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ANTIMALARIAL ACTIVITY OF FRACTIONS OF *PICRALIMA NITIDA* LEAVES (APOCYNACEAE) IN ANIMAL MODELS OF ANTIMALARIAL RESISTANCE

[1]*Azikiwe C. C. A., [2]Anowi C. F., [2]Ewuim C. C. and [3]Ifezulike C. C.

[1]Department of Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences, College of Medicine, Chukwuemeka Odumegwu Ojukwu University, Awka, Nigeria.

[2]Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria.

[3]Department of Paediatrics, Faculty of Clinical Medicine, College of Medicine, Chukwuemeka Odumegwu Ojukwu University, Awka, Nigeria.

***Corresponding Author: Dr. Azikiwe C. C. A.**
Department of Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences, College of Medicine, Chukwuemeka Odumegwu Ojukwu University, Awka, Nigeria.

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ABSTRACTS

Background: Malaria is an endemic tropical disease that accounts for several millions annual death especially, in children. Malarial drug resistance can be defined as the ability of a parasite strain to survive and, or to multiply despite the administration and absorption of drugs given in doses equal to or higher than those usually recommended. Antimalarial resistance is becoming dangerously alarming thus the need for new drug search. *Picralima nitida* is a tropical shrub used in folklore medicine for treatment of several ailments including malaria. **Aim** of the present work is to evaluate antimalarial activity of *Picralima nitida* in animal model of malarial drug resistance. **Material and method**: Materials consisted of Laboratory glassware and equipment, phytochemical reagents and appropriate malaria parasite stains, portable drinking water, animal feeds and chloroquine-resistant plasmodium bergie samples. *Picralima nitida* leaves were harvested and Taxonomy was done. The leaves were air-dried at room temperature for 14days, ground, macerated in methanol and further fractionated in aqueous, n-butanol, n-hezane and ethyl acetate. Crude and all fractions were evaporated to dryness, yields collected and stored till needed. 50 mice were procured, acclimatized, grouped into 9 (1-9) of 5animals each. Two mice were infected with malaria parasites and acted as donors. All animals in 1-9 were pre-infected with donor's blood intraperitoneally while group 1 acted as negative control. After 3days of established parasitaemia, animals in 2-9 were given the appropriate daily dose of the appropriate ACT or extract/fractions of *Picralima nitida* for 72hours. Percentage parasitaemia were evaluated and statistically extrapolated. P-values less than 0.05 were adjudged significant. **Results**: LD_{50} was taken as 770mg/ip. Phytochemistry revealed the presence of tannins, flavonoids, glycosides, alkaloids, proteins and carbohydrates. Extract/fractions exhibited statistically antimalarial activity ($p<0.002$, $P<0.001$) and were comparative to ACT. The fractions had superior activity over the aqueous. ACT and fractions of *Picralima nitida* antimalarial activities were statistically insignificant ($p>0.067$). **Conclusion:** The leaves of Pricralima posses antimalarial activity that is statistically insignificant to that of ACT that is currently the main stay in malaria chemotherapy. We recommend further characterization and clinical trial in human subjects.

KEYWORDS: *Picralima nitida*; Malaria; Antimalarial activity, ACT. Drug resistance.

INTRODUCTION

Malaria is an infectious disease caused by protozoan parasites from the Plasmodium family and can be transmitted by the bite of the Anopheles mosquito or by a contaminated needle or transfusion (Amazu *et al.*, 2009, Azikiwe *et al.*, 2011, Williams 2018).

Malaria disease has remained a global leading cause of death and disability in which about 50% of the world population is estimated to be at risk, especially in low and middle income countries (WHO, 2005). In 2009, WHO estimated about 225 million cases of malaria attack the world-over, with over 780,000 deaths (WHO, 2010).

Malaria is a common and life-threatening disease in many tropical and subtropical areas. There are currently over 100 countries and territories where there is a risk of malaria transmission, and these are visited by more than 125 million international travelers every year. Each year many international travelers' fall ill with malaria while visiting countries/territories where malaria is endemic, and well over 10000 are reported to become ill with malaria after returning home; however, underreporting means that the real figure may be considerably higher.

International travelers to countries/territories with ongoing local malaria transmission arriving from countries with no transmission are at high risk of malaria infection and its consequences because they lack immunity. Migrants from countries/territories with malaria transmission living in malaria-free countries and returning to their home countries to visit friends and relatives are similarly at risk because of waning or absent immunity (WHO, 2014)

Majority of the African population are at high risk of developing malaria disease. Malaria transmission is year-round and mostly due to *P. falciparum* in most of the countries (World Malaria Report, 2017). The sub-region had about 111 million estimated cases and 41 million reported confirmed cases. Some 19 000 malaria deaths were reported in 2016 but reporting rates were low, and the estimated number of deaths was about 224 000. Six countries accounted for 85% of the estimated cases: Nigeria (52%), Burkina Faso, Ghana, Mali and Niger (each contributing 7%) and Côte d'Ivoire (5%). (Murray *et al*., 2012).

There are five species of plasmodium parasite known to cause the malaria infection when transmitted to man. These include Plasmodium falciparum, P. malariae, P. vivax, P. ovale and P. knowlesi. P. vivax is known to be responsible for the largest number of malaria infections in the world and severe cases of attack are caused by P. falciparum (Sing et al., 2001). The other species, P. ovale and P. malariae are known to generally cause milder forms of malaria which are rarely fatal. P. knowlesi is zoonotic, prevalent in South East Asia and causes malaria in macaques but can also cause severe infections in humans. Malaria is the major tropical pathology in the tropical world. A dramatic recrudescence of this disease is ongoing due to the increasing resistance of parasites..

Despite advances in modern medicine, malaria remains a disease which is difficult to be eradicated and is therefore a major health problem, for one main reason: all anti-malarial drugs are expensive for the populations in endemic countries thus the need for an alternative traditional means of treatment and eradication (Baird, 2013).

Malaria is a complex disease that varies widely in epidemiology and clinical manifestation in different parts of the world. This variability is the result of factors such as the species of malaria parasites that occur in a given area, their susceptibility to commonly used or available antimalarial drugs, the distribution and efficiency of mosquito vectors, climate and other environmental conditions and the behaviour and level of acquired immunity of the exposed human populations (WHO 2001).

Antimalarial drug resistance has emerged as one of the greatest challenges facing malaria control today. Drug resistance has been implicated in the spread of malaria to new areas and re-emergence of malaria in areas where the disease had been eradicated. Drug resistance has also played a significant role in the occurrence and severity of epidemics in some parts of the world (WHO, 2001).

Herbs and natural remedies have been used to treat malaria and other ailments for thousands of years. Medicinal plants form the source of the two main groups (artemisinin and quinine derivatives) of modern antimalarial drugs. Again more than 80% of world population use medicinal plants (Azikiwe et al., 2006). With the problems of increasing levels of drug resistance and difficulties in poor areas of being able to afford and access effective antimalarial drugs, traditional medicines could be an important and sustainable source of treatment (Melin. *et al*., 2004). With the rising resistance to frontline drugs (artemisinin-based combinations), there is a need to accelerate the discovery and development of newer anti-malarial drugs (Tajbakhsh *et al*., 2021).

Picralima nitida is a medicinal plant from the genus *Picralima* and plant family *Apocynaceae*. It is found in tropical African countries such as Ivory Coast, Nigeria, Uganda, and Gabon, and it is popularly known as *Abeere* in the Southwestern part of Nigeria among the Yoruba people. Other common names include Akuamma plant, Osu-Igwe, Ogwashi (Igbo). It grows up to 35 meter tall, with white latex in all parts, glabrous; bole up to 60 cm in diameter; bark is hard, brittle, pale to dark greyish black or brown, smooth to slightly rough or finely striped. Leaves opposite, simple and entire; stipules absent; petiole 1–2 cm long; blade elliptical to oblong, (5–)10–26 cm × 2–13 cm, base cuneate, apex abruptly acuminate, thickly papery to thinly leathery, pinnately veined with 14–23 pairs of lateral veins. Inflorescence a terminal or sometimes axillary, compound, umbel-like cyme 6–10 cm long, 10–35-flowered; peduncle 2–35 mm long, with 3 primary branches; bracts very small. Flowers are bisexual, regular, 5-merous, fragrant or not and open during the day (Fig 1).

Fig 1: (Aerial parts) Leaves, stem and fresh growing fruits of *Picralima nitida*.

In an earlier review, Erharuyi and colleagues, 2014, demonstrated that the plant has antihypertensive, anti-inflammatory, analgesic, hypoglycaemic, antiplasmodic among other activities. In another very recent review *Picralima nitida* was shown to possess antimalarial activity among other nine African medicinal plant (Tajbakhsh *et al*., 2021), Erharuyi and colleagues, 2014,

An evaluation of its toxicity on chronic administration, *Picralima nitida* has been adjudged as not too safe in early pregnancy though like so many other drugs (Awodele *et al*., 2019).

Picralima nitida however has a protective role in metabolic oxidative stresses from free dietary radicals (De Campos *et al*., 2020) and a blend of the leaves with seeds exhibited varying degrees of analgesia and antiinflammation in animal models (Ajayi et al., 2020).

Buttressing these effects, Creed *et al*., 2021 demonstrated the characterization of sic opioidic alkaloids in *Picralima nitida* in addition to earlier isolated serpentinine bisindole alkaloids (Alcover *et al*., 2020). These morphinian alkaloids explain the antitussive, expectorant and analgesic activities earlier demonstrated by Dapaah *et al*., 2016.

MATERIAL AND METHODS

Materials consisted of Laboratory glassware and equipment, phytochemical reagents and appropriate malaria parasite stains, portable drinking water, animal feeds and chloroquine-resistant plasmodium bergie samples. *Picralima nitida* leaves were harvested and Taxonomy was done. The leaves were air-dried at room temperature for 14days, ground, macerated in methanol and further fractionated in aqueous, n-butanol, n-hezane and ethyl acetate. Crude and all fractions were evaporated to dryness, yields collected and stored till needed. 50 mice were procured, acclimatized, grouped into 9 (1-9) of 5animals each. Two mice were infected with malaria parasites and acted as donors.

ANTI-PLASAMODIC EVALUATION

This study was carried out according to the method employed by Peters et al 1983. A total of 45 mice were used. They were grouped into 9 of 5 mice each. The donor animals preinfected with malarial parasites had their blood samples collected and tested to establish confirmed parasitaemia. Blood samples were then collected from the donor animals via retro-ocular sinus. Samples were diluted with normal saline such that 0.2ml contained approximately $1x10^6$ infected red blood cells. All animals in groups 1-9 were then inoculated with *P. berghei* by single intraperitoneal administration of 0.2ml of infected blood sample. The animals were left for 72hours for parasitemia to be established. Blood sample was collected from each animal through tail-vein, thin film was made. The film was fixed with methanol, and was stained, and viewed under microscope at x100 oil immersion objective. The number of infected RBCs were noted and was termed *Basal Value or Pretreatment Value*. The animals then received treatment as follows:

Group 1 received 10ml per kg of distilled water (Negative control).
Group 2 received 37mg per kg of ACT
Group 3 received 50mg per kg of crude extract
Group 4 received 100mg per kg of crude extract
Group 5 received 500mg per kg of crude extract
Group 6 received 500mg per kg of n-hexane fraction
Group 7 received 500mg per kg of ethyl acetate fraction
Group 8 received 100mg per kg of butanol fraction
Group 9 received 200mg per kg of water fraction.

Daily dosing/treatment stopped on day 3 and day 4 thin blood films were made from the tail puncture for all animals under study, stained with Giemsia and examined for parasitemia for quantitative while Rapid test kits were used for qualitative assessment. The infected red blood cells were counted, and compared with pre-treatment value, reduction in parasitized red blood cells was a measure of anti-plasmodiceffect. The percentage curative was calculated using the formula D0 –DA/D0 X 100%,.

The data were collated and analyzed using one way analysis of variance (ANOVA). Data were tabulated as mean ± SEM (Standard error of mean). P values less than 0.05 were adjudged significant.

RESULTS

LD_{50} was taken as 770mg/ip from existing literature.
Phytochemistry revealed the presence of tannins, flavonoids, glycosides, alkaloids, proteins and carbohydrates (Table 1).
Extract/fractions exhibited statistically antimalarial activity that were dose dependent and were comparative to ACT (Table 2).
The fractions had superior activity over the crude and aqueous fractions. ACT and fractions of *Picralima nitida* antimalarial activities were statistically insignificant ($p>0.067$)(Table 2). Percentage inhibition of parasitaemia also exhibited a similar pattern (Table 3)

Table 1: Results of phytochemical Analysis of *Picralimanitida*.

Analysis	Result
Alkaloids	+++
Saponins	-
Tannins	+
Flavonoids	+++
Steroids	-
Cardiac glycoside	+

Proteins	+
Carbohydrate	+
Terpenoids	+
Reducing sugar	-

Key; + =present, +++ Richly present. - = absent

Table 2: Antimalarial activities of crude and fractions of *Picralima nitida* on malaria compared with ACT, a standard antimalarial drug (Positive control).

GROUP	DRUG/Extract	MEAN PARASITEMIA (PRE-TREATMENT)	MEAN PARASITEMIA (POST-TREATMENT)	Test of significance P. Value
1.	10ml/kg distilled water	26.70±1.79	25.80±0.96	Control
2.	37mg/kg ACT	16.90±0.51	4.60±1.19	P=0.001
3.	50mg/kg crude extract	22.75±1.47	19.10±0.88 Ns	P>0.06
4.	100mg/kg crude extract	22.42±1.10	15.58±2.16	P=0.02
5.	500mg/kg crude extract	20.92±0.55	6.08±0.79	P=0.001
6.	500mg/kg n-heaxane	18.85±0.29	13.25±0.50	P=0.001
7.	500mg/kg ethylacetate	21.35±0.34	7.25±0.32	P=0.001
8.	500mg/kg butanol	19.44±0.94	9.56±0.95	P=0.001
9.	500mg/kg water fraction	19.75±0.23	13.92±0.71	P=0.02

Table 3: Comparative percentage inhibition of the malaria parasite by ACT and extracts of the leaves of *Picralima nitida.*

GROUP	TREATMENT	%INHIBITION
1.	10ml/kg distilled water	3.3
2.	37mg/kg ACT	72.78
3.	50mg/kg crude extract	16.23
4.	100mg/kg crude extract	30.36
5.	500mg/kg crude extract	71.94
6.	500mg/kg n-hexane	29.71
7.	500mg/kg ethyl acetate	69.04
8.	500mg/kg butanol	65.82
9.	500mg/kg water fraction	29.52

DISCUSSIONS

Malarial drug resistance can be defined as the ability of a parasite strain to survive and, or to multiply despite the administration and absorption of drugs given in doses equal to or higher than those usually recommended. Antimalarial resistance is becoming dangerously alarming thus the need for new drug search. *Picralima nitida* is a tropical shrub used in folklore medicine for treatment of several ailments including malaria.

Our present work demonstrated the safety profile of *Picralima nitida* as well as its dosage dependent antimalarial activities. Earlier works by Azikiwe et al., 2011, demonstrated the acceptable standard in malaria diagnosis which was adopted in the present study. Also in a similar pattern, plasmodium bergie was used by Amazu et al., 2009 and Amazu et al., 2010 as standard in malaria parasitization and was also adopted as standard methodology in our present study.

Picralima nitida demonstrated not only a dosage dependent antimalarial activities, it buttress its superiority over ACT is tackling malaria resistance that is now becoming clearly evident against ACT. This is only but fractions of crude extracts thus one can but imagine the degree of potential potency activities of pure drug from *Picralima nitida* when fully developed.

Flavonoids and alkaloids have been documented to possess antimalarial effects. These substances are richly present in *Picralima nitida* thus its antimalarial activities could be easily extrapolated.

In conclusion the leaves of Pricralima posses antimalarial activity that is statistically insignificant to that of ACT that is currently the main stay in malaria chemotherapy.

We recommend further characterization geared towards purest active components isolation and clinical trials in humans.

REFERENCES

1. Ajayi AM, Alabi AO, Adebesin A, Ben-Azu B, Umukoro S. Aqueous extract of dry powder blend of seeds and leaves of *Picralima nitida* (Stapf) T&H Durand reduce pains and inflammation in animal models. J Basic Physiol Pharmacol, 2020; 32(2): 97-107.
2. Alcover CF, Bernadat G, Kabran FA, Le Pogam P, Leblanc K, Fox Ramos AE, Gallard JF, Mouray E, Grellier P, Poupon E, Beniddir MA. Molecular networking Reveals Serpentinine-Related Bisindole Alkaloids from Picralima nitida, a previously well investigated species. J Nat Prod, 2020; 83(4): 1207-1216. PMID 32091210
3. Amazu LU; Ebong OO; Azikiwe CCA; Unekwe PC; Siminialayi MI; Nwosu PJC; Ezeani MC; Obidiya OS; Augwo AO(2009): Effects of methanolic seed extract of Carica papaya on Plasmodium berghei infected Mice. *Asian pacific journal of tropical Medicine*, 2(3): 1-6.
4. Amazu LU; Azikiwe CCA; Njoku CJ; Osuala FN; Nwosu PJC; Ajuquo AO; Enye JC (2010). Anti-inflammatory activity of methanolic extract of the seed of Carica papaya in experimental animals. *Asian Pacific Journal of Tropical Medicine*, 12(6): 1-5.
5. Awodele O, Coulidiaty AGV, Afolayan GO, Agagu S, Omoseyindemi B, Busia K. Toxicological evaluation of *Picralima nitida* in rodents. J Ethnopharmacology, 2019, May 23; 236: 205-219; doi:PMID 30853649
6. AZIKIWE, C.C.A; Amazu, L.U; Unekwe, P.C; Ezeani, M.C & Nwosu, P.J.C(2007):Antimicrobial effect of Triclisia dityophylla, Moonseed family. Tropical. *Journal of Biomedical and allied Sciences*, 1(01): 61-65.
7. Azikiwe CCA; Ifezulike CC; Siminialayi IM; Amazu LU; Enye JC; Nwakwunite OE (2011): Comparative malaria diagnosis: Microscopy versus rapid diagnostic test kits. *Asian Pacific journal of Tropical Biomedicine*, 2012; 1-4.
8. Baird, J.K., (2013). "Evidence and implications of mortality associated with acute Plasmodiumvivax malaria", Clinical Microbiology Reviews, 26(1): 36–57.
9. Murray, C. J., Rosenfeld, L. C., Lim, S. S., Andrews, K.G., Foreman, K.J., Haring, Fullman N., Nalghavi M., Lopez, A.D. (2012). "Global malaria mortality Between 1980 and 2010: A systematic analysis". Lancet, 379(9814): 413-31.
10. Creed SM, Gutridge AM, Argade MD, Hennessy MR, Friesen JB, Pauli GF, van Rijn RM, Riley AP. Isolation and pharmacological characterization of six opioidic *Picralima nitida* alkaloids. J Nat Prod, 2021; 84(1): 71-80. PMID 33326237
11. Dapaah G, Kuffuor GA, Mante PK, Ben IO: Antitussive, expectorant and analgesic effects of the ethanol seed extract of Picralima nitida (Stapf) Th.&H Durand. Res Pharm Sci., 2016; 11(2): 100-112. PMID 27168749
12. De Campos OC, Osaigbovo DI, Bisi-Adeniyi TI, Iheagwam FN, Rotimi SO, Chinedu SN. Protective role of *Picralima nitida* seed extract in High-fat High-fructose fed rats. Adv Pharmacol Pharm Sci., 2020, Oct.; 24: 5206204: doi.PMID 33163962.
13. Erharuyi O, Falodun A, Langer P. Medicinal uses, phytochemistry and pharmacology of *Picralima nitida* (Apocynaceae) in tropical diseases: a review. Asian Pacific J Trop Med, 2014; 7(1): 1-8. PMID 24418074.
14. Ukaegbu, C.O., Nnachi, A.U., Mawak, J.D., Igwe, C.C., (2014) "incidence of concurrent Malariaand typhoid fever infections in febrile patients in Jos, Plateau State Nigeria". International Journal of Scientific and Technology Research, 2014; 3: 157-161.
15. Sinclair, D., Donegan, S., Isba, R., & Lallo, D.G. (2012) "Artesunate versus Quinine for treating malaria". Conchrane Database System Review, 2009; 3: CDC 007483.
16. Tajbakhsh E, Kwenti TE, Kheyri P, Nazeratizade S, Lindsay DS, Khamesipour F. Antiplasmodial, antimalarial activities and toxicity of African medicinal plants: a systematic review of literature. Malar J, 2021; 20: 349. PMID34433465
17. World Health Organization. WHO information consultation on recent advances in diagnostic techniques and vaccines for malaria: a rapid dipstick antigen capture assay forthe diagnosis of falciparum malaria. Bull World Health Organ, 1996; 74: 47–54.
18. World Health Organization, (2001): Drug Resistance in Malaria. Peter B. Bloland. 'Malaria Epidemiology'. Branch Centers for Disease Control and Prevention Chamble GA, United States of America WHO, Geneva.
19. World Health Organization, (2005): The world Malaria Report. WHO, Geneva. Available at: http://rbm.who.int/wmr (accessed October 2019)
20. World Health Organization, (2010): 'New Perspectives Malaria Diagnosis, Report Of A Joint.
21. WHO/USAID Informal Consultation 25-27 October 1999. World Health Organisation, (2010): Research for Diseases of Poverty Geneva, Switzerland; World heal Organization.
22. World Health Organization, (2014): Tropical Medicine and International Health is published John Wiley and Sons. Geneva, Switzerland; World health Organization.
23. Yaw A., Afrane, Guofa Zhou, Andrew K., Githeko and Guiyun Yan (2014). 'Clinical Case Definition and Malaria Attributable Fraction in the Highlands Kenya'. Malaria Journal.