control groups ($P<0.05$) (Table 5). This strongly indicates a state of renal restoration in the malaria-sick mice groups treated with the *B. vulgaris* leaf extract and is in line with the reports of Ajayi *et al.* (2017) and Anigboro (2017).

In conclusion, aqueous leaf extract of *Bambusa vulgaris* reported in this study exhibited chemoprotective effect in malarial parasitized mice in a dose dependent manner and this could be attributed to its rich content of phytoconstituents, including flavonoids and phenols. The hepatoprotection and renal function restoration observed upon the administration of the plant extract indicate to a far reaching end that *B. vulgaris* leaf extract would be a promising natural antimalarial product devoid of side effects upon use, especially when administered within the dose range of 100 – 200 mg/Kg body weight investigated in this study.

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