
An *in Vivo* Studies on the Sensitivity Pattern of *Plasmodium Bergei* to Stem Bark Extract of *Echinaceae Angustifolia DC* (Compositae)

Abdu Zakari¹, Dimas Kubmarawa², Sunday Osemeahon A³, Usman Umar Modibbo⁴, Sunday Dzarma⁵, Elisha Barde Baba⁶

¹Department of Chemical Sciences, Federal University Kashere, Gombe – Nigeria

^{2,3&4}Department of Chemistry Modibbo Adama University of Technology Yola – Nigeria

^{5&6}Department of Pharmacology, National Institute of Pharmaceutical Research and Development Idu, Abuja - Nigeria

Abstract: The methanol extract of *E. angustifolia DC* was screened for the presence of secondary metabolites and was earlier tested for the median lethal dose LD_{50} using Swiss albino mice to ascertain the *in vivo* acute toxicity and hence the practically safe dose to be used in a three model antimalarial investigation. The result of the phytochemical screening indicated the presence of alkaloids, terpenes, tannins, flavonoids, saponins and anthraquinones. The extract was found to be very toxic to the mice, which guided our choice for practically safe dose in the three models of the antimalarial evaluations. The result of the suppressive test showed a significant % suppression compared to the control with values of 57.92%, 66.62%, and 75.62% for the doses 100mg/kg, 150mg/kg, and 250mg/kg respectively. The result of the prophylactic (residual malaria infection) tests showed a significant level of inhibition compared to the control for the three doses. The curative (established malaria infection) tests also showed a significant level of parasite suppression compared to the control with % parasitemia of 0.13 ± 0.07 , 0.12 ± 0.06 , and 0.07 ± 0.04 for the doses 100, 150, and 250mg/kg respectively. The result justified the use of *E. angustifolia DC* as remedy to malaria infection by the traditional medicine practitioners in North-Eastern Nigeria.

Keywords: *In vivo* Studies, Sensitivity Pattern, *E. angustifolia DC*, *Plasmodium bergei*

1. INTRODUCTION

The fact that resistance of human malaria parasites to anti-malarial drugs has become major concern due to the shortage of novel classes of anti-malarial drugs.

It has become necessary to prevent the resistance by using new compounds that are not based on existing synthetic antimicrobial agents [3]

Potent antiplasmodial compounds isolated from medicinal plants were reported in studies carried out both *in vivo* and *in vitro*. Compounds such as quinine, quassinoids, linonoids, and artemisinin were among antimalarial natural products isolated in the past and in recent years from plants. Some plants species today have been reported to contain antiprotozoal phytochemicals and were indicated to be potential source of drugs for many tropical diseases including malaria [6].

The spread of drug-resistant parasites was reported to be the main cause of the worsening malaria situation in recent years which also led to the rising percentage mortality associated with malaria. Efforts on the development of new drugs for malaria disease control face a great challenge of resistance of the plasmodium parasite to the old and new drugs [4]

Echinacea species were reported to have a long history of medicinal use and were among the best-selling herbal preparations in several developed countries. Particular interest on *Echinaceae* spp. was in the prevention and treatment of upper respiratory tract infections. Several groups of phytoconstituents, including alkaloids and caffeic acid derivatives, were found according to studies in solvent extracts of this plant spp. Evidence was found to support some of the traditional and modern uses for *Echinacea* spp. [3]. The *in vivo* acute toxicity and *in vitro* cytotoxicity of methanol extract of *E. angustifolia DC* was reported indicating its potentials use in pharmaceutical and

toxicological studies [7]. Some clinical trials on the use of *Echinacea* preparations for the prevention and treatment of upper respiratory tract infections were reported to have high activity as compared to the control group [1].

2. METHODOLOGY

A. Plant Collection and Identification

The fresh samples of the plant was identified and collected by Clifford Emmanuel (tribe's man/research assistant) The plant was authenticated by Botanists in the Department of Biological sciences Federal University Kashere.

B. Extraction

60g of the dried and powdered sample was packed in an improvised thimble of filter paper prepared manually. The thimble was then inserted into the Soxhlet apparatus, 500ml methanol was transferred down the thimble into the pot. Extraction was carried out for 6 hours at a temperature of 75 °C. The methanolic extract was evaporated on a rotary evaporator (R110) at 40°C, altogether, 200g of each sample were extracted and labeled F01 [7]

C. Laboratory Animals used in the Research

The animal models involved in this study were the Swiss albino mice acquired from the National Veterinary Research Institute (NVRI) Vom in Jos – Nigeria. The experimental procedures relating to the animals were authorized by the Ethical committee of National Institute for Pharmaceutical Research and Development (NIPRD), Abuja-Nigeria before starting the study and were conducted under the internationally accepted principles for laboratory animal use and care (EEC Directive of 1986; 86/609/EEC) [7].

D. Volume of Extract Per Live Weight of Mice

The Volume of extract solution was calculated using the following expression

$$\text{Volume of extract solution} = \frac{(\text{weight of mice} \times \text{Dose})}{\text{Stock Concentration}}$$

E. Inoculation of the Plasmodium Parasite

Donor Mice infected with the *P. berghei* were initially acquired from the National Veterinary Research Institute (NVRI) Vom in Jos – Nigeria and reared in the animal house of NIPRD. Blood from the donor mouse infected with the *P. berghei* was used for inoculum preparation. The blood from donor mouse was obtained and diluted serially in Alsever's solution. 0.2 mL of the final suspension which contained about 1×10^6 infected RBC's was injected into mice intraperitoneally to initiate infection. The inoculated animals were then randomized into five mice per group and kept in the Animal Room, Department of Pharmacology, National Institute of Pharmaceutical Research and Development (NIPRD) Idu-Abuja in accordance with the internationally accepted principles for laboratory animals' use and care.

F. Suppressive Test

The Peter's 4 days suppressive test against chloroquine sensitive *Plasmodium berghei* NK 65 infection in mice were employed. Adult Swiss albino mice were incubated by intraperitoneal (IP) injection with standard inoculums of the *Plasmodium berghei* with 1×10^7 infected erythrocytes. The mice were randomly divided into five (5) groups of six (6) mice per group and treated for 4 consecutive days with 5ml each of 100, 150 and 250 mg extract kg⁻¹ b. wt. orally daily respectively. Two control groups were used: Positive control were treated daily with 25 mg/kg chloroquine kg⁻¹ b. wt while the negative control were given 5 ml kg⁻¹ normal saline. On day 5 of the experiment, blood was collected from the tail of each mouse and smeared on to a microscope slide to make a film. The blood films were fixed with methanol, stained with 10 % Giemsa at pH 7.2 for 10min and parasitaemia determined microscopically [5]. The percentage suppression of parasitaemia were calculated for each dose level by comparing the parasitaemia in negative control with those of treated mice i.e

$\% \text{ suppression} = \frac{\text{mean parasitemia of negative} - \text{mean parasitemia of treated group}}{\text{mean parasitemia of negative control}} \times 100$, whereas % parasitemia was evaluated using the following expression;

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

G. Evaluation of Schizontocidal Activity of the Plants Extracts on Established Infection (Curative or Rane Test)

Evaluation of the potential of the potent fractions were carried out according to modified method described by Ryley and Peters 1970. The mice were infected intraperitoneally with standard inoculums of 1×10^7 *Plasmodium bergheibergei* NK 65 infected erythrocytes on the first day (day 0). Seventy-two hours (72h) later, the mice were divided into 5 groups of six mice each. Three groups were orally treated with 100 mg/kg, 150 mg/kg and 250 mg/kg repectively. The remaining two groups served as negative and positive controls and administered 2% saline solution and chloroquine 25 mg/kg, respectively [6]

The treatment was carried out once daily for 5 days and blood smears were collected and examined microscopically to monitor the parasitaemia level.

H. Evaluation of the Prophylactic Activity of the Plants Extracts

Evaluations of the prophylactic potential of extracts of the plants were carried out according to the modified method of Peters 1967. Adult mice were randomized into 5 groups of six mice each. Group 1 were given 5 ml distilled water kg^{-1} b. wt. orally. Groups 2, 3, and 4 were given 100, 150, and 250 mg extract kg^{-1} b. wt respectively. Group 5 were however given 25mg/kg chloroquine (CQ) intraperitoneally. Treatments were initiated on day 0 and day 4 then, the mice were all infected with the parasite. Blood smears were then made from each mouse 72hrs after treatment and increase or decrease in parasitaernia were determined as described above.

3. STATISTICAL ANALYSIS

The data obtained as the mean of 5 replicates was analyzed using a univariate analysis of variance (UNIANOVA) on the SPSS package at a 95% and 99% confidence level, values of $P \leq 0.05$ and $P \leq 0.01$ were considered significant, results were expressed as % of Mean \pm SD.

4. RESULTS

Table1. Phytochemical Constituents of Methanol Extract of *Echinacea Angustifolia* DC

Test	Inference
Sapponnins	+
Terpenes	+
Tannins	+
Resin	-
Phlabotannin	-
Alkaloid	+
Flavonoid	+
Anthraquinone	+

Key: (+) = present, (-) = absent

Table2. Suppressive Effect of Methanol Extract of *Echinaceae angustifolia* DC and Chloroquine against *P. bergei* Infection in Swiss Albino Mice

Treatment	% Parasitemia	% Chemo-suppression	Significance
10% saline (control) (5mL/kg)	0.36 ± 0.057	0	NS
Extract 100mgKg-1	0.23 ± 0.03	57.92	$P < 0.01$
Extract 150mgKg-1	0.16 ± 0.06	66.62	$P < 0.01$
Extract 250mgKg-1	0.13 ± 0.07	75.62	$P < 0.01$
Chloroquine (CQ) (25mg/kg)	0.07 ± 0.02	89.39	$P < 0.01$

NS = Not Significant

Table3. Curative Effect of Methanol Extract of *Echinaceae angustifolia* DC and Chloroquine against *P. bergei* Infection in Swiss Albino Mice

Treatment	% Parasitemia	% Chemo-suppression	Sinificance
10% saline (control)	0.45 ± 0.13	0	NS
Extract 100mgKg-1	0.13 ± 0.07	71.26	$P < 0.01$
Extract 150mgKg-1	0.12 ± 0.06	74.16	$P < 0.01$
Extract 250mgKg-1	0.07 ± 0.02	83.52	$P < 0.01$
Chloroquine (25mg/kg)	0.06 ± 0.05	86.44	$P < 0.01$

NS = Not Significant

Table 4. Prophylactic Effect of Methanol Extract of *Echinacea angustifolia* DC and Chloroquine against *P. bergii* Infection in Swiss Albino Mice

Treatment	% Parasitamia	% Chemo-suppression	Significance
10% saline (control)	0.23 ± 0.02	0.00	NS
Extract 100mg/Kg	0.11 ± 0.02	49.17	P < 0.01
Extract 150mg/Kg	0.09 ± 0.02	56.56	P < 0.01
Extract 250mg/Kg	0.07 ± 0.01	60.59	P < 0.01
Chloroquine (25mg/kg)	0.06 ± 0.01	73.94	P < 0.01

NS = Not Significant

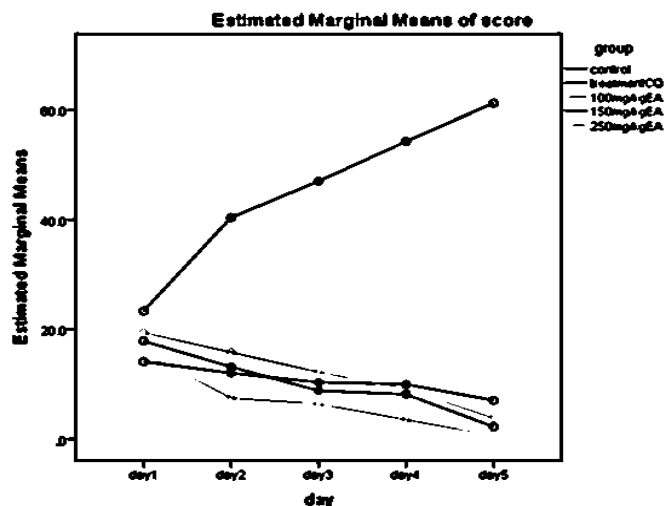


Fig1. Schizontocidal Activity of *Echinacea Angustifolia* DC Extract on Established Malaria Infection

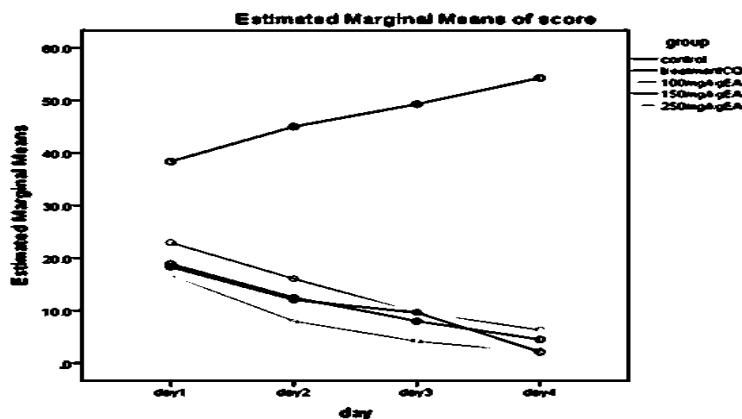


Fig2. Suppressive test result of *Echinacea Angustifolia* DC Extract on early Malaria Infection

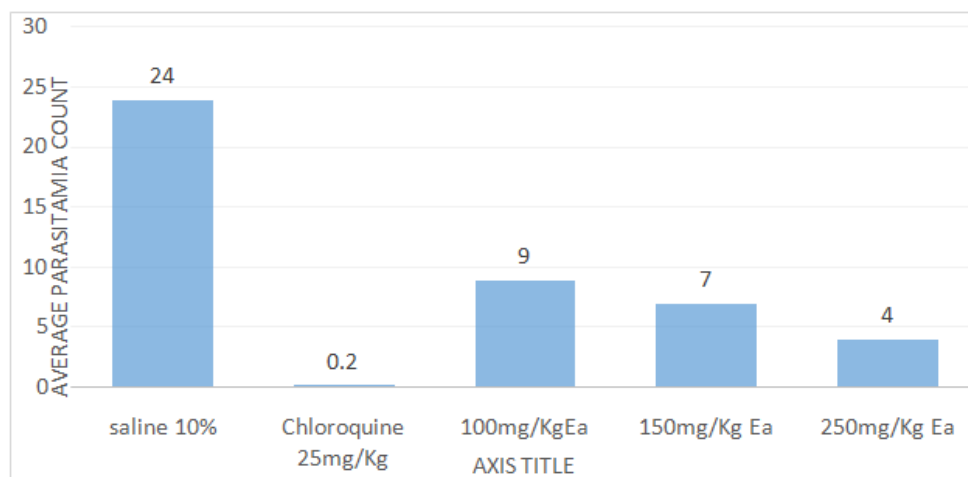


Fig3. Column Chart of Residual Malaria (Prophylactic) test result for *E. angustifolia* DC stem bark extract

5. DISCUSSION

A. Phytochemical Screening

Phytochemical screening of the methanol extract of *E. angustifolia DC* stem bark revealed that the extract contained alkaloids, terpenes, anthraquinones, flavonoids, tannins, and saponins among others. Phytochemical compounds such as alkaloids were reported to exhibit antiplasmodial activity of many plants while terpenes or terpenoids have been identified as active antiprotozoal and antimalarial agents in many pharmacological studies. Flavonoids revealed significant anti-parasitic activities against different parasite strains of malaria, trypanosome and leishmania. Anthraquinones derivatives showed antimalarial activity [2]. These phytochemicals found in the extract justified the use of the plant (*E. angustifolia DC*) by herbalists (traditional medicine practitioners) as a remedy for malaria disease.

B. In Vivo Antimalaria Studies

Significant *in vivo* oral suppression of *P. berghei* by *E. angustifolia DC* methanolextracts was investigated. Significant differences were observed in the mean survival of animals treated with the extract and untreated control. In the antiplasmodial tests, the toxicity of the plant extract guided the choice of the doses 100, 150 and 250mg/kg. Significant parasite suppression was recorded in the 3 models of the antiplasmodial tests.

C. Suppressive Anti-Malarial Activity

The *in vivo* suppressive activity of three doses (100, 150, and 250 mg/kg) of the methanol extract of *E. angustifolia* administered orally showed a dose-dependent chemosuppressive activity were 57.92%, 66.62% and 75.62% in all groups of mice (Table 2). A considerably high degree of chemosuppression was shown by the 150 and 250mg/kg doses which significantly decreased the parasitaemia of the infected mice when compared to the negative control ($P < 0.01$).

D. Curative Anti-Malarial Activity

The results indicated that the methanol extract of *E. angustifolia* exhibited dose-dependent chemosuppression in parasitaemia. This curative chemosuppression of the treated groups was statistically significant ($P < 0.01$) when compared to the control. The control group showed increased parasitaemia on the seventh day of infection (Figure 1). The chemosuppression effects for the treated groups were 71.26%, 74.16% and 83.52% for 100, 150 and 250 mg/kg, respectively (Table 2). These treated groups (100, 150 and 250 mg/kg) of mice also had longer survival times. which ranged between 23, 25 and 27 days as compared to the control with 13 days. The chloroquine-treated group had a survival time of 29 days.

E. Prophylactic Test

The methanol extract of *E. angustifolia DC* showed a dose-dependent prophylactic activity at the different doses employed resulting in significant ($P < 0.01$) reduction of parasitaemia in extract treated groups when compared to control. Values of 49.17%, 56.56% and 60.59% chemosuppressions at doses of 100, 150 and 250 mg/kg, respectively. The chemosuppression shown by the highest dose of the extract (250 mg/kg) was promising when compared to that of the standard drug (Table 4).

6. CONCLUSIONS

The stem bark extract of *E. angustifolia* demonstrated significant ($P < 0.01$) schizonticidal activity in all the three models of the antimalarial evaluations. The results of this study besides justifying the claim of traditional medicine practitioners in the region, provides a base line data for further studies on the plant. Isolation and characterization of the bioactive principles with the ultimate objective of finding novel antimalarial compounds is in progress.

ACKNOWLEDGEMENTS

The authors wish to gratefully acknowledge the financial support of the TETFund - Nigeria.

REFERENCES

- [1] Abdu Z, Dimas K (2016) *In vitro* Cytotoxicity Studies and Qualitative Investigation of Phytochemicals of Stem Bark Extracts of *Detarium microcarpum* (Caesalpinioideae), *Echinaceae angustifolia* (Compositae) and *Isobertinia doka* (Fabaceae). *National Journal of Multidisciplinary Research and Development 1*: 22-26.
- [2] Abdulelah H. Al-Adhroey,, Zurainee M. Nor, Hesham M. Al-Mekhlafi , Adel A. Amran and Rohela Mahmud; Antimalarial Activity of Methanolic Leaf Extract of *Piper betle*L. *Molecules* 2011, *16*, 107-118; doi:10.3390/molecules16010107
- [3] Barnes J, Anderson LA, Gibbons S, Phillipson JD (2005); Echinacea species (*Echinacea angustifolia* (DC.) Hell., *Echinacea pallida* (Nutt.) Nutt., *Echinacea purpurea* (L.) Moench): a review of their chemistry, pharmacology and clinical properties. *J Pharm Pharmacol* 57: 929-954.
- [4] Grace I Olasehinde, OlusolaOjurongbe, Adegboyega O Adeyeba, Obasola E Fagade, NeenaValechalsaac O Ayanda, Adesola A Ajayiand Louis O Egwari (2014); *InVitro* Studies On The Sensitivity Pattern Of Plasmodium Falciparum To Anti-Malarial Drugs And Local Herbal Extracts; *Malaria Journal* 13:63.
- [5] LaychiluhBantie, Solomon Assefa, TilahunTeklehaimanot and EphremEngidaworkBantie et al. In Vivo Antimalarial Activity of the Crude Leaf Extract and Solvent Fractions of Croton Macrostachyus Hocst. (Euphorbiaceae) Against Plasmodium BergheiIn Mice; *BMC Complementary and Alternative Medicine* 2014, 14:79<http://www.biomedcentral.com/1472-6882/14/79> Accessed on 03/02/2017.
- [6] Renata B. S. Lima Luiz F. Rocha e Silva^{1,2,3}, Marcia R. S. Melo⁴, Jaqueline S. Costa¹, Neila S. Picanço^{1,2}, Emerson S. Lima⁵, Marne C. Vasconcellos⁵, Ana Paula A. Boleti⁵, Jakeline M. P. Santos⁵, Rodrigo C. N. Amorim¹, Francisco C. M. Chaves⁶, Julia P. Coutinho⁷, Wanderli P. Tadei⁸, AntonianaU.Krettli⁷ and Adrian M. Pohlit¹(2015); In vitro and in vivo anti- malarial activity of plants from the Brazilian Amazon *Malar J* 14:508
- [7] Zakari A, Kubmarawa D (2016); Acute Toxicity (LD50) Studies Using Swiss Albino Mice and Brine Shrimp Lethality (LC50 and LC90) Determination of the Ethanol Extract of Stem Bark of *Echinaceae angustifolia* DC. *Nat Prod Chem Res* 4: 240. doi: 10.4172/2329-6836.1000240