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Effectiveness of Ethanoic Leaf Extract of *Picralima Nitida* Against *Plasmodium Berghei* in Laboratory Albino Rats

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Abstract
This study was carried out to investigate the effect of the ethanolic extract of *Picralima nitida* leaf against *P.berghei* in albino rats. Two parameters were employed to evaluate the efficacy of the extract against *P.berghei* in albino rats. A parallel test was carried out with chloroquine as the standard drug. Twenty-five albino rats were used in the experiment. The albino rats were grouped into six groups H_1, H_2, H_3, H_4. H_5 and H_6. Three groups (H_4, H_5 and H_6) served as negative, positive and standard control respectively, The other three groups (H_1, H_2 and H_3 were treated with 500,1000 and 1500mg/kg body weight of the extract for suppressive and curative activity. The extract displayed a strong anti-plasmodial activity against *P.berghei* both in the two parameters. The mean parasitemia reduction in groups H_1, H_2, H_3 were significant ($p<0.05$). The in-vivo antimalarial activities of *Picralima nitida* ethanolic leaf extract and chloroquine resistance against *Plasmodium berghei* showed that at doses of 500, 1000 and 1500mg/kg body weight. The mean parasitemia in the albino rat was reduced to 80.23%, 80.45% and 83.12 % respectively, while in the control was 71.87% 00.0% and 00.0% respectively. The results indicate that although chloroquine was still effective against the parasite, the leaf extract of *Picralima nitida* reduced the mean parasitemia more than chloroquine which was used as standard control. This means that the extract has stronger anti-malarial activity compared to the standard chloroquine drug. Further research work is recommended on the mechanism of the efficacy and haematological effect of the plant extract

Keywords: *Picralima Nitida*, Ethanolic Leaf Extract, Antimalarial, *Plasmodium Berghei*, Hematological Effect

Introduction

Malaria is an ancient disease that has been in existence for decades. Malaria occurs in Chinese documents from about 2700 BC (Abdel-Wareth et al., 2014). Malaria is a disease caused by parasites of the genus *Plasmodium*, which are transmitted to humans through the bite of female mosquitoes belonging to the genus Anopheles. Malaria poses a tremendous Public Health problem across the globe. Current estimates show that about 229 million cases are due to malaria resulting in about 409,000 deaths globally (WHO, 2021). About 3.2 billion people are at risk of malaria globally, where 97 countries have an ongoing malaria transmission with 35% of malaria deaths occurring in Nigeria and the Democratic Republic of Congo (WHO, 2016). In Angola and Ghana, about 16,000 and 13,000 deaths were attributed to malaria in 2016 (Micheal et al., 2019). Despite a reduction of 18% in the prevalence and incidence rate of the disease globally between 2010 and 2017, its impact was not felt within the African sub-region as malaria continued to be a leading cause of hospital attendance, morbidity and mortality (Adebayo et al., 2011). According to the latest World Malaria report, there were 247 million cases of malaria in 2021 compared to 245 million cases in 2020. The estimated number of malaria deaths stood at 619,000 in 2021 compared to 625,000 in 2020 (WHO, 2021). Over the 2 peak years of the pandemic (2021-2020), COVID-related disruptions led to about 13 million more cases of malaria and 63,000 more malaria deaths. The World Health Organization African Region reported in 2021 that the region was home to about 95% of all malaria cases and 96% of deaths. Children under 5 years of age accounted for about 80% of all deaths in the region (WHO, 2021). Four African countries accounted for just over half of all malaria death worldwide; Nigeria 13.3%, Democratic Republic of the Congo 12.6%, United Republic of Tanzania 4.1%, and Niger 3.9% respectively (WHO, 2022). Globally, the World Health Organization (WHO) estimated 241 million malaria cases in 2020, which was an increase, compared with 227

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million cases recorded in the previous year. The WHO African region accounted for 95% of these cases, with Nigeria leading the region accounting for 27% of recorded cases (WHO, 2021). The most common symptoms include chills, fever, sweating, headache, fatigue, anorexia, nausea, and vomiting, but in severe cases, it could lead to anaemia, haemoglobinuria, low blood pressure, acute kidney failure, hyper-parasitaemia, metabolic acidosis and hypo-glycaemia. Malaria has also been reported to affect neurological cognition in children with subsequent effects on short-term impairment of memory and language function (Vorasan et al., 2015). Children with severe anaemia may present symptoms and signs of cardiac failure, dyspnoea, tachycardia, gallop rhythm, basal crackles, hepatomegaly (enlarged liver), splenomegaly and raised jugular pressure (Wokem et al., 2008; Bartoloni & Zammarch, 2012, Auta et al., 2021). Again, severe anaemia can cause convulsion, restlessness, retinal haemorrhage and even coma. Apart from children, malaria is a major cause of maternal death and other several complications during pregnancy such as low birth weight, anaemia, spontaneous abortion, and maternal and neonatal mortality with about 200,000 newborn deaths annually (Onoja et al., 2019).

The burden of malaria is well documented and is a contributor to the economic burden of disease in communities where it is endemic and is responsible for an annual economic loss of 132 billion naira (WHO, 2009). There is a strong correlation between malaria and poverty and it has been established that malaria impedes economic growth and keeps households in poverty (Teklehaimanot & Mejia, 2008). For instance, the costs of malaria to individuals and their families include the purchase of drugs for treating malaria at home; expenses incurred in travelling to clinics and cost of treatment; lost days of work; absenteeism from school and work; expenses for preventive measures and expenses for burial in case of deaths. On the other hand, the costs to governments include maintenance, supply and staffing of health facilities; purchase of drugs and supplies; public health interventions against malaria, such as insecticide spraying or distribution of insecticide-treated bed nets and lost opportunities for joint economic ventures and tourism (WHO, 2012). The above facts have serious socio-economic implications on health outcomes and welfare with an indirect impact on economic growth (WHO, 2021).

Diagnosis should be confirmed by parasitological testing using microscopy or a rapid diagnostic test. Confirmation of the parasite's presence is by laboratory examination of the blood. Resistance remains one of the greatest threats to success in the control of malaria parasites. There are numerous factors involved in the appearance and spread of resistance. These include long-term prophylaxis with low doses of antimalarial agent, selective survival of resistant parasites in treated hosts, frequent feeding by mosquitoes from different hosts, population movements, and disruption of healthcare by wars and civil strife. The spread of chloroquine-resistant *Plasmodium falciparum* and the alarming emergence of multidrug-resistant strains have raised an urgent need to search for new anti-malarial drugs without side effects. In many tropical and sub-tropical regions plants and their materials have traditionally been used to treat malaria and other symptoms associated with the parasite (Avwioro, 2010).

Challenges associated with malaria eradication could be linked to the advent of resistant strains to several current drugs. The emergence of the ineffectiveness of chloroquine in combating malaria led to additional studies, which produced a new and effective antimalaria drug, Artemisinin (Saganuwan & Onyeyili, 2011). Despite the success recorded with the Artemisinin Combination Therapy (ACT), most malaria-endemic communities still rely on traditional herbal medicines which are often readily available and affordable. Given the problem associated with anti-malaria drugs, poverty and continuous dependence on herbal medicine by about 80% of Africans, researchers have now focused on the investigation of medicinal plants to scientifically establish the viability or otherwise of these plants in the treatment of malaria (Viroj, 2016). According to Mustofa et al. (2007), the usefulness of these medicinal plants may hold the key to another new and effective anti-malaria drug in the future. Plant natural products also play a significant role in the healthcare system of the remaining 30% to 20% of the world population who reside mainly in developed countries (Morah, 2010). Ethnobotany, the study of traditional human uses of plants, is recognized as an effective way to discover future medicines, and accordingly, the World Health Organization also described traditional medicine as one of the surest means, to achieve total health coverage for the world population (Saganuwan & Onyeyili, 2011). Similarly, the use of and search for drugs and dietary supplements derived from plants have accelerated in recent years, Pharmacologist, Parasitologists, Botanist and Natural-Products Chemist are combining the earth for phytochemicals and leads that could be developed for the treatment of various diseases (WHO,2016).

In Africa, plant extracts are employed in the treatment of different ailments due to their antibacterial, antifungal and antiparasitic properties (Sear et al., 2010). It is known that more than 400,000 species of tropical flowing plants have

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medicinal properties and these have made traditional medicines cheaper than modern medicines (Ladipo & Doherty, 2011). These plants are generally referred to as medicinal plants. Medicinal plants refer to any plant which, in one part or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. Numerous plants indigenous to Africa and Nigeria in particular have been found with amazing anti-parasitic properties. Some are well-evaluated vis-à-vis their content of specific active ingredients against the target parasites while others are not. It is therefore very essential that medicinal plants whose properties have not been fully characterized should form a top agenda of top management in developing nations whose citizens are unable to afford expensive orthodox medicine but rely on herbal medicine as an alternative. Despite various efforts to control malaria, the disease continues to be one of the greatest health problems facing Africa (Auta et al., 2021). *Picralima nitida* is a rainforest plant belonging to the Apocynaceae family. It has numerous medicinal applications in pneumonia, diabetes, hypertension, fever and malaria. Virtually all the parts have various ethnobotanical uses (Opeyemi et al., 2020). Various parts of the plant; the leaves, seeds, stem bark and roots are used by herbalists for the treatment of fever, hypertension, jaundice, gastrointestinal disorders and malaria (De Campos et al., 2020). The extract from different parts of the plant has been found to exhibit a broad range of pharmacological activities which lends credence to its ethnomedicinal uses, hence this study.

Materials and Methods

The fresh leaves of *Picralima nitida* were collected and identified in the Plant Science and Biotechnology Department, at Rivers State University. A specimen was deposited in the laboratory. The leaf was air-dried for two weeks and milled into powder form as described by (Abdel-Wareth et al., 2014). Using cold maceration two hundred grams (200g) of powdered material was weighed into a glass container, and sufficient petroleum either of one hundred and fifty to two hundred and fifty (150-250ml) was added enough to bury the powered weighed material. The glass was closed and shaken intermittently for several times a day for two to three days (2-3 days). This was filtered through a double-layer filter paper. The residue was dried at room temperature with the process repeated using ethanol solvent. At the end of the process, the two filtrates were placed in a water bath at 70^0c for forty-five minutes (45 min) to evaporate leaving the non-polar substance of the plant extracts. After five days (5 days), the ethanolic filtrate evaporates and dries completely.

Twenty-five albino rats of both sexes weighing between 150-250g, were used in the experiment and were obtained from the animal house, at Rivers State University. The animals were fed with a standard diet and water. Drug-sensitive *Plasmodium berghei* strains were obtained from Nigeria Institute of Medical Research (NIRMA) Lagos, Nigeria and maintained in the animal house by inoculating the non-infected albino rats with 0.2ml of blood from the infected every four days in the animal house. The albino rats were pre-screened by collecting blood from the tip of the tail. Thick and thin smears were made and observed to rule out the possibility of any test animal harbouring rodent *Plasmodium* species. The anti-plasmodial activity was assessed by suppressive and curative test procedures. A donor albino rat infected with the rodent malaria parasite *Plasmodium berghei* with parasitemia of about 20-40% was anaesthetized with 1% benzocaine and blood was collected through the cardiac puncture with a sterile disposable needle and syringe. The blood was diluted with normal saline. The method described by Tona et al. (2001) was used with slight modification for the inoculation of parasites. Inoculums of 0.2ml were given to each of the albino rats in groups H_4, H_5 and H_6, uninfected albino rats intraperitoneal. Evaluation of parasitemia level was done by making thin blood films of infected albino rats and then microscopically examined for parasitemia level from day 4 to day 7, the parasitized cells were counted and the percentage of parasitemia was evaluated.

Treatment with 500,1000 and 1500mg/kg body weight of ethanolic extract started immediately after the albino rats had been inoculated (early infection) as described by Auta et al. (2020). Inoculums of 0.2ml were given to each of the clean albino rats intra-peritoneal in the treated groups of H_1, H_2 and H_3, with each given 0.2ml of the extract intraperitoneal once daily for four days for the other four days respectively. A parallel test was done using chloroquine 10mg/kg, normal saline (NS) and phosphate buffer solution (PBS) to serve as a reference from Day O to DAY 3. Thick and thin films were made from the tail blood of the albino rat. The films were fixed with ethanol and stained with 4% Giemsa stain at pH 7.2 for 45 minutes. The slides were examined microscopically. Two different fields were examined on each slide and the number of infected and uninfected red blood cells were counted and the mean calculated.

The method and procedure used for the curative test were similar to the one described for the suppressive test, except that in the curative test, treatment with the extract and chloroquine(500,100 0 and 1500mg/kg and 10mg/kg) respectively

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commenced on day three and by day six, the albino rats were examined for parasite clearing. The increase/ decrease in parasitemia and the mean survival time (days) were recorded to that of the control groups.

Results

The extracts demonstrate good efficacy on the infected albino rats at all doses of the extracts as compared to the standard drugs used for the treatment of the parasite. The negative and positive all showed zero efficacies. The higher doses of the extracts gave a better suppressive treatment than the standard drug of 80.28%, 80.45%, 83.12% and 71.87% for 500mg/kg, 1000mg/kg, 1500mg/kg extract and 10mg/kg chloroquine respectively. There is a significant difference between the extracts and the standard drug $p < 0.05$ (Table 1).

Table 1: The effects of ethanolic extract of leaf of *Picralima nitida* and chloroquine infected albino rats with *Plasmodium berghei* (Suppression).

Extract	Dose mg/kg	Mean Parasitema	% Suppression
H_1	500	7.01±2.93	80.28
H_2	1000	6.95±1.00	80.45
H_3	1500	6.05±0.64	83.12
H_4	NS	28.10±2.1	00.0
H_5	BS	00.00	0.00
H_6	10mg/kg	10.00±1.15	71.87

The effect of the extract on the curative study gave a positive result which competes with the standard drug. All the infected albino rats treated with the leaf extract at all doses gave good curative results as compared with the standard drug. The negative control gave zero curative. The infected albino rat with 500, 1000, 1500 mg/kg of the extract and 10mg/kg of standard drug gave 89.88, 94.25, 94.67 and 71.89 respectively. The negative are positive controls gave zero curative 00.00% (Table 2)

Table 2: The effect of ethanolic extract of leaf of *Picralima nitida* and chloroquine on infected albino rats with *Plasmodium berghei* (curative).

Extract	Dose mg/kg	Mean Parasitema	% Suppression
H_1	500	7.01±2.93	80.28
H_2	1000	6.95±1.00	80.45
H_3	1500	6.05±0.64	83.12
H_4	10N.S	28.10±2.1	00.0
H_5	10 B.S	00.00	0.00
H_6	10 CQ	10.00±1.15	71.87

Value Express as mean + SEM $p< 0.05$

Discussion

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The in-vivo antimalarial activities of *P*icra*lima nitida* ethanolic leaf extract and chloroquine resistance against *Plasmodium berghei* showed that at doses of 500, 1000 and 1500mg/kg body weight, the extract reduced the mean parasitemia in the albino rats. This indicated that the suppressive activity of the extract drastically reduced the mean parasitemia in the albino rat by 80.23%, 80.45% and 83.12 % respectively, while in the control group treated with chloroquine, phosphate buffer solution and normal saline, the means parasitemia were 71.87% 00.0% and 00.0% respectively. Hence this suggests that the ethanolic leaf extract of *Picralima nitida* possessed some anti-plasmodial potential. Ajayi et al 2015 recorded a similar percentage of chemo-suppressive and dose-dependent effects of ethanolic fruit extract of *P. nitida* of 65.5%, 70.4%, and 73.0% respectively for 30, 70, and 115mg/kg/day doses. Osayemwenre et al. (2014) reported that the seed, fruit and stem bark extracts of *P.nitida* are active against *Plasmodium falciparum* in vitro. Francis et al. (1996) also reported the root and stembark inhibitory activities against a sexual erythrocytic form of *Plasmodium falciparum* with $1C_{50}$ values of 0.188, 0.545 and 1.581mg/ml respectively. Eyya et al. (2017) recorded a chemosuppressive of 93.4% in an ethanolic extract of preliminary phytochemical analysis and in-vivo antimalarial activity of crude extract of the leaf of African Mistletoe *Tapinanthus dodoneifolis* against *Plasmodium berghei* in mice. Tona et al. (2018) showed that *Khaya Senegalese* rootbark gave 78% suppression which supports this current work. The antimalaria activity may be due to the presence of the phytochemicals in the plant acting singly or in combination with others to exhibit the antimalaria activities (Shittu et al., 2010).

The curative activity of the ethanolic leaf extract of *P.nitida* showed a dose-dependent reduction in the mean parasitemia in albino rats more than in the groups treated with chloroquine, phosphate buffer solution and normal saline. The curative percentages for 500, 1000 and 1500mg/kg of the leaf extract were 89.88%, 94.25% and 94.65% respectively. While for groups treated with chloroquine, phosphate buffer solution and normal saline, the corresponding values were 71.87%, 00.0% and 00.0% respectively. This implies that the group treated with chloroquine was still effective in reducing parasitemia. However, the group treated with the extract of *P. nitida* leaf tends to exhibit a more drastic reduction in the mean parasitemia compared to chloroquine. This present study therefore supports the effectiveness of leaf of the plant extract of *P. nitida* as an antimalarial for use in traditional medicine for the treatment of malaria and other illnesses associated with malaria.

Conclusion

The leaf extract from *P.nitida* has been shown to exhibit moderate antimalarial against *Plasmodium berghei* in-vitro likewise chloroquine. The antimalarial activity of the plant may be due to the photochemical present. Further research work is recommended on the mechanism of the efficacy and haematological effect of the plant extract.

Recommendations

1. Further research should be conducted to determine the level of efficacy of each of the phytochemicals in the plant.
2. The government should regulate herbal therapy and integrate it with the primary health care system
3. There should be an effective law to preserve these plants.

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