Trop J Nat Prod Res, June 2022; 6(6):926-930 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Evaluation of *In vivo* Antiplasmodial Activity of the Methanol Root Bark Extract and Fractions of *Bombax costatum* (Bombacacea) in *Plasmodium berghei*-Infected Mice

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ARTICLE INFO

Article history:
Received 10 May 2021
Revised 17 June 2022
Accepted 29 June 2022
Published online 02 July 2022

ABSTRACT

Malarial infection is a disease that has defied many therapeutic and chemopreventive interventions due to persistent resistance to currently available drugs. This has led to the need to search for new Antiplasmodial drugs which are highly effective, less toxic and cost effective. This study is aimed at the determination of the *in vivo* antiplasmodial activity of the methanol extract and fractions of *Bombax costatum* in *Plasmodium berghei* infected mice. Preliminary phytochemical screening and Oral acute toxicity studies were carried out using standard protocols. Antiplasmodial activity of the methanol extract was investigated using 4-day suppressive, curative and prophylactic tests, while the fractions were evaluated using the curative test only. The phytochemical screening of the methanol extract and fractions of *Bombax costatum* revealed the presence of carbohydrate, cardiac glycosides, flavonoids, triterpenes, tannins, alkaloid, saponin and steroid. The oral median lethal dose was greater than 5000 mg/kg. The methanol extract at all tested doses (250, 500 and 1000 mg/kg) produced a significant ($p < 0.05$) dose dependent reduction in parasitaemia levels in the curative and 4-day suppressive tests compared to the standard drug (chloroquine, 5 mg/kg). However, there was no significant inhibition in the prophylactic test. The chloroform and n-butanol fractions at doses of 250, 500 and 1000 mg/kg significantly ($p<0.05$) inhibited *parasitaemia* levels compared to the hexane and ethyl acetate fractions in the curative test. This study provides evidence supporting the traditional use of *Bombax costatum in* the treatment of malaria.

Keywords: Antiplasmodial, *Bombax costatum*, Curative, *Plasmodium berghei*, Methanol extract

Introduction

Malarial infection is a disease that has defied many therapeutic and chemopreventive interventions due to persistent resistance to currently available drugs. This is driven by the most virulent specie of the plasmodium parasite, *Plasmodium falciparum*; hence it is regarded as the most deadly parasitic disease. This has led to the need to search for new Antiplasmodial drugs which are highly effective, less toxic and cost effective. This accounts for the paradigm shift to the use of medicinal plants, most especially in the tropics owing to the fact that they are sources of promising antimalarial drug.[1] Many medicinal plants have been reported to have significant Antiplasmodial activity and remain the main focus for scientists and researchers in the development of new antimalarial agents.[2] The famous and potent Antiplasmodial compounds quinine (obtained from *Cinchona* species) and artemisinin (obtained from *Artemisia annua)*were derived from plants.[3] Phytochemical compounds found present in medicinal plants including alkaloids.[4] phenolic compounds.[5] Anthraquinones.[6] and flavonoids.[7] Have been reported to possess Antiplasmodial activity.

Bombax costatum is a member of the family Bombacaceae and is commonly called red-flowered silk-cotton tree or red kapok tree.[8] The bark is thick, rough, corky, greyish brown in colour, and covered with conical pointed spines on the stem and branches. The leaves are digitally compound, ovate with 5-7 leaflets and the petioles are about 8-15 cm long. At both ends, the leaflets are partly acuminate and partly ovate with lateral nerves of 8-10 pairs. The flowers are solitary, bisexual and 5-7 cm with deep red, orange or yellow, tulip-shaped, glabrous peduncles. The calyx is cup-shaped, 12-17 mm long, truncate, 5- toothed. The plant has 5 oblong-linear petals 4.5 cm x 1.5 cm with a round apex. The fruits are contained in ellipsoidal capsule darkbrown in colour with variable shapes. The fruits are embedded in white floss called kapok and contain several small seeds.[9] In Nigeria, it is locally known as "Kurya" or "Gujjiya" in Hausa, "Joohi"or "Kuruhi" in Fulfulde and "Kutupkaci" in Nupe. Different parts of the tree are employed for the treatment of various ailments including malaria.[10] The study evaluated the *in vivo* antiplasmodial activity of the methanol extract and fractions of *Bombax. costatum* in *P. berghei* infected-mice.

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Citation: Ali BH, Mohammed I, Muhammed MA, Mohammed SY, Garba D, Busola OA, Khadijah II, Manager MM. Evaluation of *In vivo* Antiplasmodial Activity of the Methanol Root Bark Extract and Fractions of *Bombax costatum* (Bombacacea) in *Plasmodium berghei*-Infected Mice. Trop J Nat Prod Res. 2022; 6(6):926-930. doi.org/10.26538/tjnpr/v6i6.18

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Materials and Methods

Collection and identification of plant material

The root bark of *Bombax costatum* was collected from Basawa Local Government Area in Zaria; it was identified and authenticated at the Herbarium unit of the Department of Botany, Ahmadu Bello University, Zaria by comparison with herbarium reference voucher specimen. A voucher Number 1749 was collected for future reference.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Animal Ethical consideration
The study obtained permission from Ahmadu Bello University Committee on Animal use and care with approval number ABUCAUC/2021/108.

Experimental animals
Adult Swiss mice of either sex (19-25 g body weight) were acquired from Animal House facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. The animals were fed with laboratory diet and water *ad libitum* and maintained under standard conditions in cages at room temperature.

Malaria parasite
Mouse-infected chloroquine sensitive strain of *Plasmodium berghei* NK-65 was obtained from National Institute of Medical Research, Lagos. The parasite was kept alive by continuous intra-peritoneal passage in mice at the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria.

Extraction and partitioning of the plant material
The root bark of *Bombax costatum* was air dried under shade and pounded to coarse powder using mortar and pestle. The powdered root bark (2 kg) was extracted with methanol (80%v/v) by maceration for 7days. The solvent was allowed to air dry. Two hundred gram (200g) of the methanol crude extract was obtained. A portion of the crude extract (150 g) was then partitioned with n-hexane, chloroform, ethyl acetate and n-butanol to give the n-hexane, chloroform, ethyl acetate and n-butanol fractions respectively.

Table 1: Experimental design of the studies

Treatment group	Number of mice	Extract/drug/vehicle dose mg/kg
I	5	1 mL/kg distilled water
II	5	250 mg/kg extract
II	5	500 mg/kg extract
IV	5	1000 mg/kg extract
V	5	5 mg/kg chloroquine

Phytochemical screening
Preliminary phytochemical analysis of the crude methanol extract and the various solvents fractions were carried out to detect the secondary metabolites present in the plant by using the standard methods described by Trease and Evan.[11]

Acute toxicity study (LD_{50})
The oral LD_{50} of the methanol extract and the various solvent fractions in mice was conducted according to the method employed by Lorke.[12] The study was carried out in two phases; in the first phase, nine (9) mice were divided into three groups each containing 3 animals and were treated with the crude extract of the plant at doses of 10, 100 and 1000 mg/kg body weight orally and observed for signs of toxicity and death for the first 4 hours then after 24 hours. In the second phase, 3 groups each containing one mouse were giving 3 more specific doses; 1600, 2900, and 5000 mg/kg respectively based on the result of the first phase. They were observed for signs and symptoms of toxicity and death for the first 4 hours and after 24 hours. The median lethal dose was then calculated using the formula:

$$LD_{50} = \sqrt{minimumlethaldose \times maximumtolerateddose}$$

In vivo Antiplasmodial studies on the methanol extract
4-days suppressive test
The study was done according to the method described by Peters.[13] Twenty-five (25) Swiss mice (16-20g) were inoculated with standard inoculums containing approximately 1×10^7 *Plasmodium berghei* infected erythrocytes through the intra-peritoneal route. Two (2) hours post- inoculation; the mice were grouped into five (5) of five mice each. The extract at doses of 250, 500, and 1000 mg/kg was administered orally to groups 2, 3 and 4 respectively once daily for 4 days (day 0 to day 3). Groups 1 and 5 were taken as negative and positive controls and were treated with 10 mL/kg distilled water and 5 mg/kg chloroquine respectively. On day 4, thin blood smears were made on slides from the tail of each mouse. The slides were fixed with methanol, stained with 10% Giemsa for 15 minutes and examined the parasite count under the microscope. Average parasitaemia suppression was calculated using the formula below.[14]

$$\% \text{ suppression} = \frac{\% \text{ parasitaemia in control} - \% \text{ parasitaemia in treated group}}{\% \text{ parasitaemia in control}}$$

Curative test
The study was done according to the method of Ryler and Peters,[15] Twenty-five (25) mice were inoculated with standard inoculums containing approximately 1×10^7 *Plasmodium berghei* infected erythrocytes through the intra-peritoneal route. Seventy-two (72) hours post inoculation; the mice were grouped into 5 of 5 mice each. The extract at doses of 250, 500, and 1000 mg/kg was administered to groups 2, 3 and 4 respectively. Groups 1 and 5 were taken as negative and positive controls and were treated with 10 ml/kg distilled water and 5 mg/kg chloroquine respectively. Administrations was done orally once daily for four (4) days. On day seven (7) i.e. a day after last treatment, thin blood smears were made on slides from the tail of each mouse. The slides were fixed with methanol, stained with 10% Giemsa for 15 minutes and examined the parasite count under the microscope. Average parasitaemia suppression was calculated using the formula below.[14]

$$\% \text{ suppression} = \frac{\% \text{ parasitaemia in control} - \% \text{ parasitaemia in treated group}}{\% \text{ parasitaemia in control}}$$

Prophylactic Test
The prophylactic activity of the extract was evaluated using the residual infection procedure as described by by Peters,[13] Twenty-five (25) Swiss mice (16-20g) of both sexes were weight and randomly distributed into five groups of five mice each. The extract at doses of 250, 500, and 1000 mg/kg was administered to groups 2, 3 and 4 respectively. Groups 1 and 5 were taken as negative and positive controls and were treated with 10 ml/kg distilled water and 5 mg/kg chloroquine respectively. Treatment continued daily for four days and the Animals were infected with the parasite on the fifth day. Thin blood smears were prepared from each mouse 72hours post treatment and mean parasitemia in each group determined microscopically. Average parasitaemia suppression was calculated using the formula below.[14]

$$\% \text{ suppression} = \frac{\% \text{ parasitaemia in control} - \% \text{ parasitaemia in treated group}}{\% \text{ parasitaemia in control}}$$

In vivo Antiplasmodial activity of the solvent fractions
The hexane, chloroform, ethyl acetate and n-butanol solvent fractions were evaluated for their antiplasmodial effects using curative model only, following the method described for the crude extract.

Statistical analysis
Data were analyzed using SPSS version 13 for windows software. All the results were expressed as mean ± S.E.M. The differences between means were compared using one-way analysis of variance (ANOVA), followed by Dunnet's post hoc test. $P < 0.05$ was considered as statistically significant,

Results and Discussion

Phytochemical screening revealed the presence of carbohydrate, cardiac glycosides, flavonoids, triterpenes, tannins, alkaloids, saponins and steroids (Table 2). These findings are in agreement with the literature report.[16] The observed antiplasmodial activity of the root bark of *Bombax costatum* might be due to the presence of these secondary metabolites which could be acting singly or in synergy to exert the observed activity. Recently, it was found that compounds belonging to several classes of phytochemical such as flavonoids, alkaloids and terpenoids have malaria parasite inhibitory activity in different ways.[17] There are reports of flavonoids being promising anti-plasmodial compounds within clinically tolerant and non-toxic concentrations due to it ability to inhibit haem polymerization.[18]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 2: Phytochemical Constituents of Crude Methanol Extract (CME) and it solvent fractions from the root the bark of *B.costatum*

Constituents	Test	Inference				
		CME	HF	CF	EAF	NBF
Anthraquinone	Bontrager test	-	-	-	-	-
Alkaloid	Dragendoff test	+	-	+	+	-
	Mayer test		-	+	-	-
Carbohydrate	Molisch test	+	-	-	+	+
Cardiac Glycosides	Keller-Kiliani test	+	-	-	+	-
Saponin	Frothing test	+	-	-	+	+
Flavonoid	Sodium Hydroxide test	+	-	-	+	+
	Shinoda test	+	-	-	+	+
Tannins	Ferric chloride test	+	-	-	+	-
	Lead sub-acetate test	+	-	+	+	-
Triterpenes/Steroid	Liebermann-Burchard test	+	+	+	-	-
	Salkowski test	+	+	+	-	-

+ = present, - = absent, CME= crude methanol Extract, HF= Hexane fraction, CF= Chloroform fraction, EAF= Ethylacetate fraction n-BF= n butanol fraction

These metabolites are valuable in combating diseases because of their anti-parasitic efficacies and their selective mode of action.[19]

The acute toxicity studies indicated that the methanol extract and fractions of *Bombax costatum* root bark caused no mortality at dose of 5000 mg/kg. None of the mice showed any signs of toxicity, such as change of skin, eyes and mucus membranes, behavioral patterns, trembling, diarrhea, falling of the fur or coma. This suggests that oral LD_{50} of the extract and fractions is greater than 5000 mg/kg. The crude methanol extract and fractions of *B. costatum* can thus be considered as relatively safe orally.[12] this could explain the use of the plant in the management of malaria by the local people in north eastern Nigeria. The *in vivo* antiplasmodial activity of the crude methanol extract and fractions of *Bombax costatum* against *Plasmodium berghei* was investigated by evaluating the curative, 4-day suppressive and prophylactic effect of the extract. However, for all the fractions only their curative effect was investigated. *In vivo* models are usually employed in antiplasmodial studies because they take into account the possible prodrug effect and probable involvement of the immune system in the eradication of the pathogen.[20] Swiss amice were used in the study and the rodent malaria parasite (*P. berghei*) discovered by Vinkey and Lips,[21] was employed, due to the sensitivity of *P. berghei* parasite to chloroquine.[22]

The methanol extract at all tested doses produced a significant (p<0.05) dose-dependent reduction in parasitaemia levels in the curative and 4-day suppressive tests compared chloroquine (Tables 3 and 4). However, in the prophylactic test, there was no significant inhibition in parasitaemia level after extract administration (Table 5).

In vivo antiplasmodial activity of the fractions against *Plasmodium berghei* were evaluated using curative test. *In vivo* antiplasmodial activity can be classified as moderate, good and very good if an extract displays percentage chemo-suppression equal to or greater than 50% at doses of 500 mg/kg, 250 mg/kg and 100 mg/kg body weight per day.[23] Based on this classification, only chloroform and n-butanol fractions exhibited a good antiplasmodial activity (Tables 6 – 9). These findings could be due to the presence of phytochemical present in the chloroform and n-butanol fractions that were absent in the other fractions.

Table 3: Curative Effect of Methanol Extract of *Bombax costatum* in *P. berghei* infected Mice

Treatment group	Doses (mg/kg)	Mean ± SEM	Percentage suppression(%)
Distilled water (NC)	1mL/kg	30.77 ± 0.97	0.00
Methanol extract	250	19.95 ± 1.08*	35.17
Methanol extract	500	13.86 ± 1.34*	54.95
Methanol extract	1000	10.80 ± 0.96*	64.90
Chloroquine (PC)	5	7.26 ± 0.98*	76.41

n = 5; * = p < 0.05 NC: negative control, PC: positive control

Table 4: 4-days Suppressive Effect of Methanol Extract *Bombax costatum* in *P. berghei* infected Mice

Treatment group	Doses (mg/kg)	Mean ± SEM	Percentage suppression(%)
Distilled water (NC)	1 mL/kg	30.86 ± 1.11	0.00
Methanol extract	250	22.87 ± 1.59*	25.91
Methanol extract	500	14.67 ± 1.76*	52.48
Methanol extract	1000	5.40 ± 0.69*	82.50
Chloroquine (PC)	5	1.40 ± 1.01*	95.46

n = 5; * = p < 0.05 NC: negative control, PC: positive control

Table 5: Prophylactic Effect of Methanol Extract of *Bombaxcostatum*in *P. berghei* infected Mice

Treatment group	Doses (mg/kg)	Mean ± SEM	Percentage suppression (%)
Distilled water (NC)	1 mL/kg	31.40 ± 2.22	0.00
Methanol extract	250	21.40 ± 1.26*	31.83
Methanol extract	500	24.80 ± 3.02	21.02
Methanol extract	1000	24.86 ± 2.01	20.80
Chloroquine (PC)	5	7.26 ± 0.98*	86.83

n = 5; * = p < 0.05 NC: negative control, PC: positive control

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 6: Curative Effect of Hexane Fraction of *Bombax costatum*

Treatment group	Doses (mg/kg)	Mean ± SEM	Percentage suppression (%)
Distilled water (NC)	1 mL/kg	30.86 ± 1.11	0.00
Hexane fraction	250	17.00 ± 0.64*	31.96
Hexane fraction	500	15.73 ± 0.67*	49.03
Hexane fraction	1000	9.33 ± 1.46*	69.76
Chloroquine (PC)	5	4.21 ± 0.95*	86.63

n = 5; * = $p < 0.05$ NC: negative control, PC: positive control

Table 7: Curative Effect of Chloroform Fraction of *Bombax costatum*

Treatment group	Doses (mg/kg)	Mean ± SEM	Percentage suppression (%)
Distilled water (NC)	1 mL/kg	31.80 ± 2.22	0.00
Chloroform fraction	250	15.47 ± 1.12*	51.35
Chloroform fraction	500	12.60 ± 0.85*	60.37
Chloroform fraction	1000	8.53 ± 1.44*	73.16
Chloroquine (PC)	5	4.13 ± 0.51*	87.00

n = 5; * = $p < 0.05$ NC: negative control, PC: positive control

Table 8: Curative Effect of Ethylacetate Fraction of *Bombax costatum*

Treatment group	Doses (mg/kg)	Mean ± SEM	Percentage suppression (%)
Distilled water (NC)	1 mL/kg	33.20 ± 2.43	0.00
Ethylacetate fraction	250	20.31 ± 1.35	38.32
Ethylacetate fraction	500	17.47 ± 0.45	47.00
Ethylacetate fraction	1000	15.47 ± 1.05*	53.40
Chloroquine (PC)	5	7.26 ± 0.98*	78.13

n = 5; * = $p < 0.05$ NC: negative control, PC: positive control

Table 9: Curative Effect of n-butanol Fraction of *Bombax costatum*

Treatment group	Doses (mg/kg)	Mean ± SEM	Percentage suppression (%)
Distilled water (NC)	1 mL/kg	32.73 ± 1.48	0.00
Butanol fraction	250	28.66 ± 1.68	12.42
Butanol fraction	500	15.93 ± 1.09*	51.31
Butanol fraction	1000	11.67 ± 1.62*	64.35
Chloroquine (PC)	5	4.13 ± 0.51*	87.37

n = 5; * = $p < 0.05$ NC: negative control, PC: positive control

Conclusion

The methanol extract, chloroform fraction and n-butanol fractions of *Bombax costatum* root bark showed the highest antiplasmodial activities from our finding. These fractions could be targeted as potential source of lead compounds in developing new antiplasmodial agents. Therefore, further work should be done to isolate and characterize compounds from this plant that might be responsible for the observed antiplasmodial activity.

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.

Acknowledgements

The authors are grateful to Dr. I,M Maje of the Department of Pharmacology and Therapeutics and Dr. Y.Y Falla Department of Veterinary Parasitology**,** Ahmadu Bello University Zaria, Kaduna state, Nigeria.

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