Antimalarial Potential of Flavonoid-rich Extract of *Lannea acida* and Chloroquine in Mice Infected with *Plasmodium berghei*

Olusola A. O., Ogunsina O. I., Olusola A. O.

Abstract— The use of *Lannea acida* extracts for the management of infectious diseases has been accepted in traditional medicine due to the recent increase in the resistance of malaria parasites to synthetic drugs which has led to the search for alternative treatment approaches from plant sources. Flavonoid-rich extracts from *L. acida* was analyzed for antiplasmodial activity in mice. Suppressive activity of the extract was examined for five consecutive days with the different doses and chloroquine as the control drug, curative treatments were infected for five days before commencement of treatment, while the prophylactic groups were pretreated daily for five days before they were infected with inoculums of 1×107 chloroquine-sensitive *Plasmodium berghei* infected erythrocyte intraperitoneally. Four groups of five mice in each group were used. The Control group was administered with 10ml distilled water/kg body weight; experimental groups were administered with 200, and 400mg extract/kg body weight respectively, and chloroquine 5mg /kg body weight. Results obtained were analyzed using graph pad prism version 7 follow by Tukey's test was used to analyze and compare the results at a 95% confidence level. All doses of the extract produced significant, dose-dependent, chemo suppressive activity against the parasite in the suppressive, curative and prophylactic tests as compared with chloroquine treated mice. The extract also gives a prolonged mean survival time of treated mice compared to the untreated mice. The results of the study showed that the flavonoid-rich extract of stem bark of *L. acida* plant has a potent antimalarial property which could be of future importance in malaria management and antimalarial drug design.

Key words— Antimalarial, Antiplasmodial, Chloroquine, Drug, flavonoid, *Lannea acida*, *Plasmodium berghei*.

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1 INTRODUCTION

The prevalence of malaria disease remains to be one of the deadliest parasitic diseases in the world, mostly the African and Asian developing or under developed nations, because it is the major causes of mortality in children under 5 years. [1]. Mortality, currently estimated at about 405,000 people per year [2], this attributed to the resistance of the parasite to mostly used antimalarial drugs. Addition to the consequences of malaria on the health impact, the disease has a measurable direct and indirect cost, and has been known to be a major obstacle to socioeconomic development " [3], [4]".

Artemisinin Combination Therapy (ACT) has been used as first-line of treatment for uncomplicated malaria throughout Nigeria and Africa at large. ACTs has been shown to pose high efficacy against plasmodium parasites and several have been shown to be moderately effective against the early stage of infection and reduce transmission to mosquitoes. Artemether-Lumefantrine (AL) is the most widely used ACT in Nigeria and Africa continent. The Dihydroartemisinin-Piperaquine (DP) also possessed higher efficacy and its advantages of simpler dosage and a longer prophylactic period [5].

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The parasite transmission reduction is now a key component of global efforts to control and eliminate malaria diseases. [6]. The prevalence of drug resistant to malaria parasites in prevalent area has posed a great threat to the usefulness and cheapest antimalarial drugs, chloroquine (CQ) and Sulphadoxine-Pyrimethamine (SP). Presently, most malaria prevalent area in Africa including Nigeria have changed their first line antimalarial treatment from CQ or SP to artesunate/amodiaquine combination or artemether/lumefantrine combination. The ACTs used in utmost malaria prevalent countries have established high efficacy, protection against the development of resistance to each component and reduction of malaria transmission "[7], [8]". However, malaria is often referred to as the disease of poverty and the cause of poverty, the relatively high of costs, dosage complexity and the limited experience of their use in sub-Saharan Africa has affected the widespread deployment of these drug combinations. [9]

Lannea acida plants belong to the family (anacardiaceae) commonly called, awere kogun in Akoko area of ondo state, akogun in ondo town and are used in traditional medicine in the management of infectious diseases majorly malaria. *Lannea acida* is one of the most widely distributed of the Lannea species found in the hot and dry savannahs of sub-

Saharan Africa. It has a rich history of ethnobotanical and ethno pharmacological usage in the treatment of a wide range of illnesses including malaria. Barks of *L. acida* are traditionally used in Nigeria as antiabortifacient, vermifuge and to treat anal Haemorrhoids, diarrhea, dysentery, malnutrition, and debility while the leaves is used to treat rheumatism. Information provided by the traditional healer in Akoko area of Ondo state revealed that the bark aqueous or alcoholic extract is used in treatment of malaria Even though *L. acida* demonstrated biological activity that validate their medicinal roles, no phytochemical studies was performed to isolate the chemical constituents responsible for the observed activity. With this view, the present study was to evaluate the antiplasmodial activity of the bark extract of *L. acida* in *Plasmodium berghei* infected mice in order to provide scientific evidence for its continuous usage in ethno therapeutic management of malaria.

2 MATERIALS AND METHODS

2.1 Experimental Plant Material

The bark of *L. acida* plant was collected from Ugbe town, of Ondo state and was authenticated at the Plant Science Department of Adekunle Ajasin University Botanic Garden Herbarium, and a sample specimen deposited at the herbarium for future reference. The plant material was air-dried under shade for twenty-one days and ground using a Wiley laboratory mill.

2.2 Extract preparation

The back of *L. acida* was allowed to dry at room temperature. They were pulverized in mechanized laboratory grinder (Manesty, England) to fine powder. The dried back weighing 500g were soaked in 1L of absolute methanol. The mixture was thoroughly mixed and filtered after 48 hour using a Buchner vacuum filter. The filtered supernatant was evaporated to dryness with a Rotary evaporator. The weight of dried methanolic extract of the back was 47 g representing 9.4% yield in relation to the weight of the sample used.
The percentage yield of the extract was determined according to the expression provided by [10].

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of ground plant material}} \text{ X } 100$$

2.3 Extraction of flavonoid- rich fraction

A portion of the methanolic extract was dissolved in 100ml (1:4) of 1% H_2SO_4 in a small flask and was hydrolyzed by heating on a water bath until the mixture was half of its volume (30minutes). The mixture was placed on ice for 15minutes, to allow flavonoids precipitated. The cooled solution was filtered. The filtrate (flavonoids aglycone mixture) was dissolved 50mls of warm 95% ethanol (50^{o}C), the resulting solution was again filtered and the filtrate was concentrated to dryness using rotary evaporator. [11]

2.4 Animals

Both sexes of winster mice (17-20g), bred at the animal house of the institute of Advance Medical Research and Training (IAMRATS) Ibadan Oyo state Nigeria were used as the test animals for the study after receiving approval from animal ethics committee on use of laboratory animal of Adekunle Ajasin University Akungba Akoko.

2.4.1 Rodent parasite

Chloroquine sensitive rodent *Plasmodium berghei* was obtained from Institute of Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Nigeria, Oyo State, Nigeria. A standard inoculum of 1×10^7 of parasitized erythrocytes from a donor mouse in volumes of 0.2 mL was used to infect the experimental animals. The re-infected rats were kept at the animal house of biochemistry Department of Adekunle Ajasin University Akungba Akoko where the study was carried out.

2.5 Test on early malaria infection (4-day suppressive Test)

The Peter's 4-day suppressive test against chloroquine sensitive *Plasmodium berghei* infection in mice was employ [12]. Twenty-five winster mice of both sex was inoculated as described above. They were randomly grouped, having five mice in each group and administer extract daily for four (4) consecutive days. Treatment started immediately after the mice were infected with the parasite. Group 1 that served as control was administer with 10ml/kg body weight of distilled water. Groups 3 and 4 was orally administer with 200 and 400 mg extract/kg body weight daily respectively, while group 2 was administer with 5 mg chloroquine /kg body weight orally daily. On the fifth day (D_5), blood was collected from the tail of each mice and spread on a microscope slide to make a thin film. The blood films were stained with Giemsa and examined microscopically following [13]. The parasite count was recorded and the suppression of parasitemia was expressed as percentage for each dose, by comparing the parasitemia in the control group with the treated one.

$$\text{Average suppression} = \frac{\text{APC}-\text{APT}}{\text{APC}} \text{ X } 100$$

APC = Average parasitemia in the control, APT = Average parasitemia in the test group.

2.6 Test on established infection (Rane test)

Evaluation of the curative potential of *L acida* bark extract against established infection was carried out as described by [14]. Twenty-five mice were all inoculated as described above, and left untreated until the fourth day (D4) post inoculation. The mice were weigh and randomly group into five groups of five mice each. Group 1 was administered with 10ml/kg of distilled water; groups 3 and 4 received graded extract doses of 200 and 400 mg extract/kg body weight/day orally respectively, while group 2 received 5 mg chloroquine /kg body weight/day orally for four days (D_4-D_7). On Day-7 each mice were tail bleeded and a thin blood

film was made on a microscope slide. The films were stained with Giemsa stain and examine microscopically to monitor the parasitemia level. The mean survival time of the mice in each treatment group was monitored for 30 days "[15], [16]".

2.7 Repository test

The prophylactic activity of the extract was tested using the residual infection procedure described by Peters [12]. Twenty-five mice of both sexes was weigh and randomly group into five groups of five mice each. Group 1 was administered with 10ml/kg distilled water, group 3 and 4 were administer with 200 and 400 mg extract /kg body weight orally respectively, while group 2 was administer with 5mg chloroquine/kg body weight orally daily. Treatment continued daily for four days (D_1-D_4) and mice was all infected with the parasite on the fifth day (D_5). Thin blood films were prepared from each mice 72hours (D_7) post treatment and mean parasitemia in each group was determined microscopically. The mice were re-weighed on seventh day and the differences between the pre- and post-treatment body weight was recorded.

2.8 Statistical analysis

Graph pad prism version 7 was used to analyze the data obtained and these were expressed as mean ± standard error of mean follow by Tukey's test was used to analyze and compare the results at a 95% confidence level.

3 Results

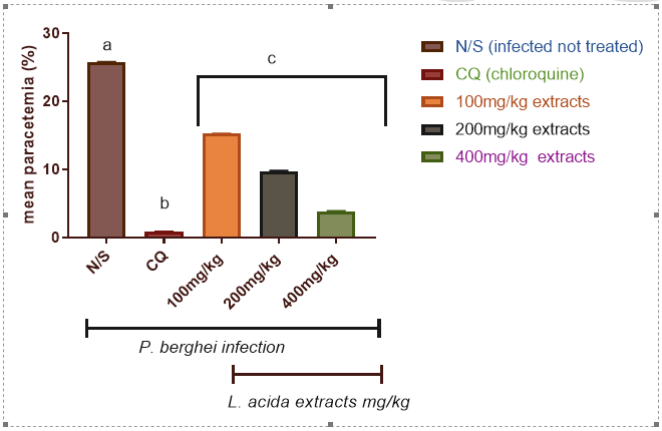

Fig 1. Suppressive activity of flavonoid-rich extract of *Lannea acida* in *P. berghei* infected mice. a-c significant different at p< 0.05 compared to the negative control

Table 1: Effect of Flavonoid-rich extract on Curative activity of Lannea acida in P. berghei infected mice

Treatment	Dose (mg/kg	Mean parasitaemia count Pre-(D3) Treatment	Post-(D7)-Treatment	D7-% Inhibition	Survival Time (Days)	Body Weight (g) D1	D7
Untreated	N/S	16.3 ± 0.19	27.6 ± 0.83d	0	7.33 ± 0.61	15.9 ± 0.17	16.73 ± 0.06
Extract	200	19.3 ± 0.19	13.3± 0.69c	51.9523			13
	400	12 ± 0.33	8.66 ± 0.19b	68.4761			23
Chloroquine	5	12 ± 0	1.66± 0.38a	93.6190			30

D3 = Day Three, D7 = Day seven, a-d significantly different compared to the control at $p < 0.05$.

Dose-dependent percentage inhibition of parasitaemia in mice placed on curative treatment with flavonoid-rich extract and chloroquine ($p < 0.05$). Each point is an average count from five infected mice (±SEM).

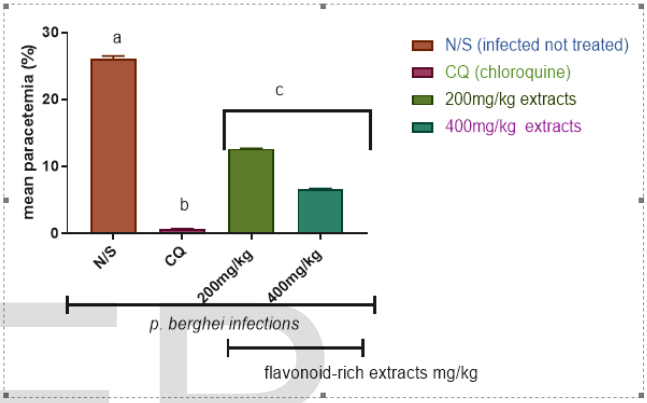

Figure 2: Prophylactics effect of methanolic bark extract of Lannea acida in *P. berghei* infected mice. a-c significant different at p<0.05 compared to the negative control

4 Discussion

The current results from the study showed that the flavonoid-rich fraction of bark extract of *Lannea acida* have a significant suppressive effect against early Plasmodium infection, curative effect against established infection and prophylactic effect against residual infection in Plasmodium berghei infected mice. The acute toxicity of *Lannea acida* bark methanolic extract was tested orally in mice following Lorke's method 1983. The Survival of mice, after oral administration of 5000 mg/kg body weight of the extract, up to 7 days, indicates that the estimated oral median lethal dose (LD_{50}) of the extract at 5000 mg/kg body weight is nontoxic. This suggests that acute oral administration of the extract is safe, and also explains the reason why the bark portion of the plant is widely used in traditional treatment of malaria

Meanwhile rodent models do not precisely give the same symptoms detected in the human plasmodium infection but they have been similar disease symptom to human plasmodium infections when infected with plasmodium berghei, which have been reported by "[17], [18]". Furthermore, several studies "[19], [16]" have employed *P.*

berghei in evaluating treatment outcome of assumed antimalarial agents, because of its high sensitivity to chloroquine, making it appropriate for this study. Constituents that bring about reduction in parasite multiplication in the host cell were considered to possess antimalarial activity [14].

The 4-day suppressive test is a regular test usually used for antimalarial screening [12]. The extract produced significant dose related chemo suppression in all the treated groups with the highest chemo suppression (73.33%) observed in the group treated with 400 mg extract/kg btw, followed by (36.66%) 200mg/kg btw, while chloroquine drug is (93.44%) respectively. The flavonoid-rich fraction of methanolic stem bark extract of *Lannea acida* also established significant dose related reduction in parasite count in both established (curative) and residual (prophylactics/repository) infection, comparable to the effect of chloroquine, which was used in this study as a standard control drug. The reduction of parasite count in the curative test is similar to the values observed in parasite count reduction in the suppressive test, but lower than the mean parasite counts in the repository test. This may possibly be due to prompt parasite clearance by the extract in early and established infection, as against a condition where the extract was primarily administered for days (Prophylactic) before inoculation with parasite. The high parasite count in the repository test may be ascribed to speedy metabolism of administered extract to inactive products [20]. The significant reduction in body weight in the curative and prophylactics group administered with extracts as well as the control group may be due to combined effect of plasmodium infection [21], and possible catabolic effect of the doses of the extract on the stored lipids. These observations indicate that the extract is potent against the malaria parasite used in this study and is consistent with the ethno medicinal use of *Lannea acida* plants, as antimalarial in Northern part of Nigeria [22].
The mechanism of antiplasmodial activities of the extract has not been elucidated, however, antiplasmodial effects of natural plant products have been credited to some of their active phytochemical components [23]. Some of these phytochemicals such as terpenoids, Quinone, saponin, alkaloids and flavonoids detected in *Lannea acida* might contributed to its antiplasmodial activity.
. The antiplasmodial effect of flavonoid-rich fraction of stem bark extract of *Lannea acida* may therefore be due to the phytochemical components (alkaloids, flavonoids, Quinone, Saponin and terpenes) or the oxidant generation potential or a combination of these mechanisms.

5 CONCLUSION

The results of this study show that the flavonoid-rich fraction of stem bark extract of *Lannea acida* plant possess moderate antimalarial activity. This result has established the rationale for the traditional use of the plants in the treatment of malaria, and showed that medicinal plants which have reputations for antimalarial properties can be screened in order to ascertain their efficacy and determine their potentials as sources of new antimalarial drugs.

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Authors' contributions
Olusola and Ogunsina performed the experiment while the manuscript was written by Ogunsina but edited my Olusola. Ogunsina analyzed and discussed the data. Both authors designed the study and reviewed the manuscript, read and approved the final manuscript.
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This study was approved by Adekunle Ajasin university Animal laboratory handling Committee.

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Not applicable.
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