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Original Research Article

Effect of *Phoenix reclinata* Jacq Methanol Leaf Extract and Fractions on *Plasmodium berghei* Infected Mice

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ABSTRACT

Malaria treatment is burdened by many challenges including growing resistance to currently used antimalarial drugs. Thus, the continuous search for safe and effective remedies is necessary. This study investigated the antiplasmodial activities of the methanol extract (ME) of *Phoenix reclinata* and its n-hexane (HF), ethyl acetate (EF), and butanol (BF) fractions. Phytochemistry of the extract and fractions was conducted. The effect of methanol extract (100, 200, and 400 mg/kg) and its solvent fractions (200 and 400 mg/kg) on parasitaemia levels was evaluated using curative, suppressive, and prophylactic anti-plasmodial models. The antipyretic effect of its solvent fractions was also determined. In the curative model, ME exhibited dose-dependent chemosuppression of 18.4%, 46.4%, and 79.6% at 100, 200, and 400 mg/kg. At 200 and 400 mg/kg, HF produced 24.5% and 28.9%, EF produced 56.8% and 85.3%, and BF produced 48% and 56.8%. In the Suppressive model, ME exhibited a dose-dependent chemosuppression of 19.08%, 45.23%, and 80% by 100, 200, and 400 mg/kg, HF exhibited 15.55% and 28.89%, EF exhibited 61.32% and 81.94%, BF exhibited 34.06% and 43.08% for 200 and 400 mg/kg. In the prophylactic model, ME exhibited a dose-dependent chemosuppression of 8.71 %, 15.59%, and 17.48 % by 100, 200, and 400 mg/kg. HF exhibited 2.66% and 8.6% for 200 and 400 mg/kg. BF exhibited anti-plasmodial suppression of 15.73% and 21.68% for 200 and 400 mg/kg. EF exhibited the highest chemosuppression in all the models.

***Keywords*:** *Phenix reclinata*, Antimalarial, *Plasmodium berghei*, Pyrexia, Parasitemia.

Introduction

Malaria is a disease caused by a protozoan parasite called *Plasmodium*. It is a disease that has defied many therapeutic and chemopreventive interventions due to persistent resistance to currently available drugs.[1] The disease is transmitted into a person's circulatory system through the bite of an infected female Anopheles mosquito via its saliva into the bloodstream, where it reproduces and matures. Typical symptoms of malaria infection include headache and fever, which can progress to death or coma in severe cases.[2] The disease is most prevalent in tropical and subtropical regions near the equator, including Sub-Saharan Africa, Asia, and the Americas.[3] According to the World Malaria Report, there were 249 million cases of malaria in 2022.[4] Five species of Plasmodium can cause disease in humans: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*. Globally, *Plasmodium falciparum* and *Plasmodium vivax* are responsible for most malaria cases. Although *Plasmodium falciparum* causes more deaths, *Plasmodium vivax* is the most widespread species and can also cause severe or fatal infections, leading to significant global morbidity and mortality.[5] *Plasmodium knowlesi* is a zoonosis that affects macaques but can infect humans.[6] In Owerri, Imo State, Nigeria, there was a 62% prevalence of malaria in 2005, with 99.7% of the identified Plasmodium species being *Plasmodium falciparum*.[7]

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Malaria parasite transmission can be reduced by preventing mosquito bites, distributing mosquito nets, using insect repellents, or implementing mosquito control measures such as draining stagnant water where mosquitoes breed and spraying insecticides.[8]

Phoenix reclinata is a large, evergreen, clumping palm that produces multiple stems up to 10 metres tall, often forming dense thickets.[9] The slender, unbranched stems can reach up to 25cm in diameter and are frequently bent over.[10] *Phoenix reclinata*, also known as the wild date palm, Arabian date palm or Senegal date palm, is a species of flowering plant in the palm family native to Western and Central African Republic extending to East and Southern Africa.[9] *Phoenix reclinata* produces edible, oblong fruit that turns orange when ripe and measures 2.5 cm in diameter. The fruits are in large pendant clusters and contain one seed each.[11] Chinwuba *et al.*[12] reported the anti-inflammatory and antipyretic properties of the methanol leaf extract of *Phoenix reclinata*, as well as some phytochemicals: alkaloids, saponins, tannins, terpenes, flavonoids, phenols and cardiac glycosides etc., in the leaf extract of the plant. Extract of *Phoenix reclinata* has been shown to exhibit a protective effect on Tenofovir Disoproxil Fumarate-induced nephrotoxicity in rats.[13] Physicochemical and nutritional analysis of *Phoenix reclinata* revealed that the pulps and the cores are weakly acidic with pH values of 6.37 and 5.74, respectively.[14] In African medicine, *Phoenix reclinata* has traditionally been used to treat various ailments, including malaria, inflammation and pain[9], and it is a major plant species exploited for malaria treatment in the Sasiga district of western Ethiopia.[15] The emergence of resistant strains of the *Plasmodium* parasite to currently used drugs necessitates the search for newer and affordable cures for malaria from medicinal plant sources.[16] Therefore, this study aims to investigate the *in vivo* antiplasmodial potentials of *Phoenix reclinata* leaf extract and its solvent fractions, and also to investigate the antipyretic properties of the solvent fractions, to validate its ethnomedicinal use for the treatment of malaria.

Materials and Methods

Plant collection and authentication

Fresh leaves of *Phoenix reclinata* were harvested at Nsukka town (coordinates 6.8429° N & 7.3733° E, about 456 meters above sea level), Enugu State in November 2021. The plant was identified and authenticated by Mr. A.O. Ozioko of the International Center for Ethnomedicine and Drug Development, Nsukka, Enugu State, Nigeria. The plant specimen was deposited at the Herbarium of the Faculty of Pharmacy, Nnamdi Azikiwe University, with a voucher number PCG/474/A/062.

Animals

Adult rats (200-250 g) and mice (18-24 g) of either sex were used in this study. All animals were procured from the Department of Pharmacology and Toxicology, Nnamdi Azikiwe University, Agulu Campus. They were housed under standard conditions and fed grower's mash. The animals were acclimatized for two weeks with access to food and water *ad libitum*. Good hygiene was maintained through constant cleaning and daily removal of excreta and spilt feed from cages. Animal experiments complied with the NIH Guidelines for Care and Use of Laboratory Animals (Pub. No.). (85-23, revised 1985). Permission was obtained from Nnamdi Azikiwe University Animal Research Ethics Committee with approval number NAU/AREC/2024/0153

Parasite Inoculation

Plasmodium berghei (rodent strain of plasmodium) was obtained from the University of Nigeria, Department of Veterinary Pathology and Microbiology. Three animals were used as infected donors and parasite reservoirs. The donor mice were monitored for infection, which included lethargy, anorexia, ruffled appearance, shivering, and heat-seeking behaviour. The inoculum consisted of *Plasmodium berghei*-parasitized erythrocytes. Each mouse was injected intraperitoneally with 0.2 mL of infected blood containing approximately $1x10^7$ *P. berghei* parasitized erythrocytes per mL. It was prepared by determining the percentage parasitemia and erythrocyte count of the donor mouse, then diluting the blood with isotonic (0.88%) saline according to the proportions indicated by both determinations[17].

Phytochemical screening

The solvent fractions of *Phoenix reclinata* were subjected to preliminary phytochemical screening for secondary plant metabolites including flavonoids, alkaloids, glycosides, terpenoids, saponins, phenolic compounds, and steroids using standard phytochemical procedures.[18]

Curative antiplasmodial activities of methanol extract and solvent fractions

The Ryley and Peter's[19] method was adopted to evaluate the curative antimalarial properties of the methanol extract (ME) on established *Plasmodium berghei* infection. Twenty-five Swiss albino mice (18-24 g) were each inoculated intraperitoneally with 0.2 mL of blood containing approximately 1×10^7. *P. berghei* parasitized RBCs on day zero. Seventy-two hours later, the mice were assigned to five (5) groups (n=5) and treated once daily via the oral route. Normal saline was administered to Group 1 (10 mL/kg/day) as a negative control, Group 2 received a standard antimalarial drug, artemether/lumefantrine (9.8 mg/kg/day), Groups 3, 4 and 5 received 100, 200 and 400 mg/kg doses of ME, respectively, for four days. Similarly, the antimalarial activities of solvent fractions (HF, EF, and BF) were investigated at 200 mg/kg and 400 mg/kg doses, with five mice in each group. Thin blood smears were made from blood collected from a snip of the tail of each mouse. The smears were fixed with absolute methanol, stained with 10 % Giemsa stain, and examined under an oil immersion microscope at 100x magnification. The number of parasitized red blood cells was counted in ten fields to determine the parasitemia level. The percentage of parasitemia (%) was calculated.

$$\% \text{ parasitemia} = \left(\frac{A-B}{A}\right) 100$$

Where A = parasitemia of the control group and B = parasitemia of the treated group.

Suppressive antiplasmodial activities of methanol extract and solvent fractions

The suppressive method described by Knight and Peters[20] was used to evaluate the anti-plasmodial activity of *Phoenix reclinata* methanol leaf extract (ME) against *P. berghei* infection. Twenty-five (25) mice of either sex (18-24 g) were infected intraperitoneally with 0.2 mL of infected blood containing about $1x10^7$ of *P. berghei* – parasitized erythrocyte per mL. The mice were assigned to five (5) groups of 5 mice each. Normal saline was administered to Group 1 (10 mL/kg/day) as the negative control, while Group 2 received the standard antimalarial chloroquine (5 mg/kg/day). Groups 3, 4, and 5 were administered 100 mg/kg, 200 mg/kg, and 400 mg/kg of ME, respectively. Similarly, the antimalarial activities of the solvent fractions (HF, EF, and BF) were compared at doses of 200 mg/kg and 400 mg/kg using 30 mice divided into six groups with five mice in each group. The animals were treated immediately after inoculation, from day zero through day 3. Thin blood smears were prepared from each mouse's tail blood, fixed methanol, and stained with 10% Giemsa stain. The stained smears were examined microscopically at x100 magnification, and the number of parasitized RBCs was counted in 10 different fields. The percentage of parasitemia (%) was then calculated.

$$\% \text{ parasitemia} = \left(\frac{A-B}{A}\right) 100$$

Where; A = parasitemia of the control group. B = parasitemia of the treated group.

Prophylactic antiplasmodial activities of methanol extract and solvent fractions

The method described by Peters[21] was used to evaluate the anti-plasmodial activity of *Phoenix reclinata* methanol leaf extract (ME) against *P. berghei* infection. Twenty-five Swiss albino mice of either sex (18-25 g) were assigned to five groups (n=5) and pretreated as follows: Group 1 received normal saline (10 mL/kg/day) as a negative control; Group 2 received standard antimalarial pyrimethamine (1.2 mg/kg/day); Group 3 received 100 mg/kg ME; Group 4 received 200 mg/kg ME; and Group 5 received 400 mg/kg ME. Similarly, the antimalarial activities of the solvent fractions (HF, EF, and BF) were compared using 30 mice at doses of 200 mg/kg and 400 mg/kg, with five mice in each group. The animals were inoculated with *Plasmodium berghei* on the fourth day after treatment through a single intraperitoneal administration of 0.2 ml of diluted infected red cells. The animals were then observed for 72 hours. Thin blood smears were prepared from each mouse's tail blood, fixed in absolute ethanol, and stained with a 10% Giemsa solution. The stained smears were microscopically examined at 100x magnification, and the number of parasitized red blood cells (RBC) was counted in 10 different fields. The degree of parasitemia (%) was then calculated.

$$\% \text{ parasitemia} = \left(\frac{A-B}{A}\right) 100$$

Where; A = parasitemia of the control group. B = parasitemia of the treated group.

Antipyretic activity

The antipyretic property of *Phoenix reclinata* was examined using the method described by Sini *et al*.[22] Twenty-five rats (200-250 g) were utilized in this experiment. They were separated into five groups (n=5). Group 1 received 10 mL/kg of distilled water, serving as the negative control, while Group 2 received 150 mg/kg of paracetamol as the positive control. Group 3 was given 400 mg/kg HF, Group 4 received 400 mg/kg EF, and Group 5 was administered 400 mg/kg BF. The normal temperature of the rats was recorded using a digital thermometer before pyrexia was induced by injecting a 15 % aqueous suspension of

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Brewer's yeast (20 mL/kg sc.). After 18 hours, rectal temperature was recorded, and corresponding groups received various treatments. Rectal temperature was recorded periodically at 30, 60, 90, and 120 minutes after drug administration.

Statistical analysis

Data obtained were expressed as MEAN ± SEM and were analysed for the significance of disparity using one-way analysis of variance (ANOVA) with repeated measures. Statistical Product and Service Solution (formerly known as Statistical Package for Social Sciences - SPSS) version 23 was employed. Values with $p < 0.05$ were regarded as significant, while values with $p > 0.05$ were regarded as non-significant.

Results and Discussion

The solvent fractions of *Phoenix reclinata* (Aracaceae) were subjected to preliminary phytochemical screening to identify the presence of various phytochemicals. The results showed the presence of glycosides, flavonoids, alkaloids, tannins, saponins, terpenoids, sterols, phenolic compounds, and terpenes (Table 1). The antipyretic effects of the leaf fractions of *Phoenix reclinata* were investigated in Brewer's assay method. The administration of Brewer's yeast elevated the temperature of the rats above the basal temperature after 18 hours following subcutaneous administration. The solvent fractions (EF, HF, and BF) produced a significant (p<0.05) decrease in rectal temperature at a dose of 400 mg/kg. The EF (400 mg/kg) exhibited a better decrease in rectal temperature (Table 2) (p<0.05).

Table 1: Phytochemistry of the solvent fractions of *Phoenix reclinata*

Phytochemical constituents	Butanol Fraction	Ethyl acetate Fraction	n-hexane Fraction
Saponin	+	+	-
Tannin	+	+	+
Flavonoid	+	+	+
Alkaloid	+	+	+
Phenolic Compounds	+	+	+
Sterols	+	+	+
Glycosides	+	+	-
Terpenes	+	+	+
Terpenoids	+	+	+
Sterols	+	+	+

(+) Presence (-) Absence

Table 2: Antipyretic activity of the solvent fractions of the methanol extract of *Phoenix reclinata*

Group	Temperature measurement (°C)					
	Basal	Pyrexia	30 min	60 min	90 min	120 min
10 ml/kg distilled water	36.4±0.01	39.7±0.03	40.1±0.03	39.6±0.02	39.8±0.02	39.4±0.03
150 mg/kg paracetamol	37.3±0.03	40.8±0.03	39.4±0.02*	39.2±0.04*	38.3±0.03*	38.0±0.04*
400 mg/kg HF	36.4±0.02	39.4±0.04	39.2±0.05	39.2±0.04	39.0±0.02	39.0±0.03
400 mg/kg EF	36.8±0.05	40.5±0.03	39.6±0.02*	39.1±0.03*	38.3±0.05*	37.4±0.02*
400 mg/kg BF	37.1±0.03	39.9±0.01	39.6±0.02*	39.2±0.03*	38.5±0.02*	38.1±0.05*

Values are expressed as mean ± SEM. n = 5; *$p < 0.05$ compared to negative control

In the curative antiplasmodial study, the methanol extract (ME) reduced the parasitaemia (p<0.05) from day 1 of treatment and achieved the highest reduction on the fifth day. ME (100, 200, and 400 mg/kg) produced percentage chemosuppression of 18.4 %, 46.4 %, and 79.6 %, respectively after treatment. The group of mice that were treated with the standard antimalarial drug, artemether/lumefantrine (9.8 mg/kg), produced 89.7 % chemosuppression (Figure 1, Figure 3). The solvent fractions (EF, HF, and BF) exhibited dose-dependent chemosuppressive effects. HF produced a chemosuppressive effect of 24.5 % at 200 mg/kg and 28.9 % at 400 mg/kg; EF produced a chemosuppressive effect of 56.8 % at 200 mg/kg and 85.3 % at 400 mg/kg, while BF produced a chemosuppressive effect of 48 % at 200 mg/kg and 56.8 % at 400 mg/kg. The order of chemosuppressive activity was EF > BF > HF (Figure 2, Figure 4). Similarly, in the suppressive antiplasmodial evaluation of methanol extract and solvent fractions of *Phoenix reclinata* on *P. berghei* infection in mice, the methanol extract (ME) significantly (p<0.05) reduced the level of parasitemia from day 1 of treatment and achieved the highest reduction on the fifth day. The parasitaemia reduction was 19.08 %, 45.23 %, and 80 % at 100, 200, and 400 mg/kg doses, respectively. The group treated with chloroquine (5 mg/kg) produced a percentage parasitemia reduction of 90.34 % (Figure 5, Figure 7). The solvent fractions (EF, HF, and BF) exhibited chemosuppressive effects as shown in Figure 6 and Figure 8. Also, in the prophylactic effect of methanol extract and solvent fractions of *Phoenix reclinata* on *P. berghei* infection in mice, administration of ME at 100, 200, and 400 mg/kg resulted in 8.71 %, 15.51 %, and 17.48 % reduction in parasitaemia levels, respectively. Pyrimethamine (1.2 mg/kg) produced a percentage parasitaemia reduction of 90.34 % (Figure 9, Figure 11) The solvent fractions (EF, HF, and BF) demonstrated dose-dependent chemosuppressive effects as shown in Figure 10 and Figure 12.

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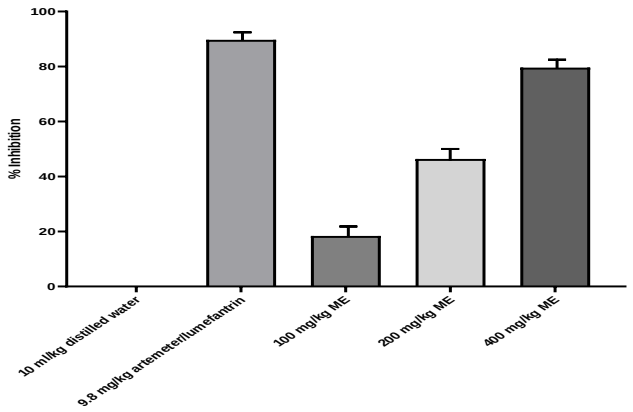

Figure 1: Percentage Inhibition of parasitaemia by methanol extract of *P. reclinata* in the curative antiplasmodial assay method

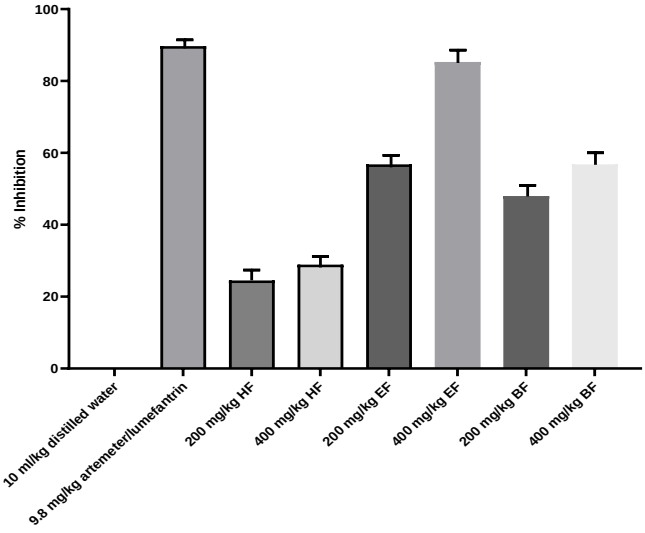

Figure 2: Percentage Inhibition of parasitaemia by solvent fractions of the methanol extract of *P. reclinata* in the curative antiplasmodial assay method

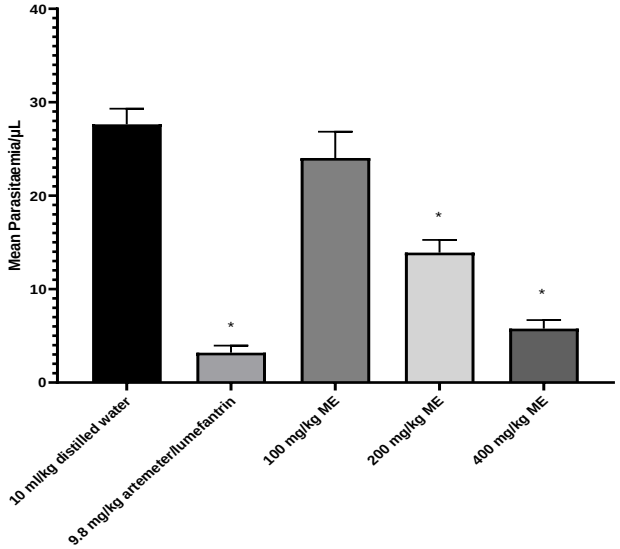

Figure 3: Curative effects of methanol extract on *Plasmodium berghei* infection in mice. Values are presented as Mean ± SEM; * p<0.05 Vs Control (Distilled water)

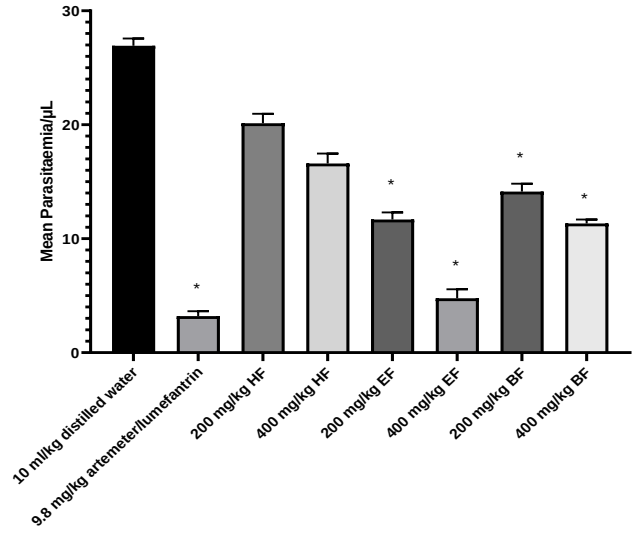

Figure 4: Curative effects of solvent fraction of methanol extract on *Plasmodium berghei* infection in mice. Values are presented as Mean ± SEM; * p<0.05 Vs Control (Distilled water).

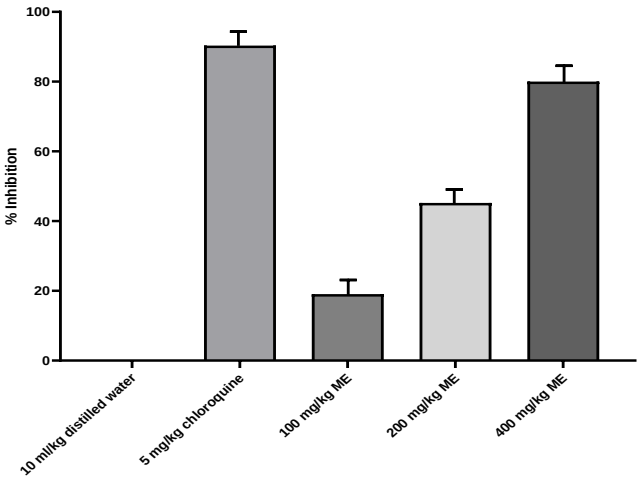

Figure 5: Inhibition of parasitaemia by methanol extract of *P. reclinata* in the suppressive antiplasmodial assay method.

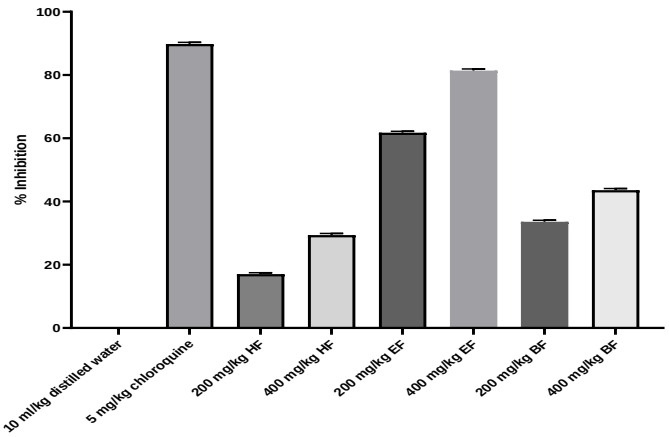

Figure 6: Percentage inhibition of parasitaemia by solvent fractions of the methanol extract of *P. reclinata* in the suppressive antiplasmodial assay method.

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Developing effective and reliable drugs to combat malaria is a critical challenge in modern parasitology.[23] This study investigated the antiplasmodial activity of the methanol leaf extract of *Phoenix reclinata* and its fractions (HF, EF, and BF). Phytochemical and antipyretic potentials of the solvent fractions were also evaluated. Many researchers have shown that most pathology observed in malaria infection and other infectious diseases can be attributed to the activation of the inflammatory system. This occurs when the balance between pro- and anti-inflammatory cytokines shifts towards systemic inflammation.[24] Considering the traditional use of *Phoenix reclinata* in treating malaria and related fevers, examining the extract and fractions for their effects on acute and chronic inflammation in rat models is crucial. The results of the anti-plasmodial tests indicated that the extract is active against the *Plasmodium berghei* malaria parasite. This effect could be due to the presence of phytochemicals widely reported for antiplasmodial activity. Tannins which are present in *Phoenix reclinata* have been demonstrated to have antiplasmodial activity on 3D7 and Dd2 strains of *P. falciparum*, suggesting the potential use of tannin extracts as an antimalarial source.[25] However, tannin mechanism of action remains unclear, they may interfere with various pathways, such as haemozoin crystallization, protein synthesis, or DNA fragmentation.[25] Flavonoids which are also one of the phytochemical constituents of *Phoenix reclinata*, have been suggested to increase the antiplasmodial activity of artemisinin and potentially slow the emergence of resistance in whole-plant preparations compared to artemisinin alone.[26] These attributes may arise from flavonoids enhancing artemisinin's solubility in water[27] or through the action of specific flavonoids, such as capsaicin, which increase artemisinin binding to haemin, a potential target of its action.[28] Yeast an exogenous pyrogen used in the induction of pyresis in this study can stimulate the release of endogenous pyrogens such as interleukin-1 and tumour necrosis factor from polymorphonuclear leukocytes, monocytes, and other cells[29], which acts on the thermoreceptive region in the preoptic anterior hypothalamus, releasing arachidonic acid, stimulating prostaglandin (PG) synthesis, and raising the set point of the temperature-regulating centre, this increases body temperature.[25] The antipyretic activity of *Phoenix reclinata* solvent fractions is likely due to inhibition of the synthesis and release of PGs in the central nervous system.[12] The key findings of this study suggest that the methanol leaf extract (ME) of *Phoenix reclinata* and its most active solvent fractions possess significant antiplasmodial activities.

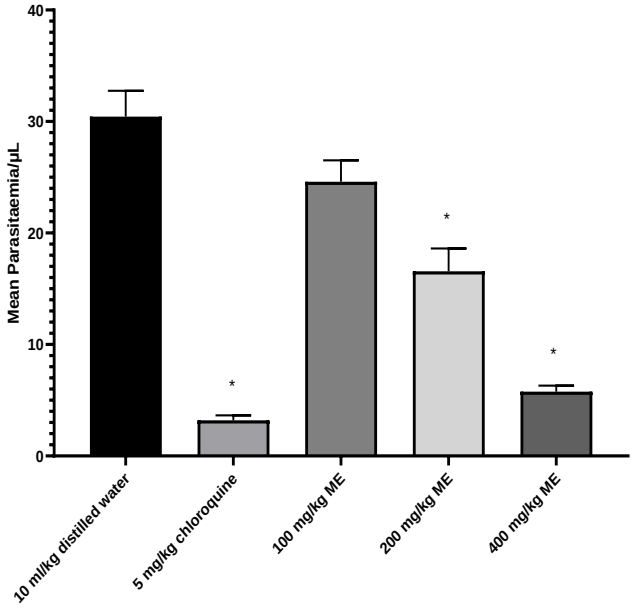

Figure 7: Suppressive effects of methanol extract on *Plasmodium berghei* infection in mice. Values are presented as Mean ± SEM; * $p<0.05$ Vs Control (Distilled water)

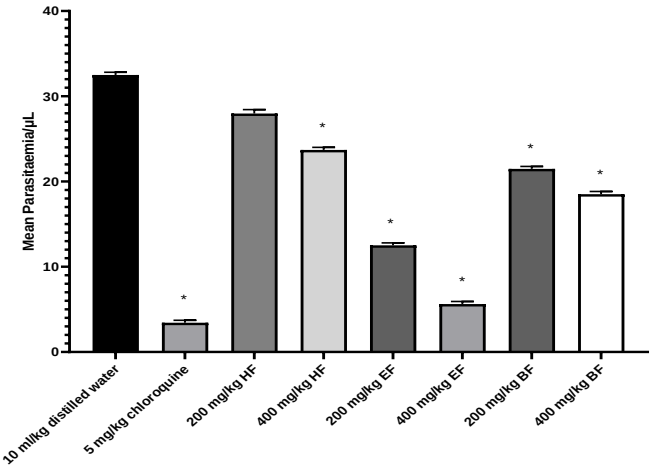

Figure 8: Suppressive effects of solvent fractions of the methanol extract on *Plasmodium berghei* infection in mice. Values are presented as Mean ± SEM; * $p<0.05$ Vs Control (Distilled water)

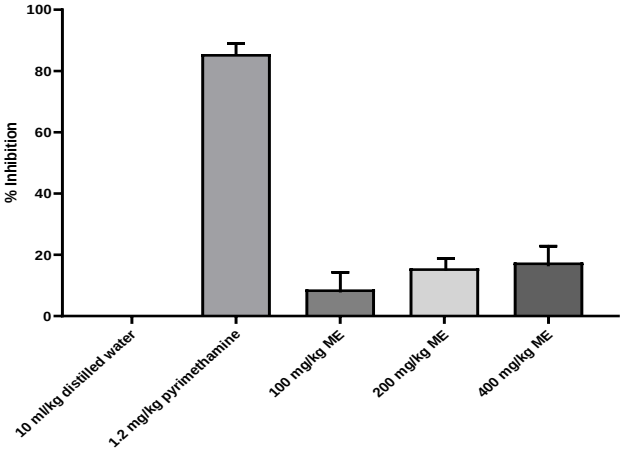

Figure 9: Percentage Inhibition of parasitaemia by methanol extract in the prophylactic antiplasmodial assay.

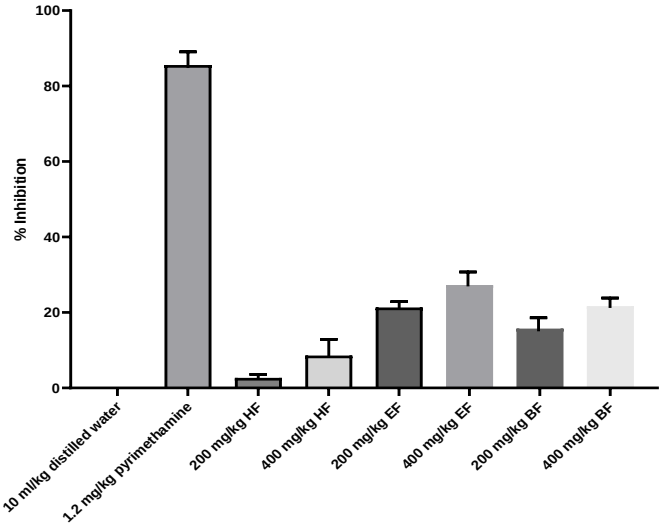

Figure 10: Percentage Inhibition of parasitaemia of the solvent fractions in the prophylactic antiplasmodial assay.

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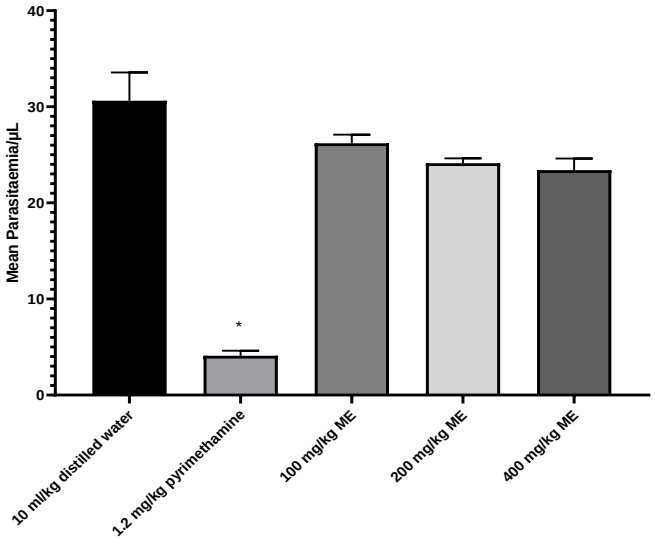

Figure 11: Prophylactic effects of methanol extract on *Plasmodium berghei* infection in mice. Values are presented as Mean ± SEM; * p<0.05 Vs Control (Distilled water)

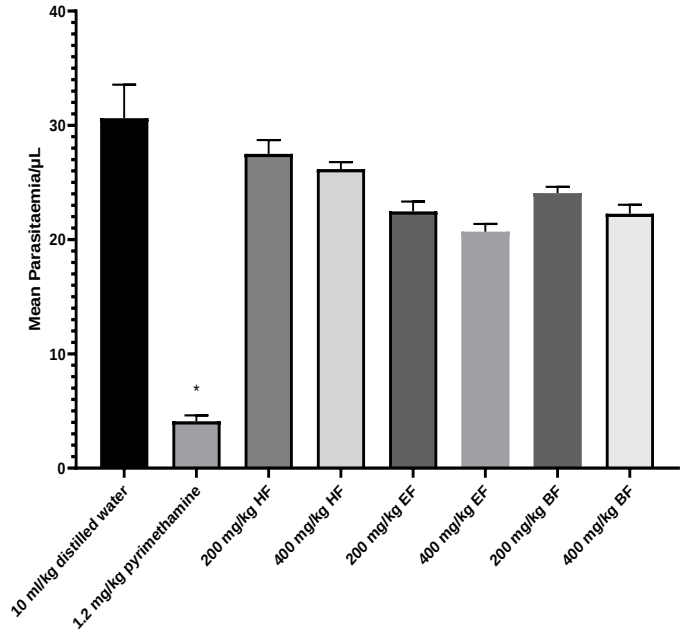

Figure 12: Prophylactic effects of solvent fractions on *Plasmodium berghei* infection in mice. Values are presented as Mean ± SEM; * p<0.05 Vs Control (Distilled water)

Conclusion

The study demonstrates that *Phoenix reclinata* extract exhibits antimalarial and antipyretic properties which may be linked to the presence of some bioactive phytochemical constituents in the plant extract and fractions. Consequently, the research supports the local use of *Phoenix reclinata* for malaria treatment and its potential for the development of new drugs to treat malaria.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Ali BH, Mohammed I, Muhammed MA, Mohammed SY, Garba D, Busola OA, Khadijah II, Manager MM. Evaluation of *In vivo* Antiplasmodial Activity of the Methanol Root Bark Extract and Fractions of *Bombax costatum*(Bombacacea) in *Plasmodium berghei*-Infected Mice.Trop J Nat Prod Res. 2022; 6(6):926-930.DOI: 10.26538/tjnpr/v6i6.18
2. Biswajit B. A short review of laboratory diagnosis of malaria. Int J Res Pharm Biomed Sci. 2013; 2(5):177-181
3. Uzoka FME, Akwaowo C, Nwafor-Okoli C, Ekpin V, Nwokoro C, El Hussein M, Osuji J, Aladi F, Akinnuwesi F, Akpelishi TF. Risk factors for some tropical diseases in an African country. BMC Public Health. 2021; 21:2261 DOI:.1186/s12889-021-12286-3
4. World Malaria Report 2023; https://www.who.int/news-room/fact-sheets/detail/malaria
5. Lacey MS. Walter TW. *Plasmodium vivax*, StatPearls Publishing. National Center for Biotechnology Information (NCBI), U.S. Nat Lib Med. 2021; 8600 Rockville Pike, Bethesda MD, 20894 USA.
6. Singh B, KimSung L, Matusop A. A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings. Lancet 2004; 363 (9414): 1017–1024.
7. Ekechukwu PA. Prevalence rate of *Plasmodium* species among the inhabitants in Owerri Metropolis. B.Sc. Project Report submitted to the Department of Medical Laboratory Sciences Imo State University Owerri, Nigeria .2005
8. Chinwuba P, Akah PA, Ilodigwe EE. *In vivo* antiplasmodial activity of the ethanol stem extract and fractions of Citrus sinensis in mice. Merit Res. J. Med. Med. Sci. 2015; 3: 140 - 146.
9. Barrow SC. *Phoenix reclinata*. Kew Bulletin 1998;53 (3), 513-575
10. Ruffo CK, Birnie A, Tengnas B. Edible Wild Plants of Tanzania. Regional Land Management Unit; 2002; Nairobi.ISBN 9966-896-60-0
11. Veronica R. *Phoenix reclinata* in The Shell Field Guide to the Common Trees of the Okavango Delta and Moremi Game Reserve. Gaborone, Botswana: Shell Oil Botswana,1992.
12. Chinwuba P, Achunike PA, Ugorji P, Nworu CS. Anti-Inflammatory, and Antipyretic Activity of Methanol Leaf Extract of *Phoenix reclinata*, Jacq (Aracaceae). Trend Nat Prod Res 2024; 5(1). 44-51. DOI:10.61594/tnpr. v5i1.2024.104
13. Charles DK, Cissy BN, Kokas I, Andrew T, Gabriel T, John K. Nephroprotective Effect of *Phoenix reclinata* Total Crude Root Extract on Tenofovir Induced Kidney Damage in Wistar Albino Rats. J. Pharm Res Int. 2017; 17(6): 1-10. DOI: 10.9734/JPRI/2017/34247
14. Hortense BS, Yolande DD, Valery NKP, Betty FM, Maxwell BAG, Patrice KL. Physicochemical and Nutritional Properties of Date (*Phoenix reclinata*) from Dabou (Côte d'Ivoire). Asian J. Crop. Soil Plan. Nutri. 2019; 5(2): 1-8.
15. Oljira Kenea OK, Habte Tekie HT. Ethnobotanical survey of plants traditionally used for malaria prevention and treatment in selected resettlement and indigenous villages in Sasiga district, Western Ethiopia. J Biol Agric Healthc.2015;5(11), 1-9.
16. Ogbeide OK, Dickson VO, Jebba RD, Owhiroro DA, Olaoluwa MO, Imieje VO, Erharuyi O, Owolabi BJ, Fasinu P, Falodun A. Antiplasmodialand Acute Toxicity Studies of Fractions and Cassane-Type Diterpenoids from the Stem Bark of *Caesalpinia pulcherrima* (L.) Sw. Trop J Nat Prod Res. 2018; 2(4):179-184. Doi: 10.26538/tjnpr/v2i4.5

17. Odetola, A. and Bassir, O. Evaluation of Antimalarial properties of some Nigerian Medicinal Plants. In: Sofowora, A. editor, Proceeding of African Bioscience Network, Fed. Min. of Science and Tech., Nigeria Society of Pharmacognosy and Drug Research. And Production Unit, University of Ife. 1980
18. Trease GE, Evans WC. Pharmacognosy. 15th Ed. London: Saunders Publishers 2002; pp. 42–393
19. Ryley JF, Peters W. The antimalarial activity of some quinolone esters. Ann. trop. med. Parasitol. 1970; 84:209-222
20. Knight DJ, Peters W. The antimalarial action of N-benzyloxy dihydro triazines. The action of cycloguanil (BRL50216) against rodent malaria and studies on its mode of action. Ann. trop. med. Parasitol. 1980; 74: pp. 393–404.
21. Peters W. Drug resistance in *plasmodium berghei*. Vinka and Lips. Exp. Parasitol. 1965; 17: 80-89
22. Sini KR, Sinha BN, Karpakavalli M, Sangeetha PT. Analgesic and antipyretic activity of *Cassia occidentalis* Linn. Ann Biol Res. 2011; 2 (1):195-200
23. Okaiyeto K, Hoppe H, Okoh AI. Plant-Based Synthesis of Silver Nanoparticles Using Aqueous Leaf Extract of *Salvia officinalis*: Characterization and its Antiplasmodial. Activity. J Cluster Sci. 2021;32:101–109. DOI:10.1007/s10876-020-01766-y.
24. Dinarello CA. Blocking interleukin-1 and tumour necrosis factor in disease. Euro Cytok Net.1997; 8: 294-296.
25. Mamadou W, Singou K, Cheickna C, Nouhoum D, Laura K, Lamine B. Antibacterial and Antiplasmodial Activities of Tannins Extracted from *Zizyphus mauritiana* in Mali. Int J of Biochem Res & Rev 2018; 24(2):1-8. DOI: 10.9734/IJBCRR/2018/45335
26. Weathers PJ, Towler M, Hassanali A, Lutgen P, Engeu PO. Dried-leaf *Artemisia annua*: a practical malaria therapeutic for developing countries? World J of Pharmac.2014; 3:39–55.
27. Mueller MS, Karhagomba IB, Hirt HM, Wemakor E. The potential of *Artemisia annua* L. as a locally produced remedy for malaria in the tropics: agricultural, chemical and clinical aspects. J. Ethnopharm.2000;73 487–493. DOI:10.1016/S0378-8741(00)00289-0
28. Czechowski T, Mauro AR, Mufuliat TF, Maria VV, Tony RL., Thilo W, Deborah AR, David H, Paul H, Ian AG. Flavonoid Versus Artemisinin Anti-malarial Activity in *Artemisia annua* Whole-Leaf Extracts. Frontiers in Plant Sci. 2019; 10: 984. DOI: 10.3389/fpls.2019.00984
29. Kumar V, Abbas AK, Fausto N. Acute and chronic inflammation. In: Kumar V, Abbas AK, Fausto N, ed., Robbins and Cotran Pathologic Basis of Disease, 7th ed. Philadelphia, Elsevier Saunders, 2005. 47–86 p.