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Antioxidant and Antimalarial Activities of Methanol Extract of *Picralima nitida* Root Bark

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ABSTRACT: Ethnomedically, the root, stem bark, seed or leaves of *Picralima nitida* are relevant in local preparations as antimalaria, antipyretic, antihypertensive and gastrointestinal agents. This study was therefore designed to determine the antimalarial and antioxidant effects of the methanol extract of the root bark of *Picralima nitida* using standard procedures. The antioxidant activity of the methanol root bark extract was evaluated using 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging method and ferric reducing antioxidant power (FRAP) assay. The antimalarial activity of the methanol root bark extract was investigated using the 4-day suppressive test in mice infected with *Plasmodium berghei*. The extract showed a concentration-dependent antioxidant activity with 50% inhibitory concentration (IC_{50}) for free radical scavenging activity of 10.19 µg/mL, while the FRAP value was 0.17 ± 0.00 mM FSE/g Extract. The extract demonstrated significant antimalarial activity with 68.33% and 67.27% parasitaemia suppression at doses of 400 mg/kg and 800 mg/kg, respectively. The present study has shown that the root bark extract of *P. nitida* has antioxidant and antimalarial activities. This study does not only validate the claimed ethnomedicinal use of the plant as antimalaria but also has shown the plant as a potential source of active antimalarial agent.

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The most important concern of mankind has been to maintain health and restore wellbeing. Over the years, different cultures and societies have developed diverse modes of traditional medical practices to maintain and restore health, contributing to improved wellbeing for a large part of the population (Ortega, 2009). In many cases, natural products remedy contribute to a greater part of traditional medicine. For thousands of years, natural products have played a very important role in healthcare and prevention of diseases. Moreover, Herbs are one of those natural product sources that have made cultural health maintenance and treatment possible. Human beings have used these herbs for the treatment of diverse ailments for thousands of years (Sofowara, 1982; Hill, 1989; Phillipson, 2001). The information about the medicinal value of plants are commonly based on the empirical knowledge of ancient people, which was passed over several generations. Nowadays, the use of plants as alternative medicine is increasingly more popular among developed societies. Historically, herbal drugs have been used as tinctures, decoction, poultices, powders, teas, and more recently, as pure compounds (Tyler, 2000). *Picralima nitida* (Apocynaceae) is an understorey tree which reaches up to 4 - 35 m in height. The plant is widely distributed in high deciduous forest of West-Central Africa where it finds great usefulness in African traditional medicine

especially in the rainforest regions of Nigeria, Ghana, Cote d'Ivoire, Gabon and Cameroon. Various parts of the plant including the seeds, fruits, fruit pulp, fruit rind, leaves, stems and roots are employed ethnomedicinally as remedy for a variety of human diseases such as malaria, diabetes, hypertension, fever, infections, pain and various inflammatory conditions (Betti, 2004). The seeds for example are used as quinine substitute in ethnomedical treatment of malaria (Ogbonna *et al*., 2013). It has also been reported to have curative effect in respiratory infections and as enema (Iwu and Klayman, 1992), as analgesic and to treat several inflammatory conditions (Ezeamuzie *et al*., 1994). Extracts and pure compounds from *P. nitida* have been shown to exhibit numerous pharmacological activities ranging from antimicrobial (Kouam *et al*., 2011; Ubulom *et al*., 2012), antiprotozoal (Bickii *et al*., 2007; Okokon *et al*., 2007), analgesic, antipyretic (Ezeamuzie *et al*., 1994; Duwiejua *et al*., 2002), anti-inflammatory (Woode *et al*., 2006), antidiabetic (Teugwa *et al*., 2013; Yessoufou *et al*., 2013), antioxidant (Erharuyi and Falodun, 2012) to cytotoxic activities (Shittu *et al*., 2010; Osayemwenre *et al*., 2011). The anti-malarial activity of the plant has been shown to be high in the roots, stem barks, and fruits, but lower in the seeds and leaves (Francois *et al*., 1996). Some indole-alkaloids have been isolated from the seeds and have shown activity against the chloroquine-resistant type of *Plasmodium falciparum* (Iwu, 1994). Some of its alkaloids possess analgesic and anti-inflammatory activities, central nervous system depressant effect as well as intestinal smooth muscles relaxant activity (Ezeamuzie *et al*., 1994; Menzies *et al*., 1998; Duwiejua *et al*., 2002; Woode *et al*., 2006). Based on its ethnomedicinal usage and previous scientific reports on its pharmacological activity, this paper set out to evaluate the antioxidant and antimalarial activities of the methanol extract of *Picralima nitida* root bark collected from a forest near Benin City, Nigeria.

MATERIALS AND METHODS

Collection and Preparation of Plant Material: Fresh *Picralima nitida* roots were collected in September, 2018 from a forest near Benin City, Nigeria. The roots were washed with water to remove earthy materials after which the bark was removed, air-dried and powdered with the aid of a mechanical grinder. The crude powdered sample was stored in an air-tight container until ready for use.

Extraction of crude powdered sample: The powdered plant material (0.7 kg) was extracted with 3.5 L of methanol by maceration at room temperature for 7 days. The extract was concentrated to dryness using a rotary evaporator at reduced pressure. The concentrated extract was weighed and the percentage yield calculated based on the initial weight of the crude powdered sample. The extract was stored in an air-tight container and kept in the refrigerator at 4^{o}C until further experiment.

Determination of Antioxidant Activity: DPPH Radical Scavenging Assay: The radical scavenging activity of crude methanol extract of *P. nitida* root bark was evaluated using method previously described with slight modification (Jain *et al*., 2008). A solution of 0.2 mM DPPH in methanol was prepared, and 1.0 mL of this solution was mixed with 3.0 mL of extract in methanol containing 0.001 - 0.2 mg/mL of the extract. The reaction mixture was mixed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as reference standard. The ability to scavenge DPPH radical was calculated by the following equation:

$$\text{DPPH RSA} = \frac{Ao - A1}{Ao}\ x\ 100$$

Where; DPPH RSA = DPPH Radical Scavenging Activity; A_o = Absorbance of DPPH radical in methanol, A_1 = Absorbance of DPPH radical + sample extract/standard; The 50% inhibitory concentration value (IC_{50}) is indicated as the effective concentration of the sample that is required to scavenge 50% of the DPPH free radical.

Ferric reducing antioxidant power (FRAP): The FRAP assay was done according to method previously described by Benzie and Strain (1996) with some modifications. The fresh working FRAP solution was prepared by mixing 25 mL of 300 mM acetate buffer (pH 3.6), 2.5 mL of 10 mM TPTZ solution in 40 mM HCl, and 2.5 mL of 20 mM $FeCl_3.6H_2O$ solution and then warmed at 37^{o}C before using. The extract (1.5 mL of 1 mg/mL) was mixed with 2.8 mL of the FRAP solution, and incubated at room temperature for 30 min in the dark. Readings of the coloured product (ferrous tripyridyltriazine complex) was then taken at 593 nm. The calibration curve was constructed using $FeSO_4.7H_2O$, at concentrations of 0.1, 0.4, 0.8, 1, 1.12, and 1.5 mM, and the absorbance values were measured as for sample solutions. Results were expressed as millimolar of ferrous sulphate equivalent per gram of extract (mM FSE/g extract).

Antimalarial assay

Animals: Twenty (20) adult Swiss albino mice with an average weight of 26 g were obtained from the Animal house of the Department of Pharmacology and

Toxicology, Faculty of Pharmacy, University of Benin. The animals were kept in clean and well maintained cages, fed with standard rodent pellets (Bendel feeds and flower mill, Ewu, Nigeria), and allowed access to water *ad libitum*. The animals were randomly grouped into five experimental groups (I to V) of four (4) animals per group.

Ethical consideration: The animals were maintained and cared for in accordance with the international guidelines for the use and maintenance of experimental animal (OECD, 2001). Ethical approval was obtained from the animal use and ethics committee of the Faculty of Pharmacy, University of Benin.

Malaria parasites and preparation of inoculum: An infected donor mouse with the chloroquine-sensitive NK65 strain of *Plasmodium berghei* was obtained from the Department of Biochemistry, Nigeria Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria. Blood (1 mL) was taken from the donor mouse by cardiac puncture after chloroform euthanasia into heparinized bottle. The parasitized blood was diluted with 3 mL phosphate buffered saline to give a working inoculum of approximately 1 x 10^8 parasitized red blood cells per mL. The dilution was based on the parasitemia of the donor mice and the red blood cell count of the normal mice so that 1 mL blood contained 1 × 10^8 infected erythrocytes (Hilou *et al*., 2006).

Antimalarial 4-day suppressive test: A four (4) day suppressive test was used for this study according to procedure previously described with slight modification (Peter *et al*., 1995). Day 0: An aliquot of 0.1 mL of the working inoculum was injected intraperitoneally into the experimental groups of mice. Two (2) hours post inoculation, the experimental groups of mice were administered graded doses of test sample (*Picralima nitida* root bark extract) via oral route using an oro-gastric tube. Groups I, II and III received 200, 400 and 800 mg/kg body weight of the extract, respectively. Group IV was administered 5 mg/kg body weight of chloroquine to serve as the positive control, while group V was untreated and was used as the negative control. Day 1 to 3: The experimental groups of mice were again administered same doses (200, 400, and 800 mg/kg) of the test extract and 5 mg/kg of chloroquine once daily by the same route as on day 0. On day 4, thick and thin blood films from all animals were made from the blood obtained by tail prick of the mice. The thin film was fixed with absolute methanol for 15 min. Both films were stained with 4% Giemsa stain (pH 7.2) for 45 min. The stain was washed from the slide by dipping in a trough of distilled water. After washing, the slides were placed in the drying rack, film side downwards, to drain and dry.

Microscopy: The blood films were examined with a microscope under the oil immersion (X 100) objective to determine the parasite density, and parasitaemia by counting 10 fields of approximately 200 red blood cells per field. For low parasitemias, up to 4000 red blood cells were counted.

The parasite density, percentage parasitaemia, and percentage suppression were calculated using the formulae:

$$\text{Parasite Density} = \frac{No.of\ parasites\ x\ 8000}{White\ blood\ cell\ count}$$

$$\%\ \text{Parasitaemia} = \frac{No.of\ parasitized\ Red\ blood\ cells}{Total\ no.\ of\ Red\ blood\ cells}\ x\ 100$$

$$\%\ \text{suppression} = \frac{MP\ (NC) - MP\ (TS)}{MP\ (NC)}\ x\ 100$$

Where; MP =$Mean\ Parasitaemia$; NC = Negative control; TS = Test sample

For treated mice, the survival time (in days) was recorded and the mean survival time calculated in comparison to untreated and standard drug treated groups. Mice still without parasitaemia on day 30 post-infection was considered cured.

Statistical analysis: Results were expressed as means ± standard error of mean (SEM) of 3 replicates. Comparison between means was done using one-way analysis of variance (ANOVA). P-value less than 0.05 were regarded as significance.

RESULTS AND DISCUSSION

Antioxidant activity:

DPPH Radical Scavenging Assay: DPPH is one of the few stable and commercially available organic nitrogen radicals. The activity is largely dependent on Hydrogen donating ability of antioxidants. The scavenging properties of antioxidants are often associated with their ability to form stable radicals (Shahidi and Wanasundara, 1992; Oke and Hamburger, 2002). The DPPH radical scavenging activity result obtained for the methanol extract of *P. nitida* showed that the extract have good DPPH scavenging effect with the 50% inhibitory concentration (IC_{50}) at 10.19 µg/mL. The DPPH radical scavenging activity (DRSA) increased with increase in concentration with the peak DRSA of 96.05% at 100 µg/mL and then 95.56% at 200 µg/mL. Figure 1 shows the scavenging activity of ascorbic acid and the sample under investigation. 50%

inhibitory concentration (IC_{50}) for the extract was 10.19 µg/mL and that of ascorbic acid was less than 1 µg/mL.

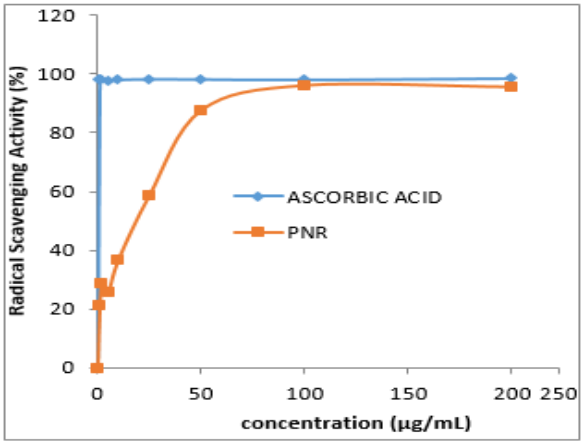

Fig 1: DPPH radical scavenging activity of crude extract of *Picralima nitida* root bark compared with standard (ascorbic acid).

Ferric reducing antioxidant power (FRAP): In the FRAP assay, a reaction time of 30 min was allowed within which it is unlikely that any process or agent will be causing inhibition of Fe^{III} reduction or causing Fe^{II} reoxidation, even when it has ferroxidase activity, due to the low pH and high chloride concentration of the reaction mixture. At low pH, when a ferric-tripyridyltriazine (Fe^{III}-TPTZ) complex is reduced to ferrous (Fe^{II}) form, an intense blue colour with an absorption maximum at 593 nm develops (Benzie and Strain, 1996). In this experiment, the absorbance value increased as the concentration increased. The FRAP value obtained was 0.17 ± 0.00 mMFSE/g Extract. Figure 2 shows the calibration plot of ferrous sulphate ($FeSO_4.7H_2O$). The ferric reducing antioxidant power (FRAP) of the methanol extract of *Picralima nitida* root bark was obtained from the equation of the calibration curve of y = 6.419x + 1.063, R^2 = 0.988 and was found to be 0.17 ± 0.00 mM FSE/g Extract.

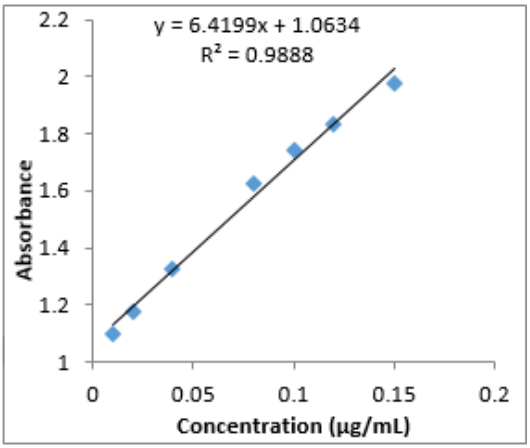

Fig 2: Ferrous sulphate calibration curve

Antimalarial activity: Plasmodium berghei parasite infects rodents and produces symptoms like those produce by *Plasmodium falciparum* in humans. The 4-day suppressive test, which primarily evaluates the antimalarial activity of candidate agents on early infections, is commonly used for antimalarial drug screening. In this method, the determination of the percentage inhibition of parasitaemia is the most reliable parameter.

The results from the study indicated that, in *P. berghei*-infected mice, the percentage parasitemia measured in the 4-day test was significantly reduced in both extract-treated groups and chloroquine-treated group, compared to mice in the negative control group. Analysis of percentage parasitaemia showed that the extract produced a dose-dependent decrease in parasitaemia on the 5th day post inoculation. The percentage parasitaemia in the extract treated group (8.20%, 4.52% and 4.60% at 200 mg/kg, 400 mg/kg and 800 mg/kg, respectively) was significantly lower, compared to the negative control (% parasitaemia = 14.27%).

The root bark extract of *Picralima nitida* displayed good chemosuppressive activity against *Plasmodium berghei*, with percentage parasitaemia suppression of 68.33% and 67.27% at doses of 400 mg/kg and 800 mg/kg, respectively. However, parasitaemia suppression in chloroquine-treated group was obviously higher with a percentage parasitaemia suppression of 98.04% at a dose of 5 mg/kg. Our present findings support previous study of the antimalarial activity of *P. nitida*.

For example, the study of Iwu and Klayman, showed that the seed, fruit rind and stem bark extracts of *P. nitida* displayed significant inhibitory activity against drug resistant clones of *Plasmodium falciparum* (Iwu and Klayman, 2002). Similarly, the work of François *et al*., also showed significant inhibitory effect of *P. nitida* root, stem bark and fruit rind extracts against asexual erythrocytic form of *Plasmodium falciparum* (François *et al*., 1996). Bickii *et al.*, also showed inhibitory effect of the methanol seed extract of *P. nitida* against *P. falciparum in vitro* (Bickii *et al*., 2007). The ethanol seed extract of *P. nitida* has also been shown to exhibit significant *in vivo* antiplasmodial activity in both 4-Day chemosuppressive and curative tests (Okokon *et al*., 2007). The present study further substantiated the continued use of the extract of *P. nitida* for the prevention and management of uncomplicated malarial in ethnomedicine.

Table 1: Parasitaemia measurement in the 4-day suppressive test of the methanol root bark extract of *Picralima nitida*.

Treatment group	Parasite density	Parasitaemia (%)	% Suppression
Control	3740.84 ± 573.42	16.78 ± 2.96	
Extract			
200 mg/kg	2502.18 ± 291.67	8.20 ± 3.34	*42.54*
400 mg/kg	2696.56 ± 875.80	4.52 ± 0.44	*68.33*
800 mg/kg	2470.71 ± 350.00	4.6675±1.26	*67.27*
CQ (5 mg/kg)	517.65 ± 86.96	0.28 ± 0.09	*98.04*

Conclusion: This experiment has shown that methanol extract of *Picralima nitida* root bark possess antioxidant activity. The extract also exhibited significant antimalarial chemosuppressive effect. These findings therefore lend credence to the ethnomedicinal use of the plant in the treatment of uncomplicated malaria.

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