

Research Article

Antimalarial activities of crude stem bark fractions of *Cylicodiscus gabunensis*

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Background: Malaria is one of the greatest health challenges worldwide threatening millions of people especially children in sub Saharan Africa. *Cylicodiscus gabunensis* (Taub.) Harms (Mimosaceae) is a tree plant of Tropical Africa used in traditional medicine for the treatment of malaria and other diseases.

Objective: The objective of this work was to evaluate the antimalarial activities of *Cylicodiscus gabunensis* stem bark fractions to ascertain the folkloric claim of its antimalarial activity.

Methodology: The stem bark fractions of *Cylicodiscus gabunensis* (n-hexane, dichloromethane, ethyl acetate and methanol; 40 mg/kg) of *C. gabunensis* were investigated for antimalarial activities against chloroquine-sensitive *Plasmodium berghei* infections in Swiss albino mice. Chloroquine (5 mg/kg) and pyrimethamine (1.2 mg/kg) were used as positive controls. Thin films made from tail blood of each mouse were used to assess the level of parasitaemia.

Results: The fractions significantly ($p < 0.01-0.001$) reduced parasitaemia in suppressive, prophylactic and curative antimalarial mouse models with the chloroform and ethyl acetate fractions exerting the most significant activities ($p < 0.05-0.01$) than other fractions. The mean survival time (MST) was significantly ($p < 0.01-0.001$) improved to 20.0 days compared to control (10.00 days).

Conclusion: The results indicate that chloroform and ethyl acetate fractions of *C. gabunensis* stem bark are the most potent antimalarial fractions with active antimalarial compounds that can be use as lead to the development of new antimalarial drug.

Keyword: Antiplasmodial, *Cylicodiscus gabunensis*, medicinal plant, ethnomedicine

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1. Introduction

Cylicodiscus gabunensis (Taub.) Harms (Mimosaceae) syn. *Erythropleum gabunensis* is a large tree, 180–200 ft in height, common in the rainforests of West and Central Africa (Hutchinson and Daziel, 1968). In folk medicine, the stem bark is used to treat body pains, fever, rheumatism, jaundice and malaria by the Ibibios of Niger Delta region of Nigeria. Biological activities reported on the stem bark include antiplasmodial (Okokon et al, 2006), antimicrobial (Kouitcheu et al, 2006a), antidiarrhoeal (Kouitcheu et al, 2006b), and antioxidant (Huang et al, 2009) activities. Okokon et al, (2006) had reported LD₅₀ value of 223.6 mg/kg and the presence of alkaloids, flavonoids, saponins, terpenes and cardiac

glycosides in the stem bark extract. The stem bark has been reported to contain triterpene saponin (Tchivounda et al, 1990a, 1991), cylicodiscus acid, a dihydroxy pentacyclic triterpene carboxylic acid (Tchivounda et al, 1990b), coumestan glycosides, coumestoside C and coumestoside D (Nchancho et al, 2009), Gabunoside, an oleanane-type triterpene saponin (Tene et al, 2011) and cyclodione, a dimeric diterpene (Tane et al, 1995). There is a little information on the antiplasmodial efficacy of the stem bark *in vivo*. The present investigation was carried out to evaluate the antimalarial activities of the various crude stem bark fractions against *Plasmodium berghei berghei* infection in mice to identify the fraction with antimalarial property in bid to localize where the active principle resides.

2. Methodology

2.1 Drugs

Chloroquine diphosphate and pyrimethamine used in this study were from Sigma-Aldrich, Germany.

2.2 Animals

The animals (Swiss albino mice) of either sex were used for these experiments. The animals were housed in standard cages and were maintained on a standard pelleted feed (Guinea feed) and water *ad libitum*. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo.

2.3 Microorganism (Parasite)

A chloroquine sensitive strain of *Plasmodium berghei* (ANKA) was obtained from the National Institute of Medical Research (NIMR), Yaba Lagos, Nigeria and was maintained by sub-passage in mice (Odetola and Basir, 1980).

2.4 Collection of plant materials

The fresh stem bark of *Cylicodiscus gabunensis* were collected in April, 2016 at a farmland in Ikono LGA, Akwa Ibom State, Nigeria. The stem bark was identified and authenticated as *Cylicodiscus gabunensis* by Dr. Margaret Bassey, a taxonomist in the Department of Botany and Ecological studies, University of Uyo, Uyo, Nigeria. Herbarium specimen (FPUU 383) was deposited at the Faculty of Pharmacy Herbarium, University of Uyo, Uyo.

2.5 Extraction

The plant parts (stem bark) were washed and air-dried on laboratory table for 2 weeks. The dried stem barks were pulverized using a pestle and mortar. The powdered stem bark (1.5 kg) was macerated for 72 hours successively and gradiently in each of petroleum ether, dichloromethane, ethyl acetate and methanol to give the corresponding fractions of these solvents. The liquid obtained by filtration was evaporated to dryness in a rotary evaporator 40°C. The crude fractions were stored in a refrigerator at 4°C until used for experiment reported in this study.

2.6 Parasite inoculation

Each mouse used in the experiment was inoculated intraperitoneally with 0.2 ml of infected blood containing about 1×10^7 *P. berghei berghei* parasitized erythrocytes. The inoculum consisted of 5×10^7 *P. berghei berghei* erythrocytes per ml. This was prepared by determining both the percentage parasitaemia and the erythrocytes count of the donor mouse and diluting the blood with isotonic saline in proportions indicated by both determinations (Odetola and Basir, 1980).

2.7 Drug administration

The drugs (chloroquine and pyrimethamine) and extract used in the antimalarial study were orally administered with the aid of a stainless metallic feeding cannula.

2.8 Evaluation of *in vivo* antimalarial activity of crude stem bark fractions of *Cylicodiscus gabunensis*

2.8.1 Evaluation of suppressive activity of the crude fractions (4-day test).

This test was used to evaluate the schizontocidal activity of the extract/fractions and chloroquine against early *P. berghei berghei* infection in mice. This was done as described by Knight and Peters (1980). Thirty six mice were randomly divided into six groups of six mice each. On the first day (D_0), the thirty six mice were infected with the parasite and randomly divided into various groups. These were administered with the crude fractions and chloroquine. The mice in groups 1- 4 were administered 40 mg/kg of n-hexane, chloroform, ethyl acetate and methanol fractions respectively. Group 5 was administered with 5 mg/kg of chloroquine (positive control), and 10ml/kg of distilled water to group 6 (negative control) for four consecutive days ($D_0 - D_3$) between 8am and 9am. On the fifth day (D_4), thin blood film was made from tail blood. The film was then stained with Giemsa stain to reveal parasitized erythrocytes out of 500 in a random field of the microscope. The average percentage suppression of parasitaemia was calculated in comparison with the controls. The mean survival time of the mice were monitored in the different groups for 30 days.

2.8.2 Evaluation of prophylactic or repository activities of crude stem bark fractions

The repository activities of the crude fractions and pyrimethamine were assessed using the method described by Peters (1965). The mice were randomly divided into six groups of six mice each. Groups 1 - 4 were administered with 40 mg/kg/day of n-hexane, chloroform, ethyl acetate and methanol fractions respectively. Groups 5 and 6 were administered with 1.2 mg/kg/day of pyrimethamine (positive control) and 10 ml/kg of distilled water (negative control) respectively. Administration of the fractions/drug continued for three consecutive days ($D_0 - D_2$). On the fourth day (D_3) the mice were inoculated with *P. berghei berghei*. The parasitaemia level was assessed by blood smears seventy-two hours later. The mean survival time (MST) of the mice in each treatment group was determined over a period of 29 days.

2.8.3 Evaluation of curative activities of crude fractions (Rane's test)

This was used to evaluate the schizontocidal activity of the crude fractions and chloroquine in established infection. This was done as described by Ryley and Peters (1970). *P. berghei* was injected intraperitoneally into 36 mice on the first day (D_0). Seventy-two hours later (D_3), the mice was divided randomly into six groups of six mice each. Groups 1 - 4 were administered with 40 mg/kg/day of n-hexane, chloroform, ethyl acetate and methanol fractions respectively. 5 mg/kg/day of chloroquine was administered to the group 5 (positive control) and group 6 was given 10 ml/kg of distilled water (negative control). The crude fractions and drugs were administered once daily for 5 days. Giemsa stained thin smears were prepared from tail blood samples collected on each day of treatment to monitor parasitaemia level. The mean survival time (MST) of the

mice in each treatment group was determined over a period of 29 days (D₀ – D₂₈).

2.9 Phytochemical Screening

Phytochemical screening of the crude stem bark fractions were carried out employing standard procedures and tests (Trease and Evans, 1989; Sofowora, 1993), to reveal the presence of chemical constituents such as alkaloids, flavonoids, tannins, terpenes, saponins, anthraquinones, reducing sugars, and cardiac glycosides and others.

3. Results

Effect on suppressive activities of crude fractions of *Cylicodiscus gabunensis*

The crude fractions showed varying degrees of chemosuppressive effect on the parasitaemia. These effects were statistically significant relative to the control ($p < 0.05 - 0.001$). The chemoinhibitory percentages ranged from 25.87 to 45.45 (Table 1). Chloroform fraction had the highest activity with chemosuppression of 65.73% followed by ethyl acetate fraction with chemosuppression of 60.13%. The crude fractions exhibited a mean survival time range of 13.66 ± 2.18 to 26.33 ± 3.66 days, while methanol fraction had MST of 26.33 ± 3.66 days. However, the effects of the crude fractions were weak compared to that of the standard drug, chloroquine, with a chemosuppression of 74.12% and MST of 30.00 ± 0.00 days. (Table 1).

Effect on repository activities of crude fractions of *Cylicodiscus gabunensis*

The crude stem bark fractions of *C. gabunensis* showed chemosuppressive effects of 25.92 – 55.55% on the parasitaemia and MST range of $16.00 \pm 1.00 - 20.00 \pm 1.73$ days during prophylactic studies. These effects were

statistically significant relative to the control ($p < 0.05 - 0.001$). Chloroform fraction had the highest activity with chemosuppression of 55.55% and MST range of 20.00 ± 1.73 days. However, these effects were weak compared to that of the standard drug, Pyrimethamine, with chemosuppression of 78.70%. (Table 2).

Antimalarial effects of crude fractions of *Cylicodiscus gabunensis* on established infection

The crude fractions showed varying degrees of schizonticidal effect on the parasitaemia. There were reductions in the percentage parasitaemia of the crude fraction/chloroquine-treated groups compared to that of the control in which prominent increases were recorded. These reductions were statistically significant relative to the control ($p < 0.05 - 0.001$) (Figure 1). The chemosuppression range of the crude fractions treated groups was 57.62-76.27% on day 7 with the chloroform fraction having the highest chemosuppression of 76.27%. The crude fractions also showed a significant ($p < 0.05 - 0.001$), mean survival time ($17.66 \pm 1.75 - 20.00 \pm 0.00$ days) on established infection, and the MST value of chloroform and ethyl acetate fractions treated groups were 20.00 ± 1.73 and 20.00 ± 0.00 days respectively. The chemosuppressive effects of the crude fractions were weak compared to that of the standard drug, chloroquine (Table 3).

Phytochemical screening

The results of the phytochemical screening of the stem bark fractions of *C. gabunensis* revealed that the presence of chemical constituents such as alkaloids, flavonoids, tannins, terpenes, saponins, cardiac glycosides all the fractions except that in n-hexane and chloroform fractions, saponins and tannins were absent. In addition, saponins were absent in ethyl acetate fraction. However, anthraquinones were absent in all the fractions (Table 4).

Table 1: Suppressive activities of stem bark fractions of *Cylicodiscus gabunensis* (4-day test).

Drug/extract	Dose (mg/kg)	Parasitaemia	% chemosuppression	Mean survival Time (days)
Distilled water	10ml/kg	27.66 ± 3.71	–	11.0 ± 1.00
n-Hexane	40	11.66 ± 2.33^b	25.87	18.0 ± 1.52^a
Chloroform	40	14.33 ± 1.20^a	65.73	17.00 ± 2.08^b
Ethyl acetate	40	6.66 ± 1.85^c	60.13	13.66 ± 2.18^b
methanol	40	10.33 ± 2.73^b	45.45	26.33 ± 3.66^b
Chloroquine	5	0.25 ± 0.06^c	74.12	30.00 ± 0.00^c

Values are expressed as mean \pm S.E.M. Significance relative to control: ap<0.05; bp<0.01 cp<0.001, n=6.

Table 2: Repository/Prophylactic activities of stem bark fractions of *Cylicodiscus gabunensis* on *Plasmodium berghei* infection in mice

Drug/extract	Dose (mg/kg)	Parasitaemia	% chemosuppression	Mean survival Time (days)
Distilled water	10ml/kg	45.66 ± 0.88	–	9.66 ± 0.88
n-Hexane	40	15.33 ± 3.71^b	25.92	17.33 ± 3.71^b
Chloroform	40	15.00 ± 2.46^b	55.55	20.00 ± 1.73^c
Ethyl acetate	40	25.00 ± 3.08^a	45.37	20.00 ± 5.00^c
Methanol	40	22.33 ± 2.91^a	37.03	16.00 ± 1.00^a
Pyrimethamine	1.2	7.66 ± 4.80^c	78.70	24.66 ± 2.72^c

Values are expressed as mean \pm S.E.M. Significance relative to control: ap<0.05; bp<0.01 cp<0.001, n=6.

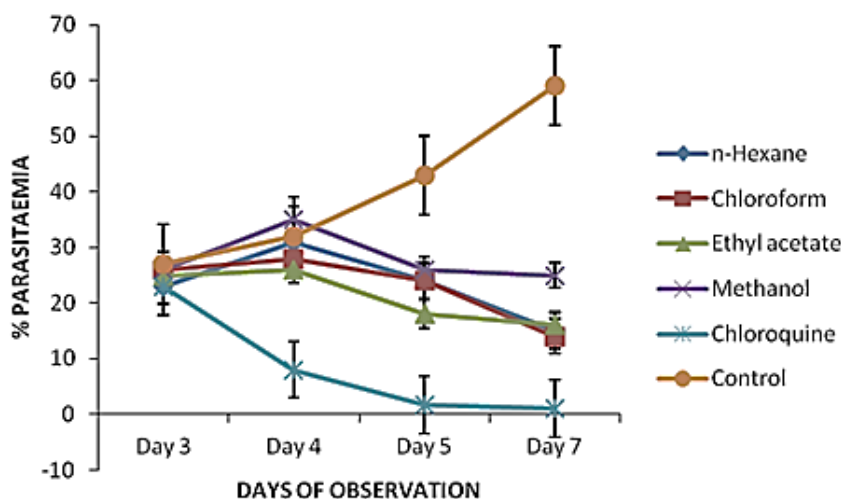


Figure 1: Antimalarial activities of stem bark fractions of *Cylicodiscus gabunensis*

Table 3: Mean Survival Time (MST) of mice receiving different stem bark fractions of *Cylicodiscus gabunensis* during established infection

Drug/extract	Dose (mg/kg)	MST (days)
Distilled water	10 ml/kg	10.00±0.57
n-Hexane	40	17.66 ± 1.75 ^a
Chloroform	40	20.00 ± 1.73 ^b
Ethyl acetate	40	20.00 ± 0.00 ^b
Methanol	40	19.33 ± 1.75 ^b
Chloroquine	5	26.00 ± 2.08 ^c

Values are expressed as mean ±S.E.M. Significance relative to control: ap<0.05; bp<0.01 cp<0.001, n=6.

Table 4: Results of phytochemical screening of stem bark fractions of *C. gabunensis*

Secondary metabolites	Crude fractions			
	n-hexane	chloroform	Ethyl acetate	Methanol
Alkaloids	+	+	+	+
Flavonoids	+	++	++	+
Saponins	-	-	-	+
Tannins	-	-	+	++
Terpenes	++	++	++	++
Cardiac glycosides	+++	+++	+++	+++
Anthraquinones	-	-	-	-

Keys: + - slightly present, ++ - moderately present, +++ - highly present, - absent

4. Discussion

The stem bark of *Cylicodiscus gabunensis* is used to treat body pains, fever, rheumatism, jaundice and malaria by the Ibibios of Niger Delta region of Nigeria. The crude stem bark fractions (n-hexane, chloroform, ethyl acetate and methanol) of *C. gabunensis* were evaluated for antimalarial activity against *P. berghei berghei* infection in mice using suppressive, prophylactic and curative models. The fractions were also screened for phytochemical constituents. The fractions were found to possess varying degrees of antimalarial potentials in the models tested with chloroform and ethyl acetate

fractions exerting the most promising activities suggesting the localization of the active ingredients in these fractions. Phytochemical study of the active fractions revealed the presence of alkaloids, flavonoids, terpenes and cardiac glycosides.

Antimalarial screening of plants has implicated alkaloids, terpenes and flavonoids in this activity (Philipson and Wright, 1990; Christensen and Kharazmi, 2001). These compounds were found to be present in the extract studied and may be responsible for the observed antimalarial activity of the extract, though the active principle is yet to be identified.

Although the mechanism of action of this extract has not been elucidated, flavonoids are known to exert antiplasmodial activity by chelating with nucleic acid base pairing of the parasite (Lui et al, 1992), thereby producing plasmocidal effect. Other modes of action include modulation of host immunity to tackle disease and inhibition of plasmodial enoyl-ACP reductase (FAB I enzyme)—a key regulator of type II fatty synthases (FAS-II) in *P. falciparum* (Teffo et al, 2010; Kirmizibekmez et al, 2004). Flavonoids may also bind parasite's serine/threonine kinase with high affinity and affect its development (Ferreira et al, 2010). Also some plants are known to exert antiplasmodial action either by causing elevation of red blood cell oxidation (Etkin, 1997) or by inhibiting protein synthesis (Kirby et al, 1989). The stem bark extract and fractions may be acting through one of these mechanisms. Besides, antioxidant potentials of some plant and natural products especially flavonoids have been found to promote schizonticidal activity by modulating the cellular signalling pathway (Al-Adhroey et al, 2011) and this has been suggested to be responsible for their antimalarial activities (Cimanga et al, 2009; Ganesh et al, 2012), as elevated free radicals levels are common features of malaria disease and are implicated in severe malaria complications. The stem bark extract of *C. gabunensis* has been reported to possess strong antioxidant activity (Huang et al, 2009). This could also contribute to the observed antimalarial activity in this study.

5. Conclusion

The results of this study support the usage of this plant in folk medicine as malarial remedy and identified chloroform and ethyl acetate as the active fractions. Further work is suggested to isolate, identify and characterize the active principles from this plant.

Conflict of Interest

The authors declare no conflict of interest.

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