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Potency of *Ficus exasparata* leaf extract on albino mice infected with *Plasmodium berghei berghei*

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ABSTRACT

This study was conducted to determine the effects of *Ficus exasparata* (sand paper plant) on albino mice experimentally infected with *Plasmodium berghei berghei*. The mice were grouped into four of five mice each. The mice in groups A, B, and C were inoculated with *Plasmodium berghei berghei* while those in the group D were not inoculated with the parasite to serve as the control group. Group A and B were treated with the ethanoic leaf extract of *Ficus exasparata* with 100mg/body weight/day and 200mg/body weight/day respectively for six days after inoculation with the parasite. The extract significantly suppressed the malaria parasite in the treated groups when compared with the control group. Phytochemical analysis of *Ficus exasparata* showed the presence of Tannins, Flavonoid, Saponins and Glycosides. The statistical tool used was Pearson Product Moment Correlation Coefficient (PPMC). The statistical analysis showed no significant difference between doses 100mg and 200mg, but there was a significant suppression of the parasite. It is therefore concluded, that *Ficus exasparata* extract is capable of treating infection with *Plasmodium berghei berghei*.

1. INTRODUCTION

Malaria is one of the greatest menace to humans that is transmitted through the bite of an infected female Anopheles Mosquito. The species of plasmodia that poses this threat are *Plasmodium falciparum*, *P. malarial*, *P. ovale*, *P. vivax*, and *P. Knowlesi* [1] of the four species that infected humans, *P. falciparum* and vivax account for 95% of infections, *P. vivax* has the widest distribution, extending throughout the tropics, subtopics, and temperate zones. *P. falciparum* is confirmed to the tropics, *P. malarial* is sporadically distributed as *P. ovale* is rare in much of the world but relatively common in Western Africa. In India, *P. vivax* and *P. falciparum* are very common, a few cases of *P. malarial* and *P.*

ovale have been reported [2]. *P. knowlesi* rarely occurs in human. It was first discovered in monkeys [3]. *P. berghei* is used in the laboratory as a practical model organism for the study of human malaria organism for the aim of developing a new management measure for the control and prevention of malaria [4]. Malaria is a serious public health problem in the world. In 2015, and estimated 214 Million new cases was recorded, 228 Million cases in 2018 worldwide resulting in an estimated 405,000 deaths with 93% of the cases and 94% of the deaths occurred in Africa of which, more than two-third of the malaria deaths occurred in children under 5 years [5]. Nigeria accounts for 27% of the total African Malaria Burden [5]. Malaria is associated with poverty and economic growth is highly hindered. Nigeria losses over 200 Billion naira annually to the battle against malaria in form of treatment costs, prevention and lose od man hours [6].

The greatest global concern now is the rapid spread of *Plasmodium falciparum* and its resistance to Artemisinin Combination Therapies (ACTs) which is used as a first line anti-malarial therapy [7]. The use of traditional herbal medicine as a possible alternative to the cure if malaria is pertinent as it is mostly available, affordable, cheap, effective with minimal side effects in clinical experience compared to other drugs [8].

Ficus exasparata - This specie belongs to the family Moraceae. It does not produce a milky sap when cut but does produce a sticky rather viscid sap. The baric is smooth, grey phyllotaxy is alternate and leaves are rather variable in morphology from being lobed to ovate and even obovate elliptic. The surface of the leaves is rough to the touch, lance the common English name, "Sand Paper tree". In Nigeria, this plant is traditionally known as Ameme in Edo State, Omeni in Etsake etc. [9]. Extracts from this plant has been used as medicine by indigenous people for the treatment of hypertension, arthritis, peptic ulcer and pre-term labour for more than 300 years [9].

MATERIAL AND METHODS

Ethical Approval

The experimental management, animal handling and care was approved by the research and ethics committee of the Department of Biology, Bayelsa Medical University, Bayelsa State Yenagoa, Bayelsa State.

Plant Leaf Collection

Ficus exasparata leaves were harvested in the month of October, 2021 from the Faculty if Science, Bayelsa Medical University Campus Yenagoa, Bayelsa State Nigeria and G Voucher Specimen of the plant was deposited in the herbarium of the Department of Biology of same University.

Preparation of Ethanoic Extract of *Ficus exasparata* leaves

The harvested fresh leaves of the *Ficus exasparata* was washed with clean water and air dried at room temperature for five days, followed by pulverization to powder form using an electric blender, then it was soaked in 80% ethanol. The 80% ethanol was prepared by measuring 20ml of distilled water into a glass jar, into which was added 80ml of ethanol, about 500g of the mixture was macerated into the 80% ethanol and allowed to stand for 24 hours to obtain the dry extract. It was evaporated to dryness in a water bath at 47 ^{o}C.

Determination of Phytochemicals

Phytochemical analysis of ethanoic leaf extract of *Ficus exasparata* was carried out using standard procedures adopted by [10] and [11] as described by [12]; [13] and [14] for the determination of tannins, flavonoid, saponins, glycosides and steroid.

Rodent Parasite

The parasite Plasmodium *berghei berghei* NK65 was brought from National Institute for Medical Research (NIMR), Lagos and maintained alive in mice.

Mice

A total of 20 albino mice for the study were obtained from the Department of Zoology and Environmental Biology, Faculty of Science, University of Calabar, Calabar. The mice were housed in standard cases in the laboratory and stabilized for seven days during which the mice were fed on commercial pellet and clean drinking water.

Experimental Design

At the commencement of the experiment, the mice were divided into four (4) groups of five (5) mice each labelled group A_1, A_2 and B_1, B_2 that will serve as the control group. The experiment was conducted in the animal house of the Faculty of Basic Medical Sciences, University of Uyo, Uyo.

Inoculation of the Mice

The mice were inoculated by intrapecitonial injection with standard inoculums of *Plasmodium berghei berghei* with $1x10^7$ infected erythrocyte five days before treatment. The mice were observed to produce clinical signs such as salivation, reduced activity, body weakness, convulsion, etc., before application of treatment.

Group A_1 and A_2 were treated for six consecutive days with 100mg and 200mg extract of *Ficus exasparata* 1kg body weight orally and daily respectively.

Two control groups B_1 and B_2 were used. Control group with B_1 were inoculated with the perascate but no treatment was given. Control group B_2 not inoculated and no treatment given[15].

Collection of Specimens for Examination

This treatment was applied once daily for six days. On the 6th day, the samples collected through cordial puncture using sterile syringes and needles. Bleed smear were made, stored with Giemsa for microscopic examination.

Determination of Parasitamia

This was obtained by counting the number of parasitized erythrocytes out of 20 erythrocytes in random field of the microscope. Percentage parasiteamia is calculated using the formula.

$$\% \text{ Parasiteamia} = \frac{\text{Total number of PRBC}}{\text{Total number of RBC}} \times \frac{100}{1}$$

where;

PRBC = Parasitized Red Blood Cells
RBC = Red Blood Cells

AV. Percentage of Parasiteamia =

$$\frac{\text{Av. \% Parasiteamia in Control - Av. \% Parasiteamia in test}}{\text{Av. \% Parasiteamia in Control}} \times \frac{100}{1}$$

RESULTS

The result as shown from the tables below revealed that seven phytochemicals were analyzed. Five were tested positive. they are; tannins, flavonoids, saponins, glycobides and steroids while phlobactannins and alkaloids are tested negative.

The percentage parasitemia for each of the albino mice used in test group A_1 has 4,4.5.4.4 and 4 with an average percentage of 4.1 at treatment level 100mg. While that of test group A_2 has 2.5, 3,2.5,2.5 and 3 with an average percentage of 2.7 at treatment level 200mg. The control group B1 shows a high parasite count with an average percentage parasitemia of 10.7 due to no treatment.

The r(cal)-0.88 is less than the critical value 0.6319 at significant level 0.05. Therefore there is no significant difference between treatment A1 at 100mg and control level. Hence the extract was effective against the parasite.

Also, the calculated value (rcal) - 0.41 which is less than the critical value 0.6319 at 0.05 significant level. Therefore, there is also no significant difference between the 200mg treatment and the control.

Table 1: Phytochemical Analysis of the Leaf of *Ficus exasparata*

S/N	PHYTOCHEMICAL	RESULT	REMARK
1	Tannins	+	Present
2	Flavonoid	+	Present
3	Saponins	+	Present
4	Phlobatannins	-	Negative
5	Glycosides	+	Present
6	Alkaloids	-	Negative
7	Steroid	+	Present

Table 2: Percentage Parasitemia for Test Group A_1

S/N	PARASITE COUNT	BODY WEIGHT	% PARASITEMIA	AV. % OF PARASITEMIA
1	8	21KG	4%	4.1%
2	9		4.5%	
3	8		4%	
4	8		4%	
5	8		4%	

Legend: *P. berghei* + 100mg /kg

Table 3: Percentage Parasitemia for the Test Group A_2

S/N	PARASITE COUNT	BODY WEIGHT	PARASITEMIA	AV. % OF PARASITEMIA
1	5	18KG	2.5	2.7%
2	6		3	
3	5		2.5	
4	5		2.5	
5	6		3	

Legend: *P. berghei* + 200mg/ kg

Table 4: Percentage Parasitemia for Control Group B_1

S/N	PARASITE COUNT	BODY WEIGHT	PARASITEMIA	AV. % OF PARASITEMIA
1	22	20KG	11	10.7%
2	20		10	
3	21		10.5	
4	22		11	
5	22		11	

Legend: *P. berghei*

Table 5: PPMC Between Treatment with 100mg and Control

Group	Mean	S.D	n	DF	T-Cal	Critical Value
Treatment	8.2	0.21	5			
Control	21.4	0.45	5	8	-0.88	0.6319

Pearson Product Moment Correlation

Table 6: PPMC Between treatment 200mg and Control

Group	Mean	S.D	n	DF	r-Cal	Critical Value
Treatment	5.4	0.49	5			
Control	21.4	0.45	5	8	-0.41	0.6319

DISCUSSION

The antiplasmodial effects of *Ficus exasparata* extract against *Plasmodium berghei* show that at doses of 100 and 200 mg/kg, the extract was effective by reducing the mean parasitamia in the albino mice. The suppressiveness of the extract lead to the drastic reduction of the mean parasitamia in the albino mice by 2.7% and 10.7% respectively. The use of plant extract has gone a long way in the combat of *Plasmodium berghei* . The work of [16] reported that the bark and seed of *Khaya sensgalensis* have been found to be active against Plasmodium falciparum in vitro. The work of [17], also reported suppressive activity of ethanoic extract of *Hyptis suoveolens* against Plasmodium berghei in mice. The ethanoic leaf extract of *Ficus exasparata* analysed contained Tannins, Flavonoid, Saponins, Glycosides and Steriod. This is in line with [18] that reported the presence of these components in *Daniella oliveri* . [19] also revealed the presence of Alkaloids, tannins, saponins, and phenolic compounds. These phytochemicals present in the extracts could be responsible for antiplasmodial activity as reported by [20] who observed that Quinine, an alkaloid, is popular for its antimalarial activities against plasmodium. [21] also concluded that pure compounds with antimalarial activities are mainly alkaloids, terpenoids, flavonoids, coumarines, phenolics, polyacetylenes, xanthones, quinones, steriods, and lignans.

CONCLUSION

The presence of these phytochemicals in the Ethanoic extract of *Ficus exsparata* in this study supports the efficacy as an antimalarial for use in traditional medicine for malaria and other illness similar to malaria. A further research is recommended on the efficacy and hematological effect of the plant.

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Competing Interests

No competing interests.

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