CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
http://doi.org/10.5281/zenodo.3339388

Available online at: http://www.iajps.com Research Article

INHIBITORY EFFECT OF ETHANOL *PICRALIMA NITIDA* SEED EXTRACT ON MALARIA PARASITE

[1]Nwankwo, N.E., [2]Egbuonu, A.C.C., *[3]Joshua, P.E., [1]Okeke, E.S., [3]Cosmas S., [4]Arazu A. V and [3]Nwodo, O.F.C.

[1]Natural Science Unit, School of General Studies, University of Nigeria, Nsukka
[2]Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Nigeria
[3]Department of Biochemistry, University of Nigeria, Nsukka, Nigeria
[4]Department of Science Laboratory Technology University of Nigeria, Nsukka, Nigeria

Article Received: May 2019 **Accepted:** June 2019 **Published:** July 2019

Abstract:

Parts of Picralima nitida are used by traditional medicine practitioners to treat illnesses such as diabetes and gastro-intestinal problems. Pulverized dried seeds of Picralima nitida were extracted using ethanol. The phytochemical composition of the seeds sample; LD_{50} and the effect of seeds extract on parasitaemia were determined. Qualitative and quantitative phytochemical screening of the seeds sample revealed flavonoids, saponins, glycosides, steroids, alkaloids, soluble carbohydrates, reducing sugars, tannins, terpenoids, phenols and hydrogen cyanides. The acute toxicity test (LD_{50}) of the extract was above 5000 mg/kg b.w, this may be relatively safe if consumed. Malaria infected mice groups treated with 20, 40 and 80 mg/kg b.w. of the extract showed significant ($p < 0.05$) reduction in parasitamia compared to normal mice in group treated with 3 % Tween 80 on days 3 and 5 post treatment. Thus, the seed extract of Picralima nitida have rich content of the determined phytochemicals, exhibited safe and significant activity against the rodent malaria parasite.

Keywords: *phytochemical, acute toxicity, parasitaemia, malaria, bioactive.*

Corresponding author:
Joshua, P.E,
Department of Biochemistry, University of Nigeria, Nsukka, Nigeria
Correspondence: ***parker.joshua@unn.edu.ng***

QR code

Please cite this article in press Joshua, P.E et al., ***Inhibitory Effect Of Ethanol Picralima Nitida Seed Extract On Malaria Parasite.,*** *Indo Am. J. P. Sci, 2019; 06[07].*

1.0 INTRODUCTION:

Picralima nitida, (family *Apocynaceae*), popularly called "*Akuamma*" by Ghanaians and "Osi-Igwe" in Igbo tribe of Nigeria. It is a tree that grows to 35 m tall with white latex. The leaves are opposite, simple and entire. Extracts of *Picralima nitida* parts (root, seed and stembark) have been reported to exhibit a broad spectrum of activity against bacterial strains [1]. These bacteria include: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis* and *Salmonella kintambo* were susceptible to the extract due to bioactive components such as alkaloids, saponins, flavonoids, steroids, tannins and terpenoids. Ubulom *et al.* [2] reported the antifungal activity of both the aqueous and ethanolic extracts of *Picralima nitida* seeds against *Aspergillus flavus*, *Candida albicans* and *Microsporum canis.* They showed the inhibitory activities against these organisms. However, the finding of the study further showed that ethanol the ethanol extract exhibited better antifungal activity than the aqueous extract. Okonta and Aguwa [3], compared the hypoglycemic effect of the glycosides of seed extract of *P. nitida* with alkaloids, and associated its effect glycosides. Acute toxicity and sub-chronic studies were performed on the seed extract of *P. nitida* [4].

Malaria parasitemia is a measure of the amount of malarial parasites in a patient's blood. It indicates the degree of infection. In blood sample, visual detection and recognition of *Plasmodium spp.* is possible and efficient via a chemical process called (*Giemsa*) staining [5]. The staining process slightly colourizes the red blood cells (RBCs) but highlights *Plasmodium spp*. parasites, white blood cells (WBC), and artifacts. *Giemsa* stains nuclei, chromatin in blue tone and RBCs in pink colour. Extracts of the seed, fruit rind and stem bark of this plant exhibited inhibitory activity against drug resistant strains of *Plasmodium falciparum* [6], even at low doses. Thus, this study was is aimed at determining the quantity of some phytochemicals in the seed of *P. nitida* and the inhibitory effect of the sample extract against malarial parasite (*Plasmodium berghei)* in rodent model.

2.0 MATERIALS AND METHODS:

Animals

The animals used for this study were albino mice of either sex weighing 20-34 g. They were obtained from the Animal Unit of Faculty of Veterinary Medicine, University of Nigeria, Nsukka.

Plant Material

The material used for this experiment was seed of *Picralima nitida* plant

Collection and Identification of *Picralima nitida* Seeds

Seeds of *Picralima nitida* were collected from Isuofia, in Aguata Local Government Area of Anambra State, Nigeria and were authenticated by Mr. Alfred Ozioko of the Bioresource Development and Conservation Programme (BDCP) Research Centre, Nsukka, Nigeria.

Extraction of *Picralima nitida* Seeds

The seeds of *Picralima nitida* were harvested and dried at room temperature (29-35 ^{0}C) for three weeks and pulverized into powdered form with a Creston high speed milling machine. The powdered seeds (1 kg) were then macerated in 5 volume (w/v) absolute ethanol at room temperature for 48 hours. The extract was filtered using muslin cloth on a plug of glass wool in a glass column. The resulting ethanol extract was finely filtered using Whatman qualitative filter paper and evaporated to dryness using rotary evaporator at an optimum temperature between 40-45^0 C (to avoid denaturation of the active ingredients). The concentrated extract was stored in the refrigerator.

Phytochemical Screening of *Picralima nitida* Seed Extract

The qualitative phytochemical screening was carried out according to the methods of Harborne [7] and Trease and Evans [8]. The quantitative phytochemical screening was carried out using the methods of Oloyed [9], Harborne [10] and Onwuka [11].

Acute Toxicity Study

The acute toxicity study was determined using method of Lorke [12].

Parasite Inoculation

The mice used for this study were inoculated with malaria parasite from already infected mice using the method of Hilou *et al*. [13].

Determination of Malaria parasitaemia

The percentage parasitemia (Mp^+) was carried out according to the method of Dacie and Lewis [14].

Experimental Design

A total of 180 albino mice of either sex weighing 20-34 g were housed in separate cages, acclimatized for one week and then divided into six groups of thirty mice each as follows:

Group 1: Normal mice treated with the vehicle, 3% Tween 80 (normal control)

Group 2: Mice infected with malaria parasite and treated with 3% Tween 80 (positive control)

Group 3: Mice infected with malaria parasite and treated with 20 mg/kg b.w. of the extract

Group 4: Mice infected with malaria parasite and treated with 40 mg/kg b.w. of the extract

Group 5: Mice infected with malaria parasite and treated with 80 mg/kg b.w. of the extract

Group 6: Mice infected with malaria parasite and treated with the standard drug, artesunate (standard control)

The route of administration (treatment) was oral with the aid of an oral intubation tube. Treatment lasted for 5 days during which analyses were carried out on days 3 and 5.

Statistical Analysis

The data obtained were subjected to ANOVA with repeated measures and t-test. Significant difference was accepted at $p < 0.05$. Results are expressed as mean ± standard error of mean (S.E.M.).This analysis was carried out using IBM SPSS Statistics version 18.

RESULTS:

Phytochemical Composition of Ethanol Seed Extract of *Picralima nitida*

Table 1 shows the qualitative and quantitative constituents of the seed extract of *P. nitida*. The phytochemical screening showed flavonoids (3.39 mg/100 g), saponins (3.33 mg/g), glycosides (5.84 mg/g), steroids (0.94 mg/g), alkaloids (2.77 mg/100 g), soluble carbohydrates (1.44 mg/g), reducing sugars (315.42 mg/100 g), tannins (66.64 mg/100 g), terpenoids (1.28 mg/g), phenols (11.75 mg/100 g) and hydrogen cyanides (0.05 mg/g).

Table 1: Phytochemical Constituents of Ethanol Seed Extract of *Picralima nitida*

Phytochemicals	Qualitative	Quantitative
Flavonoids	++	3.39 mg/100 g
Saponins	++	3.33 mg/g
Glycosides	+++	5.84 mg/g
Steroids	+	0.94 mg/g
Alkaloids	++	2.77 mg/100 g
Soluble carbohydrates	+	1.44 mg/g
Reducing sugars	+++	315.42 mg/100 g
Tannins	+++	66.64 mg/100 g
Terpenoids	+	1.28 mg/g
Phenols	++	11.75 mg/100 g
Hydrogen cyanide	+	0.05 mg/g

+++ = very abundant
++ = moderately present
+ = present in small amount

Acute Toxicity of Ethanol Seed Extract of *Picralima nitida*

The acute toxicity study (LD_{50}) of the ethanol extract of *P. nitida* on mice (Table 2) was found to be more than 5000 mg/kg body weight. No animal died and none showed any sign of toxicity within 24 hours of constant observation.

Table 2: Acute Toxicity Studies of the Ethanol Seed Extract of *Picralima nitida*

Phase I					
Group	Dosage	Mice 1	Mice 2	Mice 3	**Mice 4**
Group 1	10 mg/kg	ND and NST	ND and NST	ND and NST	**ND and NST**
Group 2	100 mg/kg	ND and NST	ND and NST	ND and NST	**ND and NST**
Group 3	1000 mg/kg	ND and NST	ND and NST	ND and NST	**ND and NST**
Group 4	Standard Control	ND and NST	ND and NST	ND and NST	**ND and NST**
Phase II					
Group	Dosage	Mice 1	Mice 2	Mice 3	**Mice 4**
Group 1	1,900 mg/kg	ND and NST	ND and NST	ND and NST	**ND and NST**
Group 2	2,600 mg/kg	ND and NST	ND and NST	ND and NST	**ND and NST**
Group 3	5,000 mg/kg	ND and NST	ND and NST	ND and NST	**ND and NST**
Group 4	**Standard Control**	**ND and NST**	**ND and NST**	**ND and NST**	**ND and NST**

Legend

ND = No death
NST = No signs of toxicity

Effect of Treatment with Ethanol Seed Extract of *Picralima nitida* on Percentage parasitaemia of Malaria-infected Mice

As shown in Table 3, the percentage parasitaemia of normal mice treated with 3 % tween 80 (normal control) was 0.00 ± 0.00 %, while infected mice treated with 3 % tween 80 (positive control) were showed the percentage parasitaemia of 39.00 ± 1.87 %. The percentage parasitaemia in mice group treated with 20, 40 and 80 mg/kg b.w. of the extract were 29.00 ± 4.16, 8.60 ± 0.87 and 6.20 ± 0.92 %, respectively on day 3 post treatment. These values decreased significantly to 10.80 ± 1.07, 6.20 ± 0.80 and 4.20 ± 1.16 %, respectively on day 5 post treatment. The percentage parasitaemia in mice group treated with 5 mg/kg b.w. of artesunate was 3.20 ± 0.72 % on day 3 post treatment which decreased to 2.00 ± 0.63 % on day 5 post treatment.

The percentage parasitaemia of extract-treated groups (groups 3, 4 and 5) of mice (on days 3 and 5 post treatment) were significantly ($p < 0.05$) lower compared to the positive control and the extract showed dose-dependent effect. When the percentage parasitaemia in mice treated with 5 mg/kg b.w. of artesunate was compared with group 3, it was significantly ($p < 0.05$) higher and increased to 17.00 % on day 3 and day 5 post treatment.

Table 3: Percentage parasitaemia for the treated Mice and the control groups

Groups	Day 3 post treatment	Day 5 post treatment
1	0.00 ± 0.00*	N.D.
2	39.00 ± 1.87	N.D.
3	29.00 ± 4.16*	10.80 ± 1.07
4	8.60 ± 0.87*	6.20 ± 0.80
5	6.20 ± 0.92*	4.20 ± 1.16
6	3.20 ± 0.74	2.00 ± 0.63

* = $p < 0.05$ compared to the positive control
The results are expressed as mean ± standard error of mean (S.E.M)
N.D. = Not Determined

Group 1: Normal mice treated with the vehicle, 3% Tween 80 (normal control)
Group 2: Mice infected with malaria parasite and treated with 3% Tween 80 (positive control)

Group 3: Mice infected with malaria parasite and treated with 20 mg/kg b.w. of the extract
Group 4: Mice infected with malaria parasite and treated with 40 mg/kg b.w. of the extract
Group 5: Mice infected with malaria parasite and treated with 80 mg/kg b.w. of the extract
Group 6: Mice infected with malaria parasite and treated with the standard drug, artesunate (standard control)

DISCUSSION:

The qualitative and quantitative phytochemical studies of ethanol seed extract of *Picralima nitida* indicated flavonoids, saponins, glycosides, steroids, alkaloids, soluble carbohydrates, reducing sugars, tannins, terpenoids, phenols and hydrogen cyanide. Tannins, phenols and glycosides were found to be very abundant. Flavonoids, saponins, alkaloids, reducing sugars moderately present while soluble carbohydrates, terpenoids steroids, and hydrogen cyanide were in small amounts. In support of the findings of this study, Ubulom *et al*. [2], screened the aqueous and ethanol extracts of *P. nitida* seed extract reported flavonoids, saponins, glycosides, tannins and terpenes as the phytochemicals present in it. Glycosides and alkaloids isolated from this plant's seed extract have shown hypoglycaemic activity and inhibitory activity on β-glycosidase, respectively [3, 15]. Five alkaloids; *akuammidine*, *akuammine*, *akuammicine*, *akuammigine* and *pseudoakuammigine* extracted from the seed of *P. nitida* exhibited opioid activity [16] which conferred antipyretic property.

The lethal dose (LD_{50}) study of the ethanol seed extract on mice was more than 5000 mg/kg body weight; an indication of non-toxic (or mininmal/ tolerable toxic) effects if consumed. Ilodigwe *et al*. [17] conducted acute toxicity studies on the ethanol leaf extract of this plant and observed that there was no death at 1000, 2000, 4000 and 5000 mg/kg of the extract, thus confirming the safety margin as observed in the seed extract. Furthermore, no sign of weakness or anorexia was observed within 24 hours of administration.

The highest mean percentage parasitaemia was exhibited by the mice group treated with 20 mg/kg b.w. of the extract, followed by group treated with 40 mg/kg b.w. of the extract and then by the least group treated with 80 mg/kg b.w. of the extract on days 3 and 5 of post treatment. The standard control showed better mean percentage parasitaemia on days 3 and 5 of post treatment compared to the extract-treated mice groups. The infected mice groups treated with extract caused a dose-dependent decrease in percentage parasitaemia, further indicating that the extract possessed anti-plasmodial activity. This correlates with the observation of Jean *et al*. [18] that among some Cameroonian medicinal plants tested for anti-malaria activity, *P. nitida* exhibited the highest activity. They also reported that on day 5 post treatment, the standard drug "artesunate" showed the highest activity against malarial parasite; an indication that artesunate is a more potent antimalarial than most crude extracts. *In vivo* antimalarial activity of *Anthocleista grandiflora* and *Faidherbia albida Del*. extracts against *Plamodium berghei* showed significant ($p < 0.05$), dose-dependent activity for suppressive, curative and prophylactic tests [19, 20]. The present finding showed a dose-dependent activity against *P. berghei* for curative test. Extract of *Artemesia turanica* exhibited inhibitory effect against *Plasmodium berghei* on the early developmental (erythrocytic) stages only [21].

CONCLUSION:

The results of this research showed that seeds of *P. nitida* are rich in phytochemicals, safe and active against malarial parasite (*Plasmodium berghei)* in rodents, thus could be very good and safe alternative for the management/treatment of malaria caused by *Plasmodium berghei*. This study recommends further research toward examining the mechanism of action of the seeds extract against malaria parasite and toxic implications following high and prolonged consumption of the seeds.

REFERENCES:

1. Nkere, C. K. and Iroegbu, C. U. (2005). Antibacterial screening of the root, seed and stembark extracts of *Picralima nitida.* African Journal of Biotechnology. 4 (6): 522-526.
2. Ubulom, P, Akpabio, E., Udobi, C. E. and Mbon, R. (2011). Antifungal activity of aqueous and ethanolic extracts of *Picralima nitida* seeds on *Aspergillu flavus*, *Candida albicans* and *Microsporium canis.* Res. Pharm. Biotech. 3 (5): 57-60.
3. Okonta, J. M. and Aguwa, C. N. (2007). Evaluation of hypoglemic activity of glycosides and alkaloids extracts of *Picralima nitida* (*Apocynaceae*) Seed. Int. J. Pharmacol. 3 (6): 505-509.

4. Ilodigwe, E. E., Urukwem, I. C., Ajaghaku, D. L., Mbagwu, I. S. and Agbata, C. A. (2013) Acute and subchronic toxicities of aqueous antidiabetic herbal decoction commonly taken in South Eastern Nigeria. Int. J. Res. Pharm. Biomed. Scs. 4 (4): 1256-1263.
5. Angulo, J. and Flandrin, G. (2003). Automated detection of working area of peripheral blood smears using mathematical morphology, U. S. Nat. Lib. Med. Anal. Cell Path. 25 (1): 39-47.
6. Osayemwenre, E., Abiodun, F. and Peter, L. (2014). Medicinal uses, phytochemistry and pharmacology of *Picralima nitida* (*Apocynaceae*) in tropical diseases: A review. Asian Pacific J. Trop. Med. 1:1-8.
7. Harborne, J. B. (1989). The flavonoids-Advances in Research Since 1980. 2nd Edn. Chapman and Hall, London, pp. 389-419.
8. Trease, G. E. and Evans, W. C. (2002). Pharmacognosy. 15th Edn. W.B. Saunders, Edinburg, pp. 137-150.
9. Oloyed, O. I. (2005). Chemical profile of unripe pulp of *Carica pagaya*. Pakistan J. Nut. 4: 379-381.
10. Harborne, J. B. (1976). Phytochemical Methods. 1st Edn. Chapman and Hall Ltd, London, pp.52-55.
11. Onwuka, G. I. (2005). Food Analysis and Instrumentation Theory and Paractice, 1st Edn, Naphthali Prints, Lagos, pp. 114-169.
12. Lorke, D. (1989). A new approach to practical acute toxicity testing. Arch. Toxicol. 54**:** 275-287.
13. Hilou, A., Nacoulma, G. and Guiguemde T. R. (2006). *In vivo* antimalarial activities of extracts from *Amaranthus spinosus* and *Boerhaavia erecta* in mice. J. Ethnopharmacol. 103: 236-240.
14. Dacie, J. V. and Lewis, S. M. (2000). Practical Haematology. 9th Edn. Churchill Livingstone, New York, pp. 429-457.
15. Akpan, E. J. and Umoh, I. B. (2004). Inhibitory activity of seed extract from *Picralima nitida*, (Staph) on β-D-glycosidase. BIOKEMISTRI; 16 (2): 72-78.
16. John, R. W. M., Stewart, J. P., Mahama, D. and Alistai, D. C. (1998). Opoid activity of alkaloids extracted from *Picralima nitida* (Fam. *Apocynaceae*). Eur. J. Pharmacol. 350: 101-108.
17. Ilodigwe, E. E., Okoye, G. O., Mbagwu, I. S., Agbata, C. A. and Ajaghaku, D. L. (2012). Safety evaluation of ethanol leaf extract of *Picralima nitida* Stapf (*Apocynaceae*). Int. J. Pharmacol. Ther. 2 (4): 6-17.
18. Jean, B., Guy, R. F. T., Jean, C. T. and Etienne, T. (2007). Antimalarial activity in crude extracts of some Cameroonian medicinal plants. African J. Trad. Compl. Alt. Med. 4 (1): 107-111.
19. Odeghe, O. B., Uwakwe, A. A. and Monago, C. C. (2012). Antiplasmodial activity of methanolic stem bark extract of *Anthocleista grandiflora* in mice. Int. J. Appl. Sc. Tech. 2 (4) 142-148.
20. Salawu, O. A., Tijani, A. Y., Babayi, H., Nwaeze, A., Anagbogu, R. A. and Agbakwuru, V. A. (2010). Antimalarial activity of ethanolic stem bark extract of *Faidherbia albida* (Del) a. Chev (*Mimosoidae*) in mice. Arch. Appl. Sc. Res. 2 (5): 261-268.
21. Mahboubeh, T., Abdolhossein, R., Hossein, N., Sabah, N. and Tofigh, T. (2013). Comparison of antimalarial activity of *Artemisia turanica* extract with current drugs *in vivo*. J. Vector Borne Dis. 50: 51-56.