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In vivo antiplasmodial activities of ethanolic exract and fractions of *Eleucine indica*

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ABSTRACT

Objective: To evaluate the *in vivo* antiplasmodial activities of the extract and fractions (*n*-hexane, chloroform, ethylacetate, butanol, aqueous) of the whole plant in *Plasmodium berghei berghei* infected mice. **Methods:** Oral administrations of the extract (200, 400, and 600 mg/kg) of *Eleucine indica* and fractions (400 mg/kg) were screened in the 4-day, repository and curative tests. Chloroquine (5 mg/kg), pyrimethamine (1.2 mg/kg) and artesunate (5 mg/kg) were used as controls. **Results:** The extract showed significant ($P< 0.05$-0.001) dose-dependent, antiplasmodial activity in the 4-day, repository and curative tests and increased the survival times of the infected mice. All the fractions exhibited significant antiplasmodial activity with the highest being ethylacetate fraction. **Conclusions:** *Eleucine indica* extract and fractions possess antimalarial activity which confirms the ethnobotanical use of this plant as a malarial remedy and opens a new highway to further investigate its potentials in the on-going fight against malaria.

1. Introduction

The burden of malaria is great as it affects the development and general well-being of many people. The disease has spread globally owing mainly to the failure of vector control programmes and spread of resistance to chloroquine and other known antimalarial drugs[1]. This trend has led to the promotion of scientific investigation of medicinal plants, especially those used traditionally in the treatment of malaria. More efforts in this area are expected to come from Sub-Saharan Africa as it remains the place where the burden of malaria is greatest[2].

Eleucine indica, L.Gaertn (Poaceae) is called nkimenang (Ibibios), and crowsfoot or goose grass (English). It is an annual growing to 0.45 m and is considered as an adventitious species that is native in the tropics and subtropical regions[3]. It is one of the medicinal plants used in the treatment of malaria fever among the Ibibios of Southern Nigeria. The whole plant decoction is used to treat malaria and to restore menstruation in females suffering from ammenorrhoea. The whole plant especially the root is depurative, diuretic, febrifuge and laxative. It is also used in the treatment of influenza, hypertension, oliguria and urinary retention as well as kidney problems in Trinidad and Tobago[4]. The plant has been reported to have phytochemical content of sterol glucoside forms and C-glycosyl-flavone possessing anti-inflammatory activities antibacterial, antioxidant and non-cytotoxic properties[5].

This present work has therefore been conducted in order to ascertain whether the whole plant has the antimalarial potentials as claimed in its ethnomedicinal use in the treatment of malaria fever.

2. Materials and methods

2.1. Plant materials

The plant material (*Eleucine indica*) was collected in April 2009 from Uyo, Akwa Ibom State, Nigeria. The plant was identified and authenticated by Dr. (Mrs.) Margaret Bassey (a plant Taxonomist) in the Department of Botany and Ecological Studies, University of Uyo, where a voucher specimen (UUH1409) was deposited. The plant material was air-dried and then oven-dried at reduced temperature (35±2)℃. It was thereafter ground into powder and cold-macerated in 70% ethanol for 72 h, and filtered. The filtrate was dried *in vacuo* using the rotary evaporator. 30 g of the dried extract was partitioned using various solvents such as *n*-hexane, chloroform, ethyl acetate, butanol and water to obtain their respective fractions. The crude extract and the

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fractions were stored in a refrigerator at -4℃ until required for use.

2.2. Phytochemical screening

The extract was screened for bioactive ingredients such as saponins, alkaloids, tannins, phlobotannins, flavonoids, anthraquinones, and cardiac glycosides[6,7].

2.3. Animal stock

Adult albino mice (20-28 g) were obtained from the Animal House of the University of Jos, Jos, Plateau State and maintained in the University of Uyo Animal House and fed with growers pellet Feed (Bendel Feeds and Flour mills Ltd, Edo State) with water given *ad libitum*. Approval for the use of animals in the study was obtained from the Animal Ethics Committee, Faculty of Pharmacy, University of Uyo, Uyo, Akwa Ibom State.

2.4. Micro-organisms

A chloroquine-sensitive strain of *Plasmodium berghei berghei* was obtained from National Institute of Medical Research in Lagos and maintained by subpassage in mice.

2.5. Inoculum preparation

The parasitized blood donor with high parasitaemia was obtained by first anaesthetizing the mouse with chloroform, and through cardiac puncture blood was collected using sterile syringe into sterile heparinised bottles. The percentage parasitaemia was determined by counting the number of parasitized red blood cells against the total number of red blood cells. The desired volume of blood then obtained from the donor mouse was suitably diluted with sterile normal saline so that the final inoculum (0.2 mL) for each mouse contained the required number of parasitized red blood cells (that is 1.0×10^7 parasitized red blood cells). Therefore the 0.2 mL of the final inoculum did contain 1×10^7 parasitized red blood cells which is the standard inoculum for the infection of a single mouse. 1 mL of the standard inoculum is expected to contain 5×10^7 parasitized red blood cells[8,9].

2.6. Drug administration

Drugs (chloroquine, pyrimethamine, and artesunate), extract and all fractions used in this antimalarial work were administered through the oral route using stainless metallic feeding cannula.

2.7. Acute toxicological study

Acute toxicological study was carried out to determine the median lethal dose (LD_{50}) using the modified method of Miller and Tainter as described by Ali *et al*[10] and Nwafor *et al*[11].

2.8. Antiplasmodial activities of the extract

2.8.1. Determination of suppressive activity of extract on early infection (4 – day test)

To determine the suppressive activity of the extract, the method earlier described with modifications[12] was adopted. The mice were each inoculated on the first day (day 0), intraperitoneally with 0.2 mL of infected blood containing about 1×10^7 *Plasmodium berghei berghei* parasitized erythrocytes. The animals were then randomly divided into six groups of six mice each. After ten minutes, the mice in the first group were orally administered with 10 mL/kg of distilled water and served as control. Groups 2, 3, and 4 received 200, 400 and 600 mg/kg of the extract orally. Group 5 received chloroquine 5 mg/kg/day as positive control while group 6 was administered with 400 mg/kg of extract conjointly with chloroquine 5 mg/kg/day. The administration of extract and drug was continued daily for 4 days (D_0-D_3) between 8:00 am and 9:00 am. On the fifth day (D_4) thin blood films were made from tail blood obtained from each mouse. The films were thereafter stained with Leishman's stain to reveal parasitized erythrocytes. The percentage parasitaemia was obtained by counting the number of parasitized red blood cells out of 500 erythrocytes in random fields of the microscope.

$$\%\ \text{Parasitaemia} = \frac{\text{No. of parasitized RBC}}{\text{Total No. of RBC counted}} \times 100$$

Average percentage chemosuppression was calculated as

$$100\times \frac{A-B}{B}$$

Where, A is the average percentage parasitaemia in negative control group and B, average percentage parasitaemia in the test group.

2.8.2. Determination of repository/prophylactic activities of extract

The methods earlier described with slight modifications[12,13] were used to assess the prophylactic activity of the extract. Mice were divided randomly into 6 groups of six animals per group. Group 1 animals received distilled water10 mL/kg. Groups 2, 3 and 4 were administered with 200, 400 and 600 mg/kg of the extract orally. Group 5 animals served as positive control and were administered with 1.2 mg/mg/day of pyrimethamine. Group 6 animals received 400 mg/kg of the extract and pyrimethamine (1.2 mg/kg/day). All the groups were treated for three consecutive days (D_0-D_2) and on day 4 (D_3), the mice were intraperitorneally injected with 1.2 mL of injected blood that contained 1×10^7 *Plasmodium berghei berghei* parasitized red blood cells (RBCs). The level of parasitaemia was assessed using thin films obtained from tail blood of each mouse 72 h after parasite inoculation. Percentage parasitaemia and the average chemosuppression were calculated as stated above.

2.8.3. Determination of effect of extract on established infection (Curative or rane test)

To assess the schizonticidal activity of the extract during established infection, the methods earlier described and modified[12,13] were used. Thirty-six mice were inoculated intraperitoneally with standard innoculum of 1×10^7 *Plasmodium berghei* parasitized red blood cells on the first day (D_0).72 h later, the mice were randomized into 6 groups of six animals per group. Group 1 received 10 mL/kg of distilled water. Groups 2, 3 and 4 animals were administered with 200, 400 and 600 mg/kg/day of the extract orally. Group 5 animals were administered with 5 mg/kg/day of chloroquine. Group 6 animals received 400 mg/kg of extract along with 5 mg/kg/day of chloroquine. All the drugs were administered to the animals once daily for 5 days. Tail blood samples from each mouse was collected daily for 5 days, stained with Leishman's stain and thin films prepared were used to monitor the level of parasitaemia. The mean survival time (MST) of each group was determined over a period of 30 days (D_0-D_{29}).

$$\text{MST} = \frac{\text{No. of days survived}}{\text{Total No. of days (30)}} \times 100$$

2.8.4. Evaluation of antiplasmodial activity of the fractions using 4-day test

Using the method earlier described[12], various fractions of *Eleucine indica* (*n*-hexane, chloroform, ethylacetate, butanol, and aqueous) were administered orally at the dose of 400 mg/kg/day to different groups of six mice per group 30 min after intraperitoneal injection of 0.2 mL of infected blood containing about 1×10^7 *Plasmodium berghei berhei*. The negative control group received 10 mL/kg of distilled water. The administration of fraction/distilled water was continued for 4 days (D_0-D_3). Thin films were prepared from tail blood of each mouse on the fifth day and the level of parasitaemia was determined by counting the number of parasitized red blood cells out of 500 RBCs in random field of the microscope. The average percentage chemosuppression was calculated as stated above.

2.9. Statistical analysis

Results were expressed as multiple comparisons of Mean±SEM. Significance was determined using One-way Analysis of Variance (ANOVA) followed by Tukey-Kramer multiple comparison post test. A probability level of less than 5% was considered significant.

3. Results

The median lethal dose (LD_{50}) was determined to be (2 090.00±0.01) mg/kg. The phytochemical screening of the extract showed that the following constituents were present: alkaloids, cardiac glycosides, tannins, flavonoids and simple sugar. Saponins, anthraquinones and phlobotannins were absent. In the suppressive test (Table 1), there was a dose-dependent decrease in the levels of parasitaemia following administration of the extract compared to control. This decrease was statistically significant (P<0.05-0.001). However, the suppressive effect was less when compared with the standard drug chloroquine. In the repository test (Table 2), the extract showed a dose-dependent decrease in parasitaemia and this decrease was significant(P<0.001) when compared to control. There was a progressive dose- and time-dependent reduction in parasitaemia when the extract was tested on established infection (Figure 1). The MST (Table 3) of extract-treated groups of mice were dose-dependently and significantly longer (25.67-2.12 days - 27.8-1.17 days) when compared to control but shorter when compared with standard drug chloroquine (29.00±0.00). The fraction having the highest chemosuppressive effect was ethyl acetate fraction (77.89%) as shown in Table 4.

Table 1

Suppressive activity of ethanolic extract of *Eleucine indica* (4-day test) (*n*=6).

Drug / extract	Dose	Parasitaemia	% Chemosuppression
Distilled water (Control)	10 mL	33.00±1.03	-
Extract	200 mg/kg	28.00±4.99	15.15
	400 mg/kg	21.50±0.76*	34.85
	600 mg/kg	11.66±2.88***	64.67
Chloroquine	5.0 mg/kg	1.33±0.61***	95.97
Chloroquine+Extract	5.0 mg/kg+400 mg/kg	15.66±2.51***	52.55

Significance relative to control: *P < 0.05 ; **P < 0.01; ***P < 0.001.

Table 2

Repository activity of ethanolic extract of *Eleucine indica* (*n*=6).

Drug / extract	Dose	Parasitaemia	% Chemosuppression
Distilled water (Control)	10 mL	14.50±0.12	-
Extract	200 mg/kg	9.66±0.11***	33.38
	400 mg/kg	9.16±0.15***	36.83
	600 mg/kg	6.33±1.23***	56.34
Pyrimethamine	1.2 mg/kg	2.16±0.60***	85.10
Pyrimethamine+Extract	1.2 mg/kg+400 mg/kg	1.66±0.42***	88.55

Significance relative to control: ***P < 0.001.

Table 3

MST of mice receiving various doses of ethanolic extract of *Eleucine indica* (Mean±SEM, *n*=6).

Drug/extract	Dose	MST (days)
Distilled water	10 mL	13.33±3.18
Extract	200 mg/kg	25.67±2.12***
	400 mg/kg	26.50±1.63***
	600 mg/kg	27.83±1.17***
Chloroquine	5 mg/kg	29.00±0.00***
Chloroquine+ Extract	5 mg/kg +400 mg/kg	28.33±0.67***

Significance relative to control: ***P< 0.001.

Table 4

Antiplasmodial activities of fractions of *Eleucine indica* during 4 -day test (*n*=6).

Drug / extract	Dose	Parasitaemia	% chemosuppression
Distilled water	10 mL	15.83±0.24	
Aqueous	400 mg/kg	7.16±0.04***	54.77
n-Hexane	400 mg/kg	7.00±0.07***	55.78
Butanol	400 mg/kg	9.66±0.13***	38.98
Chloroform	400 mg/kg	8.66±0.12***	45.29
Ethylacetate	400 mg/kg	3.50±0.76***	77.89
Artesunate	5 mg/kg	1.16±0.65***	92.67

Significance relative to control: ***P< 0.001.

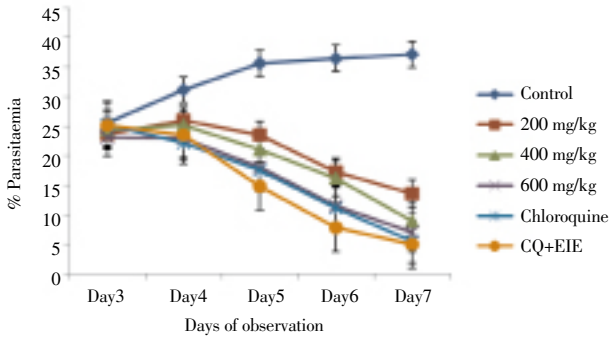

Figure 1. Antiplasmodial activity of ethanolic extract of *Eleucine indica* during established infection (curative test).
CQ-chloroquine, EIE- *Eleucine indica* extract.

4. Discussion

Based on this preliminary evaluation and screening of the antiplasmodial activity of the crude extract and fractions of *Eleucine indica*, the results showed that each of them had some degree of antiplasmodial activity against mice infected with *Plasmodium berghei*. The observed antiplasmodial activity may be associated with the presence of active compounds such as alkaloids and flavonoids. Alkaloids have been known to exhibit antiplasmodial potentials by blocking protein synthesis in *Plasmodium falciparum*[14-18]. Flavonoids have been reported to chelate with nucleic acid base pairing of the parasite[19].

It has also been reported that the leaves of *Eleucine indica* contain phenolic, possess antibacterial and antioxidant acivities[5]. The antioxidant property of this plant may represent yet another mechanism that contributes to its antiplasmodial activity. These taken together, depict the wide range of antiparasitic, anti-inflammatory, and anticarcinogenic potentials of this plant. The fact that ethyl acetate fraction showed the highest chemosuppressive effect when compared with others suggests that the active ingredients of this plant responsible for its antimalarial activity may be localized here.

The antiplasmodial activities of *Eleucine indica* extract and its fractions as observed in this work may, therefore, have resulted from one or more of these mechanisms.

The results of this study justify as well as confirm its ethnomedicinal use in the treatment of malaria among the Ibibios of Southern Nigeria. Further study is, however, necessary to identify the active principles involved and to elucidate the cellular antiplasmodial mechanisms of action.

Conflict of interest statement

The authors hereby declare that we have no conflict of interest whatsoever.

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References

[1] Hyde JE. Drug resistant malaria-an insight. *FBSJ* 2007; **274**: 4688-4696.
[2] WHO. *Global malaria control and elimination*. Geneva: WHO Press; 2008, p. 608.
[3] Haber RM, Seaman MT. Two new records from *Lebanon: Chamacyse nutans* (Lag.) Small (Euphorbiaceae) and *Eleucine indica* (L) Gaertus (Poaceae). *Turkish J Bot* 2007; **31**: 341-343.
[4] Lans CA. Ethnomedicine used in Trinidad and Tobago for urinary problems and diabetes mellitus. *J Ethnobiol Ethnomed* 2006; **13**: 45.
[5] Adel SA, Ahmad BA, Siddig IA, Chew YP, Saym M, Manal ME. *Eleucine* possess antioxidant, antibacterial and cytotoxic properties. Hindawi Publishing Corporation. *Evidence-Based Compl Alt Med* 2011; 6. Doi. 10,1093/ecam/nep091.
[6] Obasi NL, Egbuonu ACC, Ukoha PO, Ejikeme PM. Comparative phytochemical and antimicrobial screening of some solvent extracts of *Samanea saman* pods. *Afri J Pure Appl Chem* 2010; **4**(9): 206-212.
[7] Audu SA, Mohammed I, Kaita HA. Phytochemical screening of the leaves of *Lophira lanceolata* (Ochanaceae). *Life Sci J* 2007; **4**(4): 75-79.
[8] Okokon JE, Ettebong EO, Udobang JA, Obot J. Antiplasmodial and antiulcer activities of *Melanthera scandens*. *Asian Paci J Trop Biomed* 2012; 16-20.
[9] Muthaura CN, Rwukunga GM, Chhabra SC, Omar SA, Guantai AN, Gathirwa W. Antimalarial activity of some plants traditionally used in Meru district of Kenya. *Phytother Res* 2007; **21**: 860-867.
[10] Jigam AA, Abdulrazaq UT, Martins N. *In-vivo* antimalarial and toxicological evaluation of *Chrozophora senegalensis* A. Juss (*euphorbaceae*) extracts. *J Appl Pharm Sci* 2011; **1**(10): 90-94.
[11] Nwafor PA, Ettebong EO, Umoh EE, Essien GE. Anti-inflammatory and analgesic effects of ethanolic extracts of *Carpolobia ltea* leaves in mice. *Nig J Exp Appl Biol* 2008; **9**(2): 125-132.
[12] Okokon JE, Ettebong E, Bassey SA. *In vivo* antimalarial activity of ethanolic leaf extract of *Stachytarpheta cayennensis*. *Indian J Pharmacol* 2008; **40**(3): 111-113.
[13] Tekalign D, Yalemtsehay M, Abebe A. *In vivo* anti-malarial activities of *Clerodendrum myricoides, Dodonea angustifolia* and *Aloe debrana* against *Plasmodium berghei*. *Ethiop J Health Dev* 2010; **24**(1).
[14] Nergiz C, Otles S. Chemical composition of *Nigella sativa* l. seeds. *Food Chem* 1993; **48**: 259-261.
[15] Okokon JE, Etebong EO, Udobang JA, Obot J. Antiplasmodial and antiulcer activities of *Melanthera scadens*. *Asian Pac J Trop Biomed* 2011; **2**(1): 16-20.
[16] Inbaneson SJ, Ravikumar S, Suganthi P. *In vitro* antiplasmodial effect of ethanolic extracts of coastal medicinal plants along Palk Strait against *Plasmodium falciparum*. *Asian Pac J Trop Biomed* 2011; **2**(5): 364-367.
[17] Gbotosho GO, Okuboyejo TM, Happi CT, Sowunmi A. *Plasmodium falciparum* hyperparasitaemia in Nigerian children: epidemiology, clinical characteristics, and therapeutic responses to oral artemisinin-based combination treatments. *Asian Pac J Trop Dis* 2011; **2**(3): 85-93.
[18] Sundaram Ravikumar, Samuel Jacob Inbaneson, Palavesam Suganthi. *In vitro* antiplasmodial activity of chosen terrestrial medicinal plants against *Plasmodium falciparum*. *Asian Pac J Trop Biomed* 2012; **2**(Suppl 1): S252-S256.
[19] Lui KC, Yang SC, Roberts MF. Antimalarial activity of *Artemisia annua* flavonoids from whole plants and cell cultures. *Plants Cell* 1992; **11**: 637-640.
[20] Inbaneson SJ, Ravikumar S. *In vitro* antiplasmodial activity of marine sponge *Hyattella intestinalis* associated bacteria against *Plasmodium falciparum*. *Asian Pac J Trop Biomed* 2012; **2**(Suppl 1): S100-S104.
[21] Ravikumar S, Inbaneson SJ, Suganthi P. *In vitro* antiplasmodial activity of ethanolic extracts of South Indian medicinal plants against *Plasmodium falciparum*. *Asian Pac J Trop Dis* 2011; **2**(3): 180-183.
[22] Akpan EJ, Okokon JE, Etuk IC. Antiplasmodial and antipyretic studies on root extracts of *Anthocleista djalonensis* against *Plasmodium berghei*. *Asian Pac J Trop Dis* 2011; **2**(3): 36-42.