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ANTIMALARIAL ACTIVITY OF THE DIFFERENT FRACTIONS OF THE ROOT EXTRACTS OF SALACIA NITIDA (BENTH) (CELASTRACEAE) ON PLASMODIUM BERGHEI INFECTION IN MICE

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ABSTRACT

Background: Resistance to antimalarial agents is a major challenge in the treatment of malaria. The aim of the study was to evaluate the antimalarial activity of the root extract of *Salacia nitida* in *Plasmodium berghei* infection in mice. **Method:** The plant material was successively extracted with N-hexane, Dichloromethane, Ethyl acetate, and Methanol. In suppressive test *Plasmodium berghei* inoculated mice were randomly divided into eight groups of 5 mice each. Group 1, was treated with the vehicle (10 ml/kg distilled water), the treated groups received 100 and 200 mg / kg of DCM, Et Ac and MeOH respectively. Group VIII was treated with Artesunate 5 mg /kg. In the chemoprophylactic and curative test, the treated received 100,200 and 400 mg / kg. The variation in level of parasitemia, survival time and weight of the mice, were used to determine the activity of the extract. **Results:** In the suppressive test; 100 mg /kg of DCM, Et Ac. And Me OH activities were 25.58, 26.51 and 11.90% ($P<0.05$) and 200 mg/ kg. produced activities 63.95%, 28.83, 25.58% respectively and Artesunate 61.40% ($p<0.05$).In chemoprophylactic activities were: 38.02%, 55.97%, 71.81 and Artesunate 65.40% while curative activities were 28.16%, 31.36%, and 63.36% at doses of 100mg/kg, 200gm/kg, and 400mg/kg respectively of the Dichloromethane extract showed significant ($p<0.05$). **Conclusion:** The results of the study showed that the Dichloromethane root extract of *Salacia nitida* possesses good chemoprophylactic and moderate curative activity, which could be exploited in the search for malaria drugs.

KEYWORDS: Antimalarial activity, Salacia nitida, Malaria, Plasmodium berghei, Mice.

BACKGROUND

Malaria is a mosquito-borne illness that is entirely preventable and treatable. It is the major cause of morbidity and mortality in malaria endemic regions. Almost 50% of world population of over 3.2 billion people were estimated to be at a high risk of malaria illness in 2018 according to WHO (2019). And over 405,000 deaths from an estimated 216 million recorded cases of malaria illness were documented worldwide. Furthermore, about 94% of all recorded malaria deaths occurred in Africa. Globally an estimated 306000 under-five deaths occur due to malaria and Africa having the highest, an estimated 272000 deaths of children before their fifth birthday.[1]

The rising challenge of the resistance of malaria parasites particularly the *Plasmodium* species to existing anti-malarial agents.[2] And the unimpressive drug development pipeline are the major challenges in the treatment of malaria.[3] However, resistance is not the only problem experienced in the preventive and treatment strategies of malaria, high cost, toxicity, lack of malaria vaccines and logistic problems especially in malaria endemic areas are some of the major issues of concern.[4,5]

In recent times, the volume of literature showing the activity of medicinal plants (herbs), shows that medicinal plants are potential source of new effective anti-malarial agents. It becomes pertinent to validated and standardized these plants.[6] Most of these plants, have unlimited number of secondary metabolites, which constitutes the molecules of lead for development of anti-malarial Pandey.[7] *Salacia nitida* is used traditionally for the treatment of malaria amongst the Ogonis, and has antidiabetic activity.[8] Cytoprotective.[9]

METHODS

Plant Material Collection

The roots of the plant: *Salacia nitida* (Benth) were collect from Kpong in Khana Local Government Area of Rivers State, Nigeria. Plant was identified authenticated

and the voucher specimen (voucher number UPHCO291) was deposited at herbarium, of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences University of Port Harcourt.

Preparation and Extraction of plant material

The plant root was washed, dried and pulverized using a high-speed milling machine. 1850g powdered root sample was successively extracted by maceration for 72 hours with N-hexane, dichloromethane, ethyl acetate, methanol. The resulting extract was filtered into a flask using Whatman No. 1 filter paper (Whatman, England) and concentrated to dryness with a water bath at a range 40 to 50^{o}C. The dried extracts were determined and percentage yield was calculated for each sample. The extracts were then stored at 4^{o}C with aid of a standing refrigerator.[2]

Experimental Animals

Plasmodium parasites free healthy mice, male and female weighing 20 to 25g inbred at the Animal House of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Port Harcourt were used and they received free access to standard rodent's diet (livestock balanced rations; broiler finisher mash) and good drinking water. They were kept in plastic cages with softwood shavings as beddings (which has a metal cover) to ensure free passage of air within normal room temperature. All the mice used in this study were acclimatized to the working environment for a week.

Parasite

Plasmodium berghei (NK 65 strain) used, were obtained from the Malaria Research Laboratory, University of Port Harcourt. Uninfected mice were inoculated with blood from the donor *Plasmodium berghei* infected mice. These parasites were maintained by successively inoculating the blood of infected donor mice to three uninfected mice via intra-peritoneal (IP) route every four days using 0.2ml of diluted blood sample.

Drugs and reagents

Artesunate (Ipca Laboratories Ltd, For Nigeria),
Normal Saline (Juhel Pharmaceutical Ltd, Nigeria),
Giemsa (Science lab, USA). The chemical reagents used were all of analytical grade and procured from certified suppliers.

Experimental design

The animals were randomly divided into groups. Forty mice were used for suppressive test, twenty-five mice were used for prophylactic test, and thirty mice were used for curative test (with 5 mice per group).

The Preparation of Inoculum

In this preparation, the parasitemia level of the *Plasmodium berghei* infected donor mice were determined. The blood were obtained through cardiac puncture into a test tube. The obtained blood sample was diluted using normal saline of 0.9% to give 2 x 10^{7} infected RBCs in an injection volume of 0.2ml and were injected into each of the mouse intraperitoneally producing a rising parasitemia of 30 to 35%.[2]

Grouping and Administration of Drugs and Extract

In suppressive test *Plasmodium berghei* inoculated male and female mice were randomly divided into eight groups (5 mice per group), Group 1, was treated with the vehicle (10 ml / kg of distilled water), Group II, III and IV were treated with 100 mg / kg of DCM, Et Ac and Me oH respectively. Group V, VI and VII were treated with 200 mg / kg in the order as above, Group VIII was treated with Artesunate 5 mg /kg.

In the chemoprophylactic and curative test: Group I, was treated with 10 ml / kg of distilled water, while II, III and IV were treated with 100, 200 and 400 mg / kg respectively, While V, was treated with Artesunate 5 mg / kg. The safety data of the plant was obtained from Dooka and Ezejiofor.[8] The antimalarial activity of the extracts was determined using variation in level of parasitemia, survival time and weight of the mice.

Four days Suppressive Antimalarial

The four-day suppressive test is a preliminary test used to determine the efficacy of new compounds after four daily doses, by comparing the blood parasitemia and the survival times of both the treated mice and untreated. After the process of inoculation of the parasites. A total of forty mice were randomly divided into 8 groups, The treatment began three hours after infection and continued for three days consecutively (i.e. D_0 to D_3). The fifth day (D_4), thin films of blood were been made from each mouse's tail and placed on the slide of a microscope (citoplus, China) as described by Fidock but with little modification by.[2,10]

Chemoprophylactic Antimalarial Test

The fraction of the extract (DCM extract) which was found to be active in the four-day suppressive test was further evaluated in the prophylactic test. Twenty five mice were randomly grouped into five. Treatment were carried out for a period of 4days (D_0 to D_3) consecutively. And a standard inoculum of 0.2ml was further given by intra-peritoneal route to each mouse on day 5 (D_4). After a period of seventy two hours (D_7) of infection, thin blood smears were prepared from the tail of mice on a microscopic slide as described by Fidock et al but with modification.[10]

Rane's Curative Antimalarial Test

A total of 30 mice were infected with parasitized erythrocytes, seventy two hours after infection the mice were grouped randomly into six groups of five mice and were orally treatment once a day for four days consecutively (D4 to D7) post inoculation during which the level of parasitemia were monitored regularly.[11]

Peripheral Blood smear Preparation

In the two models (suppressive and prophylactic) a thin blood smears were made from the tail of each mouse on the fifth day (D_4), eighth day (D_7) and (D_7) respectively. The blood drop on the microscopic slides was drawn evenly through a second slide to obtain a thin film of blood and allowed to dry at room temperature. The slides were then fixed with 100% methanol and stained with 10% Giemsa Armor for 15 minutes, at pH 7.2.

Determination of Parasitemia

By examining the stained slides under the microscope for each of the mouse, the parasitemia levels were determined by means of counting the number of parasitized erythrocytes randomly in a microscope's field.

Parasitemia suppression percent then calculated using the relation by Fidock.[10]
As follows;

$$\%\ Parasitemia = \frac{Number\ of infected\ RBCs}{Total\ number\ of\ RBCs} x\ 100$$

$$The\ \%\ suppression = \frac{(\%\ parasitemia\ of\ negative\ control - \%\ parasitemia\ of\ treated\ group}{\%\ parasitemia\ of\ negative\ control} x\ 100$$

Determination of Mean Survival Time

The Mean Survival Time is used to evaluate the antimalarial activity of plant extracts. Plant extract which results in a higher survival time compared to the infected non-treated mice was considered as being active visa-visa.[12] In this study, the mortality was monitored on a daily bases and the Mean Survival Time was determine from the number of days from time of infection until deaths for the mice treated and that of the control group of the programmed period, and the survival time were determined using the relation given below:
MST = (The Sum of Survival time of the mice in the group (days**)** / (The total number of mice present in the group)

Determining the body weight loss

Loss of body weight is a key feature of rodent malaria infection. The change in body weight parameter was used to determine the effectiveness of the plant extract. Prior to infection (D_0) and after treatment (D_4), for suppressive test, the body weight of each mouse was taken using a sensitive electrical balance (ADP 720L, Adam Equipment). The change % body weight of the extract treated group were compared with the non-treated group.

Statistical analysis

All the data obtained in this study were expressed as mean ± SEM(standard error of mean) for the experimental groups and the Data obtained were analyzed with one - way variance (ANOVA) analysis and Students T-test with the aim of comparing the mice parasitemia between the given doses then through treatments with the negative control using P-value of ($p<0.05$). as a measure of significant difference between means.

Ethical consideration

The ethical clearance for the study was requested for and obtained from the university of Port Harcourt Ethics Committee UPH/CEREMD/REC/04. All the experimental animals were handled and cared in accordance with the internationally accepted laboratory animals' use, care and welfare guideline.[13]

RESULTS

The Percentage Yield of the plant

The percentage yield of the roots of *Salacia nitida* with the various solvents: N-hexane 0.0011%, Dichloromethane 0.7784%, Ethyl acetate 0.6486 and Methanol 3.47%.

Four Day Suppressive Antimalarial Test

The dichloromethane, ethyl acetate and methanol extracts of the roots of Salacia nitida showed suppressive activity against *P. berghei* infection in mice with 25.58, 26.51and 11.90% at 100 mg / kg and 63.95, 28.83 and 25.58% at 200 mg / kg.

Table 1: Four-day suppressive activity of dichloromethane, ethyl acetate and methanol extracts of the roots of *Salacia nitida* against *P. berghei* infection in mice.

Treatments	Doses	% parasitemia	% chemosuppression	Mean Survival Time (Days)
Distilled water	10 ml/kg	21.5 ± 0.10	0.00	10.25 ± 5.66
Dichloromethane Extract	100 mg/kg	16.0 ± 0.20	25.58*	30.00 ± 0.00
"	200 mg/kg	7.8 ± 0.10	63.95*	30.00 ± 0.00
Ethyl acetate extract	100 mg/kg	15.8 ± 0.13	26.51*	22.5 ± 1.25
"	200 mg/kg	15.3 ± 0.15	28.83*	28.75 ± 4.79
Methanol extract	100 mg/kg	19.0 ± 0.20	11.90*	16.24 ± 3.12
"	200 mg/kg	16.0 ± 0.12	25.58*	13.25 ± 4.40
Artesunate	5 mg/kg	5.3 ± 0.09	77.40*	25.25 ± 2.80

*The values above are expressed as a Mean ±SEM; n = 5.; * = P < 0.05.*

The chemoprophylactic antimalarial test

The dichloromethane extract of the roots of *Salacia nitida* showed chemoprophylactic activity against *P. berghei* infection in mice with 38.02, 55.97 and 71.81% inhibition at doses of the extract: 100, 200 and 400 mg / kg respectively.

Table 2: The chemoprophylactic activity of the dichloromethane roots extracts of *Salacia nitida plant* on *P. berghei* infected mice.

Group	Doses mg/kg	% parasitemia	% inhibition
Distilled water	10ml	14.65 ± 1.53	0.00
Dichloromethane	100	9.08 ± 1.03	38.02*
"	200	6.45 ± 1.75	55.97*
"	400	4.13 ± 0.18	71.81*
Artesunate	5	3.07 ± 0.29	79.46*

The values above are expressed as a Mean ± SEM; n = 5.; Statistical significant * = P < 0.05

Curative Antimalarial Test

The dichloromethane extract of the roots of *Salacia nitida* showed Curative activity against *P. berghei* infection in mice with 28.16, 31.36 and 63.36% inhibition at doses of the extract: 100, 200 and 400 mg / kg respectively.

Table 3: Curative activity of the dichloromethane root extracts of *Salacia nitida* against *P berghei* infection in mice.

Treatment	Doses	% parasitemia		
		Pre-(D3) treatment	Post-(D7) treatment	% inhibition
Distilled water	10ml/kg	11.94 ± 0.16	12.50 ± 0.19	0.00
Dichloromethane extract	100mg/kg	12.10 ± 0.22	8.98 ± 0.95	28.16*
"	200mg/kg	11.84 ± 0.28	8.58 ± 0.48	31.36*
"	400mg/kg	11.96 ± 0.24	4.58 ± 0.68	63.36*
Artesunate	5mg/kg	12.44 ± 0.19	3.92 ± 0.17	68.8*

*The values above are expressed as a Mean ± SEM; n = 5. ; Statistical significant * = P < 0.05*

Percentage Body weight change

The four-day suppressive test model showed loss of bodyweight in the ethyl acetate and methanol extract treated groups while in the dichloromethane group there was an increased in bodyweight as compared to negative control as shown in Table 4.

Table 4: The effect of the dichloromethane, ethyl acetate and methanol extracts of the roots of *Salacia nitida* on the body weight of *Plasmodium berghei* infected mice using the suppressive test.

Treatment	Dose mg/kg	Body Weight Loss in (gm)		
		D_0	D_4	%Weight Loss
Distilled water	10ml	24.45 ± 1.65	24.20 ± 2.23	-1.02
Dichloromethane	100	27.40 ± 1.88	28.33 ± 0.51	3.39
"	200	26.50 ± 1.89	27.73 ± 0.03	4.64
Ethyl acetate	100	23.42 ± 0.43	23.68 ± 0.70	1.10
"	200	19.18 ± 0.52	15.60 ± 0.97	-0.18
Methanol	100	14.83 ± 0.21	14.80 ± 0.44	-0.2
"	200	14.95 ± 0.29	14.93 ± 1.20	-0.1
Artesunate	5	19.28 ± 1.36	19.50 ± 0.58	1.14

The values above are expressed as a Mean ± SEM; n = 5.; P < 0.05

DISCUSSION

The anti-malarial activity of the root extract of S*alacia nitida*, used in traditional medicine in the southern part of Nigeria, against *Plasmodium berghei* infected mice in models: 4-day suppressive, prophylactic and curative malaria test are reported.

The root extracts of S*alacia nitida*, were found to reduce parasitaemia level. There was a significant changed in percentage parasitemia suppression of the extracts treated groups from those used as the negative control group. This shows that S*alacia nitida* extract possess anti-malarial activity which is in support of the use of the plant by folks in the South-South and South-East of Nigeria as an as antimalarial herb. The *Salacia nitida* parasitaemia suppression is above 30%, such a compound is said to be active.[14,15] *Pseudocedrela*

kotschyi.[14] *Brassica nigra*.[2] And *Phytolacca dodecandra*.[16]

In chemoprophylactic model, dichloromethane root extracts of *Salacia nitida* showed a significant prophylactic activity of 55.97 and 71.81% chemosuppression against the residual infection at doses; 200 and 400mg /kg respectively. This result is in support of previous studies on medicinal plants applied in the treatment of malaria- fever which include *Agelanthus dodeneifolius* and *Languas galangal*, reported to showed chemosuppression of 52.6% and 56.00% respectively. The report of Deharo et al.[17] on the classification of *in-vivo* anti-malarial activity, dichloromethane *Salacia nitida* root extract showed very good chemoprophylactic anti-malarial activity.

In the curative model, dichloromethane extract showed significant curative activity at a dose of 200mg/kg as 52.96% inhibition and for the 400 mg/kg the percentage inhibition was 63% inhibition. This finding were in accordance with previous works reported by Pinnmai et al.[18] where the percentage inhibition of *Phyllanthus emblica (a plant used as antimalarial) was* 69.46% for the 400mg/kg However, not as high as that of *Piliostigma thonningii* as reported by[19] and *Acacia nilotica*[20] which were 81.36% and 79.5% suppression respectively.

For suppressive, prophylactic and curative models, the reference drug used was Artesunate, the current antimalarial drug.[13] Showed 61.40, 65.46 and 44.64% chemosuppression for suppressive, prophylactic and curative tests respectively, All lead to decreased in parasitemia and recovery from severe malaria, The artemisinins exhibit their antimalarial activity by the production of free radicals that result in the iron-catalyzed cleavage of the artemisinin endoperoxide bridge in the food vacuole of the parasites or by the inhibition of the calcium ATPase. of the parasites The chemosuppression indicated by Artesunate in this research agreed with previous studies on *Smilax krausiana (*used as anti-malaria*)* by Okokon *et al*[21] and *another* medicinal plants known as *Terminalia avicennioides*.[22]

The anti-plasmodial activities found in many plants are as a result of bioactive secondary metabolites present in the crude extracts of the plant materials.[23] The bioactive secondary metabolites such as flavonoids, tannins, carbohydrates, reducing sugars, anthraquinones gotten from the phytochemical analysis.[10] Hence, the anti-plasmodial activity of this plant could be as a result of a single or combined action of these metabolites.

The anti-malarial activity of these plant could be via antioxidation and the scavenging of free radicals[13] and immunomodulatory response,[24] Or by intercalation in the DNA and subsequent inhibition of protein synthesis and interferences of the malaria parasite invasion of the new RBCs or via various unknown mechanism as reported by Rasoanaivo et al.[25]

The mean survival time was also determined, an important parameter which evaluates anti-malarial activities of plant extracts and any plant material that extents the survival time of the treated infected animals compared to the non-treated negative control is said to be active.[16]

In this work, mice treated with 200 and 400 mg /kg of dichloromethane extract and Artesunate significantly lived longer when compared with non-treated. This could be due to the anti-malarial activities of the plant extract, Body weight loss is one major symptoms of *Plasmodium berghei* infection in mice which may result from the depressant action on the appetite of the animal, disturbed metabolic function or hypoglycemic effect of the parasite.[26] Loss of body weight is usually a key parameter that helps in the evaluation of anti-malarial activity of plant extracts. Mice treated with 100 mg/kg and 200 mg/kg of dichloromethane extracts significant gained weight compared with non-treated and that of the positive control. The weight gained with dichloromethane extract was greater compared to negative control group. This means that plant might be endowed with vitamins such as thiamine B_1 and other metabolites, that may have maintained or stimulate appetite and growth.

The LD_{50} calculated was estimated as $\geq$ 5000 mg /kg, there was no mortality at all dose levels used.[27] The absence of death following the oral administration of *Salacia nitida* extract at 5000 mg/kg observed in mice implies that the extract is practically non-toxic.[10] It has previously been noted that any chemical that exhibited an LD_{50} more than 5000 mg/kg is practically non-toxic based on Hodge and Sterner Toxicity Scale.[28]

CONCLUSION

The result obtained in this research indicated that the dichloromethane extract of *Salacia nitida* root have good chemosuppressive and curative activities and a high chemoprophylactic activities against *Plasmodium. berghei* infected mice. Therefore, the plant extract showed good antimalarial properties that support the folk use of the plant in treatment of malaria.

The plant should be further investigated with the focus on isolating and characterizing its active anti-malarial constituents which will be a useful potential source of lead molecules and new anti-malarial for prevention and/or treatment of malaria infections.

List of abbreviations

Dichloromethane =(DCM), Ethyl acetate (At Ac) and Methanol (Me OH).

DECLARATIONS

Ethics approval and consent to participate

The ethical clearance for the study was requested for and obtained from the university of Port Harcourt Ethics Committee. All the experimental animals were handled and cared in accordance with the internationally accepted laboratory animals' use, care and welfare guideline.

Consent for publication

All authors approved the submitted version of the manuscript and we are accountable for all aspects of the work, in that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Availability of data and material

Data available is original experimental data included in the body of the article.

Competing interests

All the authors declare that we have no conflicting interests.

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Authors' contributions

All authors participated in the study design and write up. The actual laboratory works were conducted by DB with the supervision of KH. The manuscript was prepared by DB and KH.

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