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EVALUATION OF ANTIMALARIAL ACTIVITY OF *BOSCIA SENEGALENSIS* (CAPPARACEAE) AGAINST *PLASMODIUM BERGHEI* INFECTION IN MICE

Famoriyo OP[1], Yakubu MI[2*], Bello OS[4], Jimoh AA[2], and Abbas MY[3]

1. Department of Pharmacology and Therapeutics, University of Lagos, Nigeria
2. Department of Pharmacology and Toxicology, Kaduna State University, Kaduna, Nigeria
3. Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria
4. Dept. of Pharmacology, College of Health Sciences, Usmanu Danfodio University, Sokoto

Corresponding author: Email: mustycin@yahoo.com; Phone: +2348032874478

ABSTRACT

Aims: This study aimed at evaluating the antimalarial potential of the methanol stem bark extract of *Boscia senegalensis* in mice.

Study design: Up and Down procedure using Organization for Economic Cooperation and Development (OECD) guideline for LD_{50} determination, suppressive and curative tests for the antimalarial study.

Place and Duration of Study: this study was conducted in the department of Pharmacology, College of Health Sciences, Usmanu Danfodio University, Sokoto from February to May 2013.

Methodology: The oral median lethal dose of the methanol stem bark extract of *Boscia senegalensis* was determined using the OECD guideline 425: Up and Down Procedure. Phytochemical screening of the extract was done using standard methods. *In vivo* antiplasmodial activity was assessed by suppressive and curative tests using *Plasmodium berghei* infected mice, according to the method previously described by Peters and Robinson.

Results: Phytochemical screening revealed the presence of alkaloids, terpenoids, saponins and anthraquinones, and the oral median lethal dose of the extract was estimated to be greater than 3,000 mg/kg. The extract demonstrated a dose-dependent significant reduction of parasitemia ($p<0.05$) in suppressive and curative test**s**, produced a significant ($p<0.001$) increase in mean survival time of *P. berghei* infected mice in dose dependent manner in curative test compared to the negative control and the standard drug (choloquine 5 mg/kg/day).

Conclusion: The methanol stem bark extract of *Boscia senegalensis* possess significant antimalarial activity and may be a potential source of a new antimalarial drug or lead compound.

*__Keywords__: *Boscia senegalensis*, LD_{50}, Antimalarial activity, *Plasmodium berghei*

INTRODUCTION

Malaria is a life-threatening parasitic disease that is transmitted through the bite of infected female anopheles' mosquitoes [1]. It is one of the world'**s** deadliest infectious diseases [2]. Malaria is a febrile illness characterized by cycles of chills, fever, pain and sweating. It has a significant burden on public health and economic stability with direct impact on households' income, wealth and productivity of both the sick and the caregivers [3]. Malaria is caused by five different species of *Plasmodium* parasite; *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae, and Plasmodium knowlesi*, and two of these species *P. falciparum* and *P. vivax* pose the greatest threat to human [1]. According to World Health Organization, *P. falciparum* accounted for 99.7% of estimated cases of

malaria in W.H.O African Region for the year 2017 [4].

According to World Malaria Report, 2018, there were 219 million cases of malaria in 2017 and children under five years of age accounted for 61% (266,000) of all malaria deaths worldwide, 92% of the cases and 93% of malaria deaths occurred in Sub-Saharan Africa, five countries accounted for nearly half of all malaria cases worldwide: Nigeria (25%), the Democratic Republic of the Congo (11%), Mozambique (5%), India (4%) and Uganda (4%), and the total funding for malaria control and elimination in 2017 was US$ 3.1 billion, [4]. According to this report, Nigeria has one of the highest burdens of malaria in the world. Situated between 4° and 13° Northern Latitude, Nigeria has a suitable climate for malaria transmission throughout the country. In Nigeria, malaria accounts for 60% of outpatient visits, 30% hospitalization with children under five years of age mostly affected [6]. The combination of favourable weather conditions for the breeding of mosquitoes, abundant numbers of efficient mosquito vectors and a large reservoir of infected persons, poverty and systemic inadequacies in Nigeria and other developing countries made these countries malaria endemic region [7]. Despite the great efforts and huge financial contribution made globally towards malaria eradication and elimination, it continues to be a major public health challenge in developing countries, especially countries in Sub-Saharan Africa [6]. More so, the increasing *Plasmodium* resistance to antimalarial drugs such as primaquine and chloroquine over the few decades has risen the mortality and morbidity due to malaria in Africa and created a major setback in the fight against malaria and its attendant complications [8, 9, 10]. The *Plasmodium* resistance to the anti-folates and 4-aminoquinolines antimalarial drugs led the WHO to recommend the current artemisinin-based combination therapy (ACT) to treat *P. falciparum* malaria infections [5, 11]. However, the reported incidence of decreased sensitivity of *Plasmodium* parasites to the ACT drugs is now of great concern and challenge to the global goal of malaria elimination and eradication [2, 12]. Therefore, there is urgent need for effective, safe, affordable and accessible alternative new anti-malarial agents [13, 14].

Medicinal plants have been used to treat malaria for thousands of years and are the source of the two main groups of modern antimalarial drugs; quinine and artemisinin derivatives, both of which have served as chemical leads to several synthetic antimalarial analogues [11]. In Africa, the use of indigenous herbal plants still plays an important role in malaria treatment and these plants might be interesting sources for the detection of novel antiplasmodial compounds [15]. *Boscia senegalensis* is an evergreen shrub usually 1-2m tall, indigenous to Mauritania, Sudan, Burkina Faso, Niger, and Nigeria. It is commonly called Hemmet (Arabic), Anza (Hausa, Northern Nigeria) and is used traditionally as anthelminthic, in treatment of syphilis, pruritis, schistosomiasis in Senegal, Sudan, Niger and Nigeria [16]. There is a folkloric claim of its use as antimalarial herb by the Hausas in Northern Nigeria (undocumented). However, there is no available scientific data to validate the antimalarial properties of *Boscia senegalensis*. Consequently, it is important such folkloric claim is investigated in order to establish its efficacy, safety and to

determine its potential as sources of new antimalarial drugs. The aim of this study was, therefore, to evaluate the anti-malarial activities of stem bark extract of *Boscia senegalensis* in Swiss albino mice.

MATERIALS AND METHODS

Experimental animals and Parasites

Male Swiss albino mice (28–31g) obtained from the animal unit of National Veterinary Research Institute, Vom-Jos, Nigeria were used for the study. The animals were kept in standard cages and maintained under standard hygienic conditions with free access to water and food, all experiments were carried out in accordance with the standard protocols of National Institute of Health [17] Guidelines for use and care of laboratory animals. Chloroqine sensitive *Plasmodium berghei* NK65 (donated by Malaria Research Reference and Reagent Resource (MR-4, USA) was obtained from the Institute for Medical Research and Training, College of Medicine, University of Ibadan, Nigeria.

Preparation of plant extract

The stem bark of *Boscia senegalensis*, Pers (Capparaceae) was collected from Achida area of Sokoto, Northern Nigeria in February, 2013. The plant material was authenticated by Mallam Muhammed Musa at the Herbarium section, Department of Biological Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria by comparing with existing specimen and it was allocated the Voucher Specimen Number (900537). The plant materials were washed, the bark peeled off, thinly sliced, dried under shade, size reduced to powder using mortar and pestle and extracted with 70% **v/v** methanol using Soxlet extractor (Electrothermal Engineering, UK). The extract was then transferred to small aluminum plate, evaporated and concentrated to dryness in a water bath at 37^{o}C to obtain the dry crude extracts of the plant. The dried crude methanol extract was stored in a refrigerator at 4^{0}C in air tight plastic container until used for this study.

Phytochemical Screening

Phytochemical screening of the methanol stem bark extract of *Boscia senegalensis* was carried out according to the methods described by Trease and Evans [18].

Acute toxicity study

The limit test dose, Up and Down procedure of Organization for Economic and Cultural Development [19], was employed. Five female non pregnant mice used for the study were randomly selected and kept in a separate cage for 5 days prior to the dosing. Food (but not water) was withheld from the animals 3 hours before the study. Freshly prepared methanol extract of the plant at 3000mg/kg body weight were administered orally to the mice (one after the other after every 48 hours) and observed intermittently for signs of toxicity such as difficulty in movement, weakness, loss of appetite, and mortality for the next 24 hours. The animals were allowed access to food and water 1 hour after the extract administration. All the five mice were observed thereafter for the next fourteen days for any delayed toxic effect.

In *Vivo* Antimalarial Test

Parasite inoculation

Swiss Albino mice previously infected with *P. berghei* having variable parasitemia were used as donor. The parasitemia (usually 20 – 30%) of the donor mice was first determined. The donor mice (one donor mouse is usually used for the same experiment to ensure

uniformity) were then humanly sacrificed and blood sample was collected by severing the jugular vein in petri dish having 0.5% trisodium citrate (TSC) added as anticoagulant. The blood was then diluted with physiological saline (0.9%). The dilution was made based on the percentage parasitemia of the donor mouse and the RBC count of normal mice ($4.5x10^9$ RBC/ml) [20] in such a way that 1ml blood contains $5x10^7$ infected erythrocytes. A standard inoculum of approximately 1 $x10^7$ *P. berghei* infected red blood cells (RBC) was administered by intraperitoneal route (0.2 ml) to each test mouse.

Suppressive Test

The screening was based on the 4-day parasitaemia suppression test against *P. berghei* infection in the mice as reported by Peters [21]. Three hours after inoculation of the parasite, the mice in the treatment groups were administered with the extract in doses of 50, 100 and 200 mg/kg for four consecutive days. The two control groups were administered with distilled water and chloroquine phosphate at 5mg/kg/day daily for four consecutive days. All drugs were intraperitoneally administered. Twenty-four (24) hour after last treatment (i.e. 96-hour post infection), thin blood films were prepared from the blood collected from the tail of each mouse. The films were air dried, fixed in methanol for 30 seconds, and stained with 10% Giemsa for 30 minutes. The slide was rinsed carefully and thoroughly under running tap water and left to stand in an upright position to dry [22]. Prepared slides were then viewed under the x100 objective (oil immersion) light microscope (Kyowa XSZ – 21) with special ocular and condenser sufficiently close to give a good contrast. Photomicrograph of the fields were taken with electronic eye piece (YJEYE01 -130) connected to a computer via USB cable. The parasitemia was determined by counting minimum of three fields per slide with 100 RBC per field [23]. The percentage suppression of parasitaemia was calculated for each dose level by comparing the parasitaemia in infected controls with those treated. According to the modified method of Peters and Robinson [24], percent parasitemia and inhibition were calculated as:

$$\%\ Parasitaemia = \frac{Number\ of\ parasitized\ RBC\ \times 100}{Total\ number\ of\ RBC}$$

$$\%\ Parasite\ inhibition = \frac{parasitemia\ in\ control\ -\ parasitemia\ in\ treated\ groups}{parasitemia\ in\ control} \times 100$$

Curative (Rane's) test

The method of Peters [21] method was used. 25 mice were infected with 0.2 ml of the standard inoculums, weighed and labeled on Day 0. Seventy-two (72) hours post inoculation, the mice were divided into 5 groups of five mice each. They were intraperitoneally treated daily with three different doses of the extract of *B. senegalensis*, (50 mg/kg, 100 mg/kg and 200 mg/kg), Chloroquine (5 mg/kg) as positive control and an equal volume of Normal Saline (negative control) for five days (D1 – D5). Thin films stained with Giemsa stain were then prepared from the tail blood of each mouse daily for 5 days to monitor the parasitaemia level. The mean survival time for each group was determined arithmetically

by finding the average survival time (days) of the mice (post inoculation) in each group over a period of 28 days (day 0 to day 27).

Data Analysis

Data were analyzed with One-way Analysis of Variance (ANOVA) followed by Tukey-Kramer multiple comparison post hoc test. Results were expressed as mean ± SEM and considered statistically significant at ($P \leq 0.05$).

RESULTS AND DISCUSSION

Phytochemical Screening

The result of the preliminary phytochemical screening of ethanol stem bark extract of *B. senegalensis* revealed the presence of alkaloids and terpenoids in high quantities, saponins was moderate and anthraquinones in trace amount as shown in Table 1. Several classes of plant secondary metabolites are responsible for antimalarial activity, but the most important and diverse biopotency has been observed in alkaloids, quassinoids and sesquiterpene lactones [25]. The extract of *B. senegalensis* contain alkaloids, saponins, terpenoids and anthraquinones classes of secondary metabolites. These classes of compounds have been reported to have shown antiplasmodial activity in other plants [26, 27]. Thus, the antimalarial activity observed in this study could have resulted from these secondary metabolites acting singly or synergistically. According to Saxena et al., [25], alkaloids are one of the major classes of compounds possessing antimalarial activity. In fact, one of the oldest and most important antimalarial drugs, quinine, belongs to this class of compound and is still relevant till today.

Table 1: Phytochemical constituents present in methanolic stem bark extract of *Boscia senegalensis*

Phytochemical constituents	Inference
Terpenoids	+++
Alkaloids	+++
Cardiac glycosides	-
Saponins	++
Anthraquinones	+
Flavonoids	-
Carbohydrate	+++
Proteins	+++
Tannins	-

Keys: + = Present - = Absent

Acute Toxicity of the extract (Median lethal dose (LD_{50})

The extract of *B. senegalensis* was tolerated by the mice when administered orally up to a dose of 3,000 mg/kg body weight, no death was observed. This shows that the median lethal dose of *B. senegalensis* crude extract was estimated to be greater than 3,000 mg/kg body weight. However, it was observed that the mice showed weakness and refusal to feed for about half an hour after treatment with the extract but the animals later regained strength and began to feed normally. Although medicinal plants are assumed to be safe, many of them are potentially toxic [28]. In this study, no death or signs of toxicity was observed and the median lethal dose (LD_{50}) of *B. senegalensis* crude extract was estimated to be greater than 3,000 mg/kg. According to OECD 425 guidelines [19], any substance with an LD_{50} > 2000mg/kg but < 5000mg/kg is considered to be of relatively

low acute toxicity hazard. Thus, the fact that *Boscia senegalensis* did not cause mortality up to a dose of 3000 mg/kg which is much higher than the minimum effective dose tested (100 mg/kg) is an indication that the plant is safe following oral administration in mice. This could explain the safe folkloric use of the plant in Africa.

Effect of the extract on Parasitemia and Chemosuppression

The extract produced a dose dependent chemosuppressive effect at various doses employed in this study. The chemosuppression were 30.69, 56.77 and 83.17% for 50, 100 and 200mg/kg/day doses respectively as shown in Table 2. The chemosuppression produced by the extracts were significant (P=0.05) at 50, 100 and 200 mg/kg/day as compared to normal saline and the standard drug (chloroquine 5 mg/kg/day). The chemosuppression of 83.17% produced by the extract at 200 mg/g is comparable to a chemosuppression of 87.79% produced by the standard drug (choloquine 5 mg/kg/day). The effect of the extract on chemosuppression of the plasmodium parasite was aslo demonstrated in plates 3,4 and 5 as shown below.

The results of this study shows that the methanol stem bark extract of *B. senegalensis* possess dose-dependent antiplasmodial activity as evident from the parasite suppressive test. The extract exhibited a dose-dependent increase in chemosuppression of the parasites and the highest dose tested (200 mg/kg) produced 83.2% suppression compared to 87.8% suppression produced by the standard drug – chloroquine phosphate. There was no statistical difference between the reductions in parasitaemia at 200 mg/kg dose of the extract and Chloroquine 5 mg/kg (standard drug) as both of them produced similar chemosuppression. According to Carvalho et al., [29], a compound is considered to be active when reduction in parasitemia is greater than or equal to thirty percent. Based on this assertion, methanol stem bark extract of *B. senegalensis* is active against malaria infection. The strong chemosuppressive effect of the extract observed in the 4-day suppressive test demonstrate the potentials of this plant as possible a source of an antimalarial drug molecule.

This finding is in agreement with previous studies where several medicinal plant extracts have been reported to produce a similar level of parasitemia suppression. *Stachytarpheta cayennensis* [30], *Nigella sativa* [26], *Croton zambesicus* [27], *Anonna senegalensis* [28], *Boscia angustifolia* [31] with a percentage suppression of 78.2, 55.88, 80.7, 76.3 and 60.12 respectively. However, relatively lower antiplasmodial activities than the present result has been reported on *Asparagus africanus* [32], *Amarantus spinosus* [15], *Withania somnifera* [33] and *Clerodendrum myricoides* [34] with percentage parasitemia suppression of 27.84, 30.94, 33.75, and 31.7 respectively. The suppression of malaria parasites by the extract was also demonstrated in Plates (1, 2, 3, 4 & 5).

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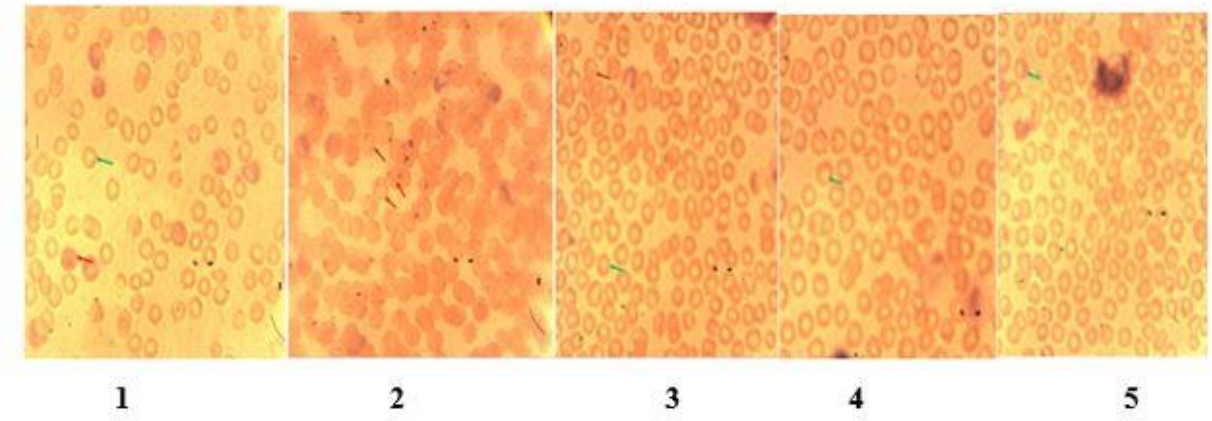
Table 2: Effect of methanol stem bark extract of *B. senegalensis* on parasitemia and chemosuppression of *P. berghei* infected mice

Drug/Extract	Dose (mg/kg)	Average % Parasitaemia	Average % Suppression
Boscia senegalensis Extract	50	21.00 ± 2.59*	30.69
Boscia senegalensis Extract	100	13.10 ± 1.26**	56.77
Boscia senegalensis Extract	200	5.50 ± 0.65**	83.17
Chloroquine Phosphate	5	3.70 ± 0.72**	87.79
Normal Saline	0.2 ml	30.30 ± 3.77	-

Parasitemia expressed as mean ± SEM, n=5, F = 26.197and *P*= 0.001 when compared to control.

*= *P*=0.05, **= *P*=0.001



Plates (1, 2, 3, 4 & 5): Photomicrographs of thin blood film of *P. berghei* infected mice treated with 5 mg/kg chloroquine (1), Normal saline (2), 50 mg/kg (3), 100 mg/kg (4) and 200 mg/kg (5) of methanol stem bark extract of *Boscia senegalensis* in Suppressive test

Showing RBC infected with *P. berghei* parasite
Showing normal RBC

Effect of the extract on Mean Survival Time

On established infection, it was observed that there was a daily increase in the parasitemia of the negative control group. However, there was a daily reduction in the parasitemia levels of the extract treated groups as well as the positive control (chloroquine) as summarized in Figure 1. The extract produced a significant mean survival time (*P*=0.001) in the treated mice as compared to the negative control in a dose dependent manner as shown in Table 3. However, the mice in all groups were not cured from the infection. The methanol stem bark extract of *B. senegalensis* prolonged the mean survival time of the mice indicating that the extract suppressed *P. berghei* in the mice. However,

neither the extract nor the standard drug produced a total parasite clearance. It can be noted that there was significant difference (P= 0.05) in the mean survival time produced by all doses of the extract tested and the standard drug (chloroquine phosphate). The present result on mean survival time is in agreement with similar studies done on extracts of *Stachytarpheta cayennensis* [30] and *Boscia anguastifolia* [31]. In established infection, the extract exerted significant suppression of parasitemia. The result of the curative test showed that the extract at 200 mg/kg produced a response which was similar to that of the standard drug (chloroquine 5 mg/kg/day). As shown in Figure 1 below, all treated groups produced different responses; the 200 mg/kg extract and standard drug brought about reduction of parasitemia after the second dose, it was on the day three for the 100 mg/kg extract while at 50 mg/kg extract, reduction of parasitemia was obtained on day four. However, none of the doses completely cleared the infection. This delay in the onset of action for the observed antimalarial activity at the lower doses of the extract may be an indication that a loading dose is required for the extract [35]. The result obtained in this study is similar to that of *Stachytarpheta cayennensis* [30], comparable to other stud**ies** done on root bark extract of *Gardenia ternifolia* [35] but unlike the 100% parasitemia suppression produced by hydroethanolic extract of the stem of *Baphia pubescens* [36]. The pronounced antimalarial activity observed in the established infection test (curative test) as shown in Figure 1 and plates (8, 9 & 10) demonstrate the potentials of this plant as possible a source of an antimalarial drug molecule.

Table 3: Mean survival time of *P. berghei* infected mice receiving various doses of methanol stem bark extract of *Boscia senegalensis*

Drug/Extract	Dose (mg/kg)	Mean survival time (day)
Boscia senegalensis Extract	50	12.40 ± 0.68
Boscia senegalensis Extract	100	18.60 ± 1.21**
Boscia senegalensis Extract	200	26.00 ± 0.20**
Chloroquine Phosphate	5	27.8 ± 0.20**
Normal Saline (control)	0.2ml	8.20 ± 0.37

Parasitaemia expressed as mean ± SEM, n=5, F = 96.622 and **= P = 0.001, significant when compared to control.

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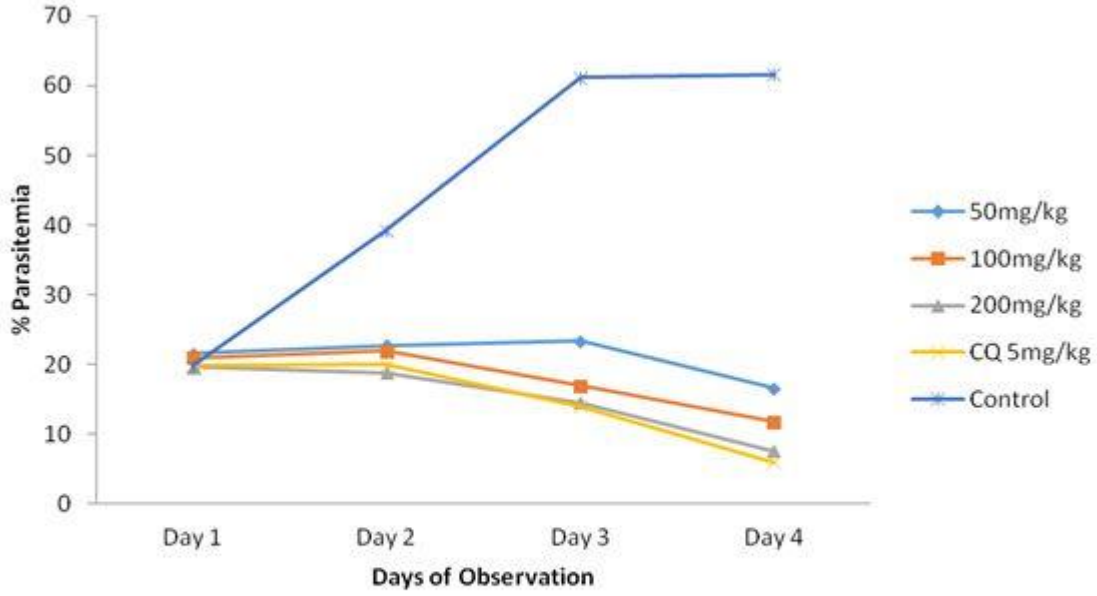
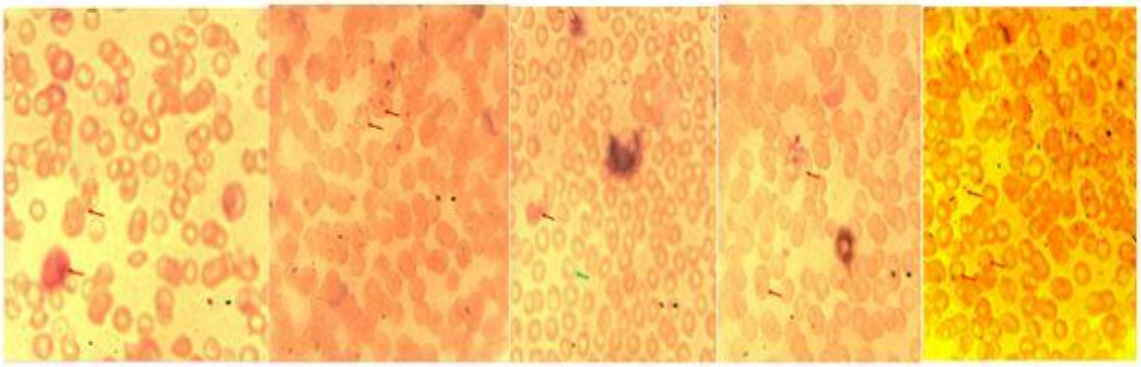


Figure 1: Effect of *B. senegalensis* on established infection (curative test)
CQ= Chloroquine phosphate 5 mg/kg/day, Control= Normal saline, n= 5



Plates (6,7, 8, 9 & 10): Photomicrographs of thin blood film of *P. berghei* infected mice treated with 5 mg/kg Chloroquine (6), Normal saline (7), 50 mg/kg (8), 100 mg/kg (9) and 200 mg/kg (10) of methanol stem bark extract of *Boscia senegalensis* in curative test

Showing RBC infected with *P. berghei* parasite
Showing normal RBC

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CONCLUSION

The present study indicated that methanol stem bark extract of *Boscia senegalensis* possess significant antimalarial activity and justify its use in ethnomedicine in the treatment of malaria. Therefore, the plant could be a valuable source of lead compounds for the development of new antimalarial agent.

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Conflict of interests

The authors of the manuscript declare that they have no conflict of interest.

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