

Antimalarial Activity of Crude Extract of *Buddleja Polystachya* Fresen (*Buddlejaceae*) Against *Plasmodium Berghei* in Mice

Nurahmed Mohammed^a, Mohammedberhan Abdulwuhab^b, Faiz Mohammed^{c*}

^{ac}School of Medicine, College of Medicine and Health Sciences, Dire Dawa University, Dire Dawa, Ethiopia

^bSchool of Pharmacy, College of Medicine and Health Sciences, Gonder University, Gonder, Ethiopia

Abstract: Malaria is one of the most serious widespread diseases encountered by human and it is the second leading cause of death from infectious diseases next to HIV/AIDS in Africa. People, in several parts of the world, use different traditional medicines for treating malaria and it has been reported that the roots, leaves, and flowers of various species of *Buddleja* are used for the same purpose. The aim of the present study was, therefore, to evaluate *in vivo* and phytochemical screening of secondary metabolites, safety and antimalarial activity of the crude extract of *Buddleja polystachya* in mice. The animals were divided into 5 groups. The first group served as negative control and was administered with vehicle (0.2ml of distilled water). Group two (B100), three (B200) and four (B300) were administered 100, 200 and 300 mg/kg *Buddleja polystachya* extract, respectively. Group five served as positive control group and was administered with chloroquine (10mg/kg/day). Antimalarial activity was evaluated using 4-day suppressive assays against *P. Berghei* by orally administering the mice with either vehicle, various doses of the *Buddleja polystachya* extract or chloroquine, while safety study was performed using acute toxicity studies by administering 2000mg/kg of the *Buddleja polystachya* extract to non-infected mice. The effect of crude extract was measured and analyzed using One-way ANOVA and paired *t*-test. The result showed that the extracts did not cause symptoms of toxicity at the given doses. Preliminary phytochemical screening of the leaves powder of the plant indicated the presence of major antimalarial secondary metabolites. The extract of *Buddleja polystachya* leaves exerted dose dependent parasitemia suppression, although the active responsible principles are yet to be identified, which need further studies to elucidate the antimalarial mechanism of their action. It can be concluded that crude extracts of *Buddleja polystachya* showed strong activities against *P. berghei* indicating that it contains some chemical constituents that possibly lead to antimalarial drug development

Keywords: Acute Toxicity; Anti-malarial activity; *Buddleja polystachya*; Malaria; Parasitaemia; *Plasmodium berghei*; Phytochemical Screening.

*^w here is the correspondence author?

I. Introduction

Malaria is one of the most serious and widespread diseases encountered by human. It is still a significant problem around the world due to the development of insecticide-resistant mosquitoes, drug-resistant *Plasmodium* parasites, and economic/ political instability in areas of the world [1-3]. It is a particular burden to pregnant women and children below 5 years of age. It can cause maternal anaemia, premature/still births, and low-weight babies [4].

Strategies for preventing and controlling malaria involve four key tools. Reduction of human-mosquito contact, reduce the total number of mosquitoes in an area, identifying and treating infected persons, and preventive treatment (chemoprophylaxis). Mefloquine and atovaquone-proguanil are the recommended chemoprophylactic antimalarial drugs in Ethiopia [5, 6]. Subsequently, treatment strategies of malaria aim to terminate the acute blood infection, to cure the clinical symptoms, to clear hypnozoites from the liver, and to prevent the spread of infection [7]. Various pharmacological agents available for this purpose are chloroquine, mefloquine, quinine, primaquine, pyremethamine, artemisinin derivatives like artesunate, artemether, arteether and amino alcohols like lumefantrine and halofantrine along with tetracycline, doxycyclines and sulfadoxime etc. [8]. Traditional medicinal systems of the world rely on plants as effective sources for treating malaria or lessening its crisis. It has been estimated that about 1200 species of plants are now currently in use in the traditional systems of various countries for the treatment of malaria [9]. In Ethiopia, for instance, it is estimated that about 80% of the population is still dependent on traditional medicine which essentially involves the use of plants. A number of studies have been conducted *in vivo* evaluation of the anti-malarial activity of Ethiopian traditional medicinal plants: Root of *Clerodendrum Myricoides*, Leaves of *Dodonea Angustifolia*, *Aloedebra*, *Dodonea Angustifolia* seeds, *Asparagus africanus* roots, leaves of *Clerodendrum* species, *Annona squamosa* (*Annonaceae*) etc. [10- 14].

A number of potential new anti-malarial metabolites from plant and marine sources have been reviewed. These include alkaloids, terpenes, quassinoids, flavonoids, limonoids, chalcones, peptides, xanthenes,

quinones, coumarins. It has been shown that extract of *Acacia nilotica* (L.)Willd, for instance, possess IC₅₀ (50% inhibitory concentration) values of less than 5 microgram/ml in vitro against *P. falciparum* of CQ (Chloroquine Phosphate)-sensitive, CQ-resistant and pyrimethamine-sensitive strain [15, 16].

***Buddleja polystachya* Fresen.**

The genus *Buddleja* is a flowering plant and, in fact, the name *Buddleia* is in honour of the Reverend Adam Buddle. It comprises about 100 species native of the tropical, America, Asia and Africa [17, 18]. It has been reported that several *Buddleja* species have been used in traditional medicine in many parts of the world, and previous phytochemical investigations led to the isolation of flavonoids, iridoids, phenylpropanoids, sesquiterpenoids and saponins [17, 18].

B. polystachya which is known as (“Anfare”) in Amharic, Madera (Afargna), Madere (Tigrigna), Metere (Eritrea) belongs to family *Buddlejaceae* (sometimes named *Buddleia* in the scientific and popular literature and) previously it was included in the *Loganiaceae*. It is a multi-branched shrub that grows to < 5 m, but can occasionally reach 12 m in favourable conditions. This tree is endemic to the semi-arid highlands flanking the red Sea in Eritrea, Ethiopia, Saudi Arabia, Somalia and Yemen [19].

It has been reported that the roots, leaves, and flowers of various species of *Buddleja*, in several parts of the world, have been used in folk medicine. It used as antiprotozoal, for liver diseases, bronchial complaints, dysentery, eye or skin inflammation and wound healing; as diuretic, sedative, analgesic, anti-inflammatory, antimalarial, antimicrobial anti hyperglycaemic, antispasmodic, and antioxidant agent [17-23]. In North Gondar of Ethiopia, for instance, the juice of *B. polystachya* leaves is given orally for ten days in the morning and evening to cure malaria [24]. Additionally, in west Ethiopia fresh leaves of this plant is used for treatments of eye disease in live stock [25]. There are also reports that the plant used for treatments of headache and migraine [26]. Although a great number of Ethiopia people rely mainly on traditional medicines, generally, and *B. polystachya* leaves, particularly, for their primary health care needs against malaria, the effectiveness of these traditional medicines has not been scientifically evaluated. Consequently, this study intended to investigate the acute toxicity and antimalarial properties of *B. polystachya* leaves.

Hypothesis Crude extract of the leaf of *B. polystachya* possess some antimalarial effect.



Figure 1: Morphological view of *B. polystachya* Fresen.

II. Materials and Methods

1.1. Plant material preparation

Fresh leaf of *B. Polystachya* (100 g) was collected during the month of November, 2013 from University of Gondar maraki campus, 750km northwest to Addis Ababa, Ethiopia. The plant was identified by a taxonomist, and a voucher specimen (001) was deposited in the National Herbarium, College of Natural Sciences, Addis Ababa University.

Leaves of *B. polystachya* was thoroughly washed with distilled water to remove dirt and dried under shade and crushed into coarse powder. The powdered plant material was macerated in 80% of methanol for 72 h with occasional stirring. The filtrate was separated from the mark using Whatman Number 1 filter paper and the mark was re-macerated three times. The filtrates were combined and dried in an oven at the temperature not exceeding 40 °C. The dry extract was blue black dried powder and solid at room temperature. It was weighed by analytical balance and the yield was 34.85%. It was then transferred into vials and kept in desiccators until use [27, 28].

For this study, 80% of methanol, instead of water, was used to get greater percent of extract yield based on previous studies conducted [21]. Importantly, methanol serves as a less medium for the occurrence of the micro-organisms, it is more efficient in cell wall and seed degradation as well as it has low or no enzyme activity as compared to water [29]. Additionally, alcoholic or hydro alcoholic extract of plant materials contain a wide variety of polar (and moderately polar) compounds [30, 31].

1.2. Preliminary phytochemical screening

Standard tests were employed to detect the major secondary metabolites such as phenolic compounds, saponins, flavonoids, Tannins, Sterols, Resins, Anthraquinones, alkaloids, Cardiac glycosides and Terpenoids [29, 32]. Look Annex 1.

1.3. Experimental animals

A total of 30 adult albino mice of both sex were used for the whole experiment and they were obtained from colonies in the animal unit of the University of Gondar, College of Medicine and Health Sciences, Department of Pharmacology. They were 6-8 weeks of age and had weights ranging from 20 to 36 g. They were housed in plastic cages with standard wood chip bedding and had access to food and water *ad libitum*. Light was in its natural cycle (for a 12 h on and 12 h off). Care and handling of the mice was performed according to the guidelines given by OECD and ILAR [33-35].

1.4. Acute toxicity test

The crude extract was evaluated for its toxicity in *P. berghei* non-infected young female, nulliparas and non pregnant, Swiss albino mice aged six to eight weeks and weighing 28-34g. Oral Acute toxicity study was conducted based on the limit test as per the internationally accepted protocol drawn by OECD [36]. The mice were kept for week prior to dosing to acclimatize and then fasted for 3h food but with normal supply of water. The fasted body weight (BW) of each animal was determined and the doses were calculated according to the body weight. The test substance was administered at 2000mg/kg by oral route. After the administration, food was withheld for a further 2 hours. Animals were observed continuously during the first 30 min after dosing and observed periodically (with special attention given during the first 4 hours) for the next 24 hours and then daily thereafter for 14 days. All observations were systematically recorded with individual records being maintained for each animal within the 24 hours test period. Clinical signs observed included increased motor activity, anaesthesia, tremors, rolling, colonic convulsions, tonic extension, lacrimation, pilo-erection, salivation, muscle spasm, writhing, depression, stimulation, blanching, and cyanosis. Following the results from the first mouse, other two mice were recruited and fasted for 3 h and administered a single dose of 2000 mg/kg and observed in the same manner. These observations continued for further 14 days for any signs of overt toxicity, and individual weights of animals were recorded before the administration of drug on 1st day of the study and thereafter on the 7th and 14th day of the experiment [36].

1.5. Parasite

P. berghei ANKA (Antwerp/Kasapa) strain (Chloroquine sensitive) were obtained from EHNRI and the parasites were subsequently maintained in the laboratory by serial blood passage from infected mice to non-infected ones on weekly basis.

1.6. Plasmodium berghei infection

CQ-sensitive strain of the rodent parasite *P. berghei* was kept alive by continuous intra peritoneal (IP) passage in mice on a weekly base. Percent parasitaemia of the donor mouse was first determined (about 20 to 30% Para) and blood was collected by gentle cardiac puncture from the donor mouse using syringe after it has been sacrificed using Ketamin 0.2ml. Then, 1ml of blood was diluted with 4ml of physiological saline (0.9%) which contain 3.8% Tri sodium Citrate (TC) to maintain isotonic condition, such that 1ml of the dilution contains 5×10^6 of infected erythrocytes. Therefore, each mouse was infected on day zero (D0) intraperitoneally with 0.2ml dilution of infected blood (standard inoculums) containing approximately 1×10^6 *P. Berghei* parasitized red blood cells (10 million parasites in 0.2) [37].

1.7. Antimalarial activity (4-day suppressive) test

1.7.1. Grouping and dosing of animals

The animals were randomly divided into 5 groups, each comprising five animals. Group I served as negative control and was administered 0.2ml of distilled water (d_{H2O}). Group II-IV were administered three dose levels of the extract: Low-100mg/kg (B100), Moderate-200mg/kg (B200) and High-300mg/kg (B400). Group V served as positive control and treated daily with 10mg/kg of CQ. All the extract and the drug were administered orally for four consecutive days (starting from day 0 in a 24hr schedule) using oral gavage to insure safe ingestion. The various doses for the extract were selected based on previous reports [12, 21, 38] and then administered based on both their body weight and group.

1.7.2. Parasitological study

On day 4 (96hours) post infection, drop of blood was collected from the mice by venesection of the tail and transfereed onto the edge of a microscopic slide and drawn evenly across a second slide to make a thin and

thick blood film then allowed to dry at room temperature, fixed with methanol, stained with 10% Giemsa stain for 15 minutes. Slides were viewed using light microscopy with oil immersion (100x magnification). Parasitemia is determined microscopically by counting in random 4 fields of approximately 100 erythrocytes per field. The parasite count was performed by an experienced technician (blinded) and Percent parasitemia was calculated according to the following formula [39, 40].

$$\% \text{ paristemia} = \frac{\text{Total number of PRBC}}{\text{Total number of RBC}} \times 100 \dots\dots\dots (1)$$

Where: - PRBC: Parasitized red blood cell; RBC: red blood cells

Average (Av.) percentage of parasitemia suppression (APPS) was calculated as follows:

$$\text{APPS} = \frac{\text{Av. \% of parasitemia in control} - \text{Av. \% of parasitemia in test}}{\text{Av. \% of parasitemia in control}} \times 100 \dots\dots\dots (2)$$

1.7.3. Determination of body weight

The body weights of the mice were measured to observe whether the test extract of *B. polystachya* leaves extract prevent body weight loss that is commonly reduced with increasing parasitaemia in infected mice. The weights were taken on D0 (after infection is initiated) and D4 (Day four) [13].

1.7.4. Determination of mean survival time

Survival time was recorded to observe the effect of the extract for improvement in survival days. The animals were fed ad libitum and was observed for about 28 days. Any death that occurred during this period was noted to determine the mean survival time (MST) [11].

$$\text{MST} = \frac{\text{sum of days of survival of animals in group}}{\text{total number of animals in group}} \dots\dots\dots (3)$$

1.8. Statistical analysis

Data obtained from the study are presented as mean ± standard error of mean. Data Analysis was performed using Statistical Package for Social Science (SPSS), version 20. Comparison of levels of parasitaemia, body weight and survival times of the *P. berghei* infected mice in non infected mice values between the control and extract treated groups were determined by one-way ANOVA followed by post hoc (Tukey method), while multiple comparison and paired t-test were employed to test significance for the difference between initial and final results within the same group. The significance was set at p<0.05.

III. Results

1.9. Phytochemical study

The result of preliminary phytochemical screening of the hydro alcohol leaf extract of *B. polystachya* is found in Table 1 and it revealed that the extract contains phenolic compounds, saponins, flavonoids, tannins, sterols, resins, anthraquinones while alkaloids, cardiac glycosides and terpinoids compounds were absent. The test was done by using specific chemicals based on Annex 1.

Table1: Phytochemical screening result of the leaf extract of *B. polystachya*.

Test for secondary metabolite	Observed Results
Flavonoides	+
Saponins	+
Phenols	+
Tannins	+
Sterols	+
Resins	+
Anthraquinones	+
Cardiac glycosides	-
Alkaloids	-
Terpinoids	-

Key: + Present, - absent

1.10. Acute toxicity

Acute toxicity study of the hydro alcohol extract of *B. polystachya* was conducted through oral administration of a single dose of 2000 mg/kg. Gross physical and behavioural observation of the experimental mice revealed no visible signs of behavioural, neurological, autonomic or physical changes. Besides, the extract

did not cause mortality. The body weights of all mice had shown normal increments instead, which was found to be statistically insignificant ($P > 0.05$).

1.11. Antimalarial activity (suppressive) tests

Antimalarial suppressive test results of hydro alcohol extract of the leaves of *B. polystachya* against *P. berghei* in Swiss albino mice is shown in Table 2. When compared with the respective negative control group (Group I), the oral doses of the extract B100, B200 and B400 induced a statistically significant ($P < 0.05$) suppression of parasitaemia (40.00 %, 54.17 % and 66.67% respectively) on day four of post infection. Among the extract tested, the highest level of inhibition (66.67%, $P < 0.001$) was observed in B400, while the lowest suppression (40%, $P < 0.01$) was obtained in B100. These groups (B100, B200 and B400) were observed with significant ($P < 0.05$) lower percent parasitaemia levels of 14.4 ± 2.71 , 11.0 ± 1.00 and 8.00 ± 1.22 % respectively when compared with Group I (24.0 ± 1.87 %). The difference in parasitaemia reduction among extract treated groups, nevertheless, was statistically insignificant ($P > 0.05$). In the same assay, on day 4, Chloroquine had a chemo suppression of 100% at the dose level of 10 mg/kg/day and showed significant suppression ($P < 0.05$) when compared with extract treated groups. Percent parasitaemia level was directly proportional with concentration gradient of an extract.

Table 2: *In vivo* antimalarial suppressive test of methanol extract of the leaves of *B. polystachya* against *P. berghei* in Swiss albino mice (n=5)

Drug/extract	N	Dose mg/kg/day	Average % parasitemia	Average % parasitemia suppression	P value
Vehicle (Group I)	5	0.2 ml	24.00 ± 1.87	0.00	—
<i>B. polystachya</i> (Group II)	5	100 (B100)	$14.40 \pm 2.71^{A2, B3}$	40.00	0.004
<i>B. polystachya</i> (Group III)	5	200 (B200)	11.00 ± 1.00^{A3}	54.17	0.000
<i>B. polystachya</i> (Group IV)	5	400 (B300)	$8.00 \pm 1.22^{A3, B1B2}$	66.67	0.000
Chloroquine (Group V)	5	10	0.00^{A3}	100	0.000

Key: Data are expressed as means \pm SEM for five mice per a group; A =the mean value is significant when compared with vehicle treated group; B = mean value is significant when compared with Chloroquine treated group. 1=the mean value is significant ($P < 0.05$); 2= the mean value is significant ($P < 0.01$); 3= the mean value is significant ($P < 0.001$).

1.12. Determination of body weight

Between D0 (day zero) and D4 (day four), there was significant decrease in body weight in B200 ($P < 0.05$) and B400 ($P < 0.01$), and insignificant decrease ($P > 0.05$) for B100, Group I and Group V. In addition, analyses of variance conducted between the extract treated groups in comparison with the respective control have showed insignificant deviation. Extract of *B. polystachya* leaves did not prevent a loss of body weight in infected mice with increasing parasitemia.

Table 3: Effect of hydro alcohol extract from the leaves of *B. polystachya* on body weight of *P. berghei* infected Swiss albino mice (n=5).

Drug/extract	Dose mg/kg/day	WeightD0	WeightD4	P – value
Vehicle (Group I)	0.2ml	24.4 ± 1.35	22.28 ± 1.00	0.121
<i>B. polystachya</i> (Group II)	100 (B100)	27.06 ± 0.68	24.26 ± 0.96	0.147
<i>B. polystachya</i> (Group III)	200 (B200)	27.06 ± 0.36	$22.82 \pm 0.81^*$	0.021
<i>B. polystachya</i> (Group IV)	400 (B300)	27.68 ± 0.66	$25.68 \pm 1.09^*$	0.002
Chloroquine (Group V)	10	24.74 ± 0.77	23.98 ± 0.43	0.188

Key: Data are expressed as means \pm SEM for five mice per group; Weight D0: Weight pre-treatment on day zero; Weight D4: weight post-treatment on fifth day * = statistically significant ($P < 0.05$) between D0 and D4 within group.

1.13. Determination of mean survival time

Majority of the treated groups lived longer than the corresponding negative control. B100, B200 and B400 had mean survival time of 7.4 ± 0.75 , 10.0 ± 0.84 and 10.2 ± 0.73 respectively, while Group I (the negative control) lived 6.8 ± 0.37 , as shown in Table 4. Although all the extract treated groups lived longer than the negative control group, significant difference was observed with B200 and B400 ($P < 0.05$ and $P < 0.01$).

respectively) when compared with the Group I. Likewise, Group V (28.0 ±0.00) lived significantly longer than all first four groups (P<0.001)

Table 4: Effect of leaves crude extract on mean survival time

Drug/extract	N	Dose mg/kg/day	Mean survival days	P - value
Vehicle (Group I)	5	0.2 ml	6.8 ±0.37	-
<i>B. polystachya</i> (Group II)	5	100 (B100)	7.4 ±0.75 ^{B3 C1}	0.958
<i>B. polystachya</i> (Group III)	5	200 (B200)	10.0 ±0.84 ^{A1, B3}	0.013
<i>B. polystachya</i> (Group IV)	5	400 (B300)	10.2 ±0.73 ^{A2, B3}	0.008
Chloroquine (Group V)	5	10	28.0 ±0.00 ^{A3}	0.000

Key: Data are expressed as means ± SEM for five mice per group: A= the mean value is significant (P<0.05) when compared with Group I, B=the mean value is significant (P<0.05) when compared with Group V, C = the mean value is significant (P<0.05) when compared with Group III. 1=the mean value is significant (P < 0.05); 2= the mean value is significant (P < 0.01); 3= the mean value is significant (P < 0.001).

IV. Discussion

Typical acute toxicity (such as changes in general behaviours, variations in body weight and mortality) with the test extract was not observed during the given period of test [10, 41]. Since the maximum dose level recommended by OECD is 2000 mg/kg body weight, further dosing to estimate the median Lethal Dose (LD₅₀) of the plant was not performed. Actually, at the end of 14 days no death and no adverse effects of extract was recorded at 2000mg/kg, which was about 5 times the maximum effective dose tested (400 mg/kg). Based on the result, it can be stated that *B. polystachya*'s LD₅₀ value is greater than 2000mg/kg and this plant can be classified under category 5 in accordance with Globally Harmonised System of Classification and Labelling of chemicals [10, 42]. The rodent malaria model is the prominent step to screen most of the in vivo antimalarial activities of extracts and metabolites, even though it is not exactly similar to that of the human plasmodium infection [10, 44]. Importantly, pharmacological agents such as Chloroquine, Halofantrine, Mefloquine and more recently artemisinin derivatives have been studied using this model. More importantly, it is also cost effective to conduct preliminary pharmacological screening studies of crude extract in rodent model than primate model [13]. The *in vivo* model was employed for this study since it takes into account the possible prodrug effect and the possible involvement of the immune system in eradication of infection as compared to an *in-vitro* study [44]. In anti-malarial drug discovery process, there are number of established in-vivo murine plasmodium models, although plasmodium berghei was used in this study since it is an appropriate parasite that is most commonly used. Likewise, as this parasite is sensitive to and significantly suppressed by chloroquine, this drug was employed as a standard [44]. Interestingly, this 100% suppression effect of chloroquine was observed in this study.

The four-day suppressive test is a standard test commonly used for early antimalarial screening and the determination of percent inhibition of parasitaemia is the most reliable parameter [45]. In this study, the suppression activity of all doses of the extract was statistically significant in spite of its inconsistency with increasing dose on day 4. The extract of *B. polystachya* had shown highest level of parasitemia suppression (66.67%, P<0.001) in the higher dose with relatively longest survival time compared to the negative control group and the remaining two extract doses. Subsequently, it can be suggested that 400mg/kg of extract might be the optimal effective therapeutic dose. Interestingly, the present result is in line with hydro methanolic leaf extract of *Calpurnia aurea* (Fabaceae) [28]. The lower efficacy of the extracts (at all doses used) than Chloroquine may be in part due to unpurified/crude nature, non selectivity, pharmacokinetics and pharmacodynamic parameters of the crude extract [46]. Similarly, the *vitro* antibacterial properties of the methanol extract of *B. polystachya* was not as effective as the standard drugs (Ampicillin and Gentamicin), although it showed some activity against bacterial strains used [21].

Although mechanism of action of these secondary metabolites was not evaluated in the present study, its antimalarial activity might be attributed to the presence of phytochemical constituents (such as saponins and flavonoids) in many plants that produce a definite antiplasmodial activity which finally modify physiological action on the human body relate to malarial infection [47]. Species from the genus *Buddleja* have been reported to exhibit moderate antiplasmodic activity for H-hypoxanthin up take in cultured *plasmodium falciparum*. The active antiplasmodial constituents of *Buddleja polystachya* leaves may be novel or similar to those active constituents of *Buddleja globosa* [38].

It is a good step that the result had clearly showed that this extract contains saponins, polyphenols, tannins & flavonoids and these chemical compounds may be acting in synergy with one another to exert the observed antimalarial activity of the extract [12]. Like other antimalarial agents, the mechanism of action of this plant might be due to antioxidant property, immunomodulatory effects, intercalation with the parasite DNA,

protein binding, enzyme inhibiting properties, and anti inflammatory activity [11, 46, 48, 49]. Moreover, the confirmed *in vitro* antibacterial activity of the species within the genus *Buddleja* support the present study in which the genus is rich in active antibiotic phytochemical ingredients that could probably suppress parasitaemia of the malaria parasites. Consequently, the extract could have elicited its action through any of the above mentioned mechanism or by some other means yet to be determined.

Regarding the effect on body weight, it has been observed that the extract's preventive effect on loss of weight decreased with increasing dose. This might indicate that the plant could have an increased anorexic effect with escalating dose. This appetite suppressing activity of the extract might be ascribed to saponins, flavonoids, and phenolic compounds [43]. Importantly, this result is consistent with previous reports on hydro methanolic extract of *Calpurnia aurea* (*Fabaceae*), *Dodonaea angustifolia* and other plants [11, 28, 50]. The result of the present study on body weight, however, was not similar with that of Chloroform and Butanol fractions of *D. Angustifolia* [13].

The extract prolonged the mean survival time of the mice indicating that it suppresses *P. berghei* as well as reduces the overall pathologic effect of the parasite on these mice [12]. This study is similar with other study made on the effect of hydro alcoholic extraction of *D. Angustifolia* on mean survival time [11]. Similarly, like other *in vivo* antimalarial studies, the longest mean survival time of the mice was strongly associated with the maximum parasitemia inhibition [51].

Importantly, a compound is considered as an active antimalarial agent when it reduces parasitaemia level by $\geq 30\%$ which gives a sufficient ground to claim that the result of the parasite suppression of the present study showed antimalarial activity [37]. More importantly, an *in vivo* anti-plasmodial activity can be classified as moderate, good and very good if an extract displayed percent parasitemia suppression equal to or greater than 50% at a dose of 500 mg, 250 mg and 100 mg/kg per day respectively [52]. Based on this classification, the parasitemia chemosuppression of this extract was fairly below 50% at B100, while the optimum doses (B200 and B400) has provided a drastic parasite suppression ($>50\%$). Subsequently, it can be concluded that the hydromethanolic leaf extract of *B. Polystachya* exhibited a good anti-plasmodial activity.

V. Conclusion

This study collectively indicates that the hydro alcoholic extract of *B. polystachya* plant is not toxic and does not cause major acute toxic symptoms on acute toxicity test bases. The extract also possesses promising anti-plasmodial activity, which can be more evaluated for its profound activities by further researches. It contains phenolic compounds, saponins, flavonoids, tannins, sterols, resins and anthraquinones, while alkaloids, cardiac glycosides and terpenoids compounds are absent.

VI. Limitations of the study

1. The use of only polar extract instead of non polar extract. This means some of the compounds which may have a therapeutic effect in an extract may be absent in the test samples.
2. Unavailability of culture conditions or *in vitro* assay which may reduce the use of animals and also gives more representative (confirmatory) outcomes in the antiplasmodial assays than study *in vivo* alone.

Conflict of Interest

We declare that there are no conflicts of interest to disclose.

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Annex 1

Phytochemical Screening Test (Qualitative Method)

1. Test for Flavonoids: Weigh 1g of extract dry powder and dissolve 10ml of distilled water shake gently and filter with whatman filter paper no.1, add 5ml of 10% ammonia solution and add 1ml of concentrated sulphuric acid. A yellow coloration that disappears on standing indicates the presence of flavonoids.
2. Test for tannins: weigh 0.5g of the extract dry powder and dissolve in 10ml of distilled water and boiling for 20 minutes at 100 °C in a water bath and filter using Whatman filter paper and add 3 drops of 0.1% of ferric chloride to the filtered solution. A brownish green or blue black coloration indicate the presence of tannins.
3. Test for saponins: weigh One gram of extract dry powder and dissolve in 5ml distilled water, boiling for 20 minutes at 100 °C in a water bath and filter using Whatman filter paper and add 3ml distilled water to the filtrate, shake vigorously for about 5 minutes. Frothing which persisted on warming is taken as an evidence for the presence of saponins.
4. Test for cardiac glycosides (Keller-Killani test): weigh 0.5g of dry powder extract and dissolve in 5ml of distilled water, treated with 2 ml of glacial acetic acid, add one drop of 0.1% ferric chloride solution and finally add 1ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.
5. Test for terpenoids (Salkowski test): weigh 1g of dry extract powder, dissolve in 10ml distilled water to form 10% w/v solution of the extract, add 2ml of chloroform and shake, then add 3ml concentrated sulphuric acid carefully to form a layer. A reddish brown colouration of the interface formation indicates positive results for the presence of terpenoids.
6. Test for sterols (Liebermann-Burchard test): weigh 0.2g of dry extract powder, dissolve in 10ml chloroform and shake to ensure dissolution, add 2ml of 70% acetic acid to the solution cool in ice in refrigerator for 15 minutes and add 2ml of concentrated sulphuric acid carefully. A blue green ring indicates the presence of sterols.
7. Test for resins: weigh 0.5g of dry extract powder, dissolve in 10ml of 95% ethanol, boil it for 20 minutes at 100 °C in a water bath and add 5ml distilled water. The formation of a precipitate indicates the presence of resins.
8. Tests for anthraquinones (Borntrager's test): weigh 1g of dry powder extract, dissolve in 10 ml of benzene shake, filtered with whatman filter paper, add 5ml of 10% ammonia solution to the filtrate and shake. The presence of a pink, red or violet colour in the ammonical (lower) phase indicates the presence of free anthraquinones.
9. Test for phenols: weigh 0.2g of dry powder extract, dissolve in 5ml of 95% ethanol, and then add 2 drops of 1M ferric chloride solution. The appearance of intense colour indicates the presence of phenols.
10. Test for alkaloids: Weigh 1g of methanolic dry extract and dissolve 10ml of acid alcohol and boil for 20 minutes at 100 °C in a water bath and filter using Whatman filter paper, take 5ml of the filtrate, add 2ml 10% ammonia, 5ml chloroform and shake gently to extract the alkaloidal base. Extract the chloroform layer with 10ml of 70 % acetic acid and divide the solution in two portions in a test tube and add 2 drops of Mayer's reagent to one portion and 2 drops of Dragendorff's reagent to the second portion. The formation of cream with Mayer's reagent and reddish brown precipitate with Dragendorff's reagent is regarded as positive for the presence of alkaloids.