

In Vitro* Phytochemistry and Antiplasmodial Activity of Leaf Extract and Fractions of *Nauclea diderrichii

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Abstract

The current investigation deals with phytochemical screening and *in vitro* antiplasmodial activity of crude ethanol leaf extract and three fractions of crude ethanol leaf-extract of *Nauclea diderrichii*. Phytochemical test to screen bioactive compounds was carried out via standard protocols which uncovered the presence of alkaloids, saponins, steroids, phenols, tannins, flavonoids, glycosides and carbohydrates, extraction was done using absolute ethanol to afford the crude extract (Nd-ET) while maceration was done using solvents of different polarity gradient (petroleum ether, chloroform and ethyl acetate) to afford the remaining fractions (Nd-F₁, Nd-F₂ and Nd-F₃). The antiplasmodial activity of the crude-extract and those of crude-extract-fractions against plasmodium falciparum unveil promising percentage elimination at all concentrations, with ethanol crude extract (Nd-ET) and ethyl acetate fraction (Nd-F₃) having the highest, with 75.50% and 72.65% at 625µg/ml, and 87.83% and 86.33% at 5000µg/ml, respectively. These results clearly indicated that the active compounds present in the crude leaf extract/fractions of *Nauclea diderrichii* are highly potent eliminators of plasmodium falciparum and validate their popular usage in folk medicine in Gusau Local Government, Zamfara State, Nigeria, for the treatment of malaria.

Introduction

From time immemorial, medicinal plants have been used to flavour and conserve

Received: September 16, 2019; Accepted: November 2, 2019

Keywords and phrases: *Nauclea diderrichii*, alkaloid, antiplasmodial, ethanol crude, phytochemical, toxicity.

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food, treat health disorders and to prevent diseases including epidemics and infectious disease with increasing number of reports on pathogenic microorganisms resistant to antimicrobials in clinical used (Singh [16]). Assurance of the safety, quality, and efficacy of medicinal plants and herbal products has now become a key issue in industrialized and in developing countries (Singh [16] and Khond et al. [21]). The knowledge of their healing effects has been transmitted over the centuries within and among human communities. This healing effect is due to active compounds produced by the medicinal plants during secondary metabolism (Singh [16] and Dar et al. [12]). Secondary metabolites produced either as a single compound or in combination as a group of related compounds within the natural products (extract/fraction) are usually responsible for the biological activity of medicinal plants and as potentials for drug discovery (Soderberg [22]).

Natural environment is furnished with plants and plant's products which have been found to be of great medicinal and nutritional importance. There are many experimental pieces of evidence indicating the use of plants for medicinal purposes, hence, the plant kingdom has become a target for the search of biologically active lead compounds for the complementary/alternative management of human ailments (Osigwe et al. [11]).

Nauclea diderrichii is an ever green tree belonging to the Rubiaceae family. It is endemic in West and Central Africa and has been extensively studied for its anti-malaria (Lamidi et al. [5]), anti-plasmodial (Valentin et al. [6]), anti-leishmanial (Di Giorgio et al. [1]) and anti-trypanosomal (Nwodo et al. [8]) activities.

In folkloric medicine in Nigeria and other part of the world, particularly West Africa, *Nauclea diderrichii* is used in the management of a wide range of ailments ranging from endocrine disorders, infections, wound healing, anti-malaria, anemia, stomach ache, indigestion, fever and jaundice. In Nigeria, bark preparations are used against fever and malaria, and as antiperiodic, appetizer and others (Orwa et al. [10]), it is also used for its anti-diabetic activity with good toxicological profile (Theophine et al. [17]). The bark and leaf decoctions of the plant are taken in Sierra Leone and Ghana against stomach ache, diarrhea, malaria, as a wash for measles and as a foot wash after long walks. In Côte d'Ivoire, the bark is sometimes used to treat bacterial infection. In Gabon, a bark infusion is drunk against fever. In Congo, bark decoctions are taken or the leaf pulp is rubbed in for the treatment of fever, stomach problems, gonorrhoea, hepatitis and menstruation problems, while a bark infusion is taken as a vermifuge. In various

countries leaf infusions, leaf decoctions and the leaf pulp are drunk or used in washings, baths or embrocation to treat fever. In Guinea leaf preparations are applied on tumours. The ripe infructescence is also eaten as a cough medicine in Sierra Leone (*Nauclea diderrichii* (PROTA) [7]).

The aim of this investigation is to evaluate the phytochemical contents and to carry out anti-plasmodial test on the crude ethanol leaf-extract (Nd-ET) and three fractions (Nd-F₁, Nd-F₂ and Nd-F₃) of the crude ethanol extract of *Nauclea diderrichii* at different concentrations.

Materials and Methods

Botanic description of the plant (Nauclea diderrichii)

Nauclea diderrichii is a plant with English name (African peach) and trade name (Opepe). It is an evergreen tree that reaches a height of 30-40 m and a diameter of 0.9-1.5 m; bole cylindrical, slender, straight and branchless and a broad spherical crown with thick foliage. The shining leaves are 15 cm long and bigger when young, elliptic, and acute at the ends, keeled towards the base, and stipulate, with a pair of distinct leafy stipules at the base. It is mostly deciduous except at the ends of shoots and the nodes are often occupied by ants. The flowers are small, green-white-yellow and tubular, in solitary terminal heads (unbranched), 3 cm across; stalks only about 1 cm. The fruit is yellow, fleshy, in a globose head deeply pitted between the deeply fused calyx lobes (Orwa et al. [10]).

Traditional description of the plant (Nauclea diderrichii)

The plant, *Nauclea diderrichii* is commonly known locally as Opepe (Nigeria), Opepi (United Kingdom), Kusia (Ghana), Badi (Ivory Coast), Bilinga (Gabon), Akondoc (Cameroon), Kilingi (Uganda). It is a medium sized tree up to 30 m tall with bole up to 80 cm in diameter. The Heartwood is orange or golden yellow, darkening on exposure; sapwood whitish or pale yellow, clearly defined. Texture rather coarse; grain usually interlocked or irregular; lustrous; without characteristic odor or taste (*Nauclea diderrichii* (PROTA) [7]). It is well known as “Tuwon Biri” in Hausa land, North-western, Nigeria.

Sample collection and authentication

The fresh leaves of *Nauclea diderrichii* were plucked directly from the plant at Mada district, Gusau Local Government Area, Zamfara State, Nigeria. It was identified and authenticated at the Department of Plant Biology, Bayero University, Kano, Nigeria, with Herbarium Accession Number 0434.

Sample preparation and extraction

The fresh leaves of *Nauclea diderrichii* were washed with clean water and air dried under shade at ambient temperature for about a week in the absence of any form of contaminant. It was then pulverized mechanically (using mortar and pestle) to form a fine powder. After pulverization the powdered sample was stored in an air tight container, in cool and dry place away from light and was later subjected to ethanol extraction (Bargah [13]).

Extraction process

About 1000 g of the powdered leaf sample of *Nauclea diderrichii* was percolated with 3.0 L of absolute ethanol with shaking at regular interval for one week, after which the extract was separated from the debris by filtration. The filtrate was then concentrated using a Rotavapor (R110 at 40°C) and was coded Nd-ET (ethanol crude extract), weighed and kept in a cool and dry place away from any form of contaminant (Sarkar et al. [15]).

Maceration of crude (Nd-ET) extract

The crude ethanol extract (Nd-ET) was then macerated with petroleum ether, chloroform and ethyl acetate to afford the remaining fractions, coded Nd-F₁, Nd-F₂ and Nd-F₃ respectively. Each fraction obtained was weighed and concentrated at room temperature.

In vitro phytochemical screening

Preliminary phytochemical tests for the screening and identification of bioactive chemical constituents like alkaloid, carbohydrates, flavonoid, glycoside, phenol, saponin, steroid, and tannin present in the crude leaf extract and three extract-fractions of *Nauclea diderrichii* were carried out using standard procedures adopted from Harborne [4] and Gurav et al. [3].

***In Vitro* Antiplasmodial Activity**

Preparation of stock solution and other concentrations

The stock solutions of the ethanol-crude-extracts (Nd-ET) and those of crude-extract-fraction (Nd-F₁, Nd-F₂ and Nd-F₃) were prepared by dissolving 10 mg of each extract in 1 mL of dimethyl sulphuroxide (DMSO) to produce 10000 µg mL⁻¹. Other Lower concentration solutions of 5000, 2500, 1250 and 625 µg mL⁻¹ were obtained from the stock solutions by serial double dilution.

Preparation of culture media (RPMI 1640)

The media was prepared by dissolving 10.4g of the powdered material into 1 Litre of distilled water, 1 mL of gentamicin was added as a stabilizer and then autoclaved at 121°C for 15 minutes.

Anti-plasmodial activity

About 0.1 mL of the test solution, 0.1 mL of healthy rabbit blood, and 0.2 mL of the culture media were all added together in a vial containing 0.1 mL of the parasitemia erythrocytes and mixed thoroughly. The mixture was incubated for 24hrs at 37°C. the incubation was carried out in a glass bell jar containing a lighted candle to ensure the supply of required quantity of CO₂ (about 5%), O₂ (about 2%) and N₂(g) (about 93%) (Trager and Jensen [23]). Alongside a positive control, 2000 µg/mL Artemether Lumefantrine and a negative control, culture media and positive erythrocytes were also incubated.

After 24hrs incubation, a drop of the incubated sample was smeared on a microscopic slide and stained Giemsa's staining technique. The stained microscopic slides were viewed under light microscope and the numbers of cleared RBC are counted, this was repeated 3 times and the mean number erythrocytes appearing as discoid cells for each concentration were recorded. The activity of the test samples was calculated as percentage elimination using expression below (Mukhtar et al. [20]).

$$\text{Percentage (\%)} \text{ elimination} = \left(\frac{N}{N_x} \right) \times 100, \quad (1)$$

where N =Total number of RBC cleared, N_x =Total number of parasitized RBC, "RBC" =Red Blood Cells.

Results and Discussions

Fractionation of Nauclea diderrichii leaf extract

The results of fractionation of the crude extract (Nd-ET) are shown in Table 1.

Table 1. Weight, color and texture of fractions of *Nauclea diderrichii* leaf extract.

Fractions	% Yield	Weight (g)	Color	Texture
Nd-F ₁	12.72	1.22	Golden green	Sticky
Nd-F ₂	05.21	0.5	Dark green	Sticky
Nd-F ₃	02.81	0.27	Dark brown	Sticky

Phytochemical screening

The results of the phytochemical test for the screening of potent compounds imbedded in the crude leaf extract of *N. diderrichii* and those of fractions can be seen in Table 2.

Table 2. Phytochemical analysis result of *Nauclea diderrichii* leaf extracts.

Phytochemical	Nd-ET	Nd-F ₁	Nd-F ₂	Nd-F ₃
Alkaloids	+	+	+	+
Saponin	+	+	+	+
Steroids	+	-	-	-
Phenol	+	-	-	+
Tannin	+	-	+	+
Flavonoid	+	-	-	+
Carbohydrate	+	+	+	+
Glycosides	+	-	-	+

Key: (+) = Present and (-) = Absent

As we can see from Table 2, the ethanol crude extract (Nd-ET) contained all the tested phytochemicals, while in the fraction of ethyl acetate (Nd-F₃) steroid has not been detected, and in both fractions of Petroleum ether (Nd-F₁) and Chloroform (Nd-F₂), phenol, glycoside and steroid have not been detected.

Literatures from previous studies, also confirmed the presence of phytochemical ingredients like alkaloids, cardiac glycosides, saponins, phenolic compounds, tannins, phytosterols, carbohydrates, flavonoids and terpenoids, imbedded in the leaf extract of *Nauclea diderrichii* (Isa et al. [19] and Ette et al. [18]).

Antiplasmodial assay of *Nauclea diderrichii* leaf extracts

The anti-plasmodial activities of the crude leaf extract and those of the three fractions (crude leaf extract-fractions) of *Nauclea diderrichii* uncovered promising percentage elimination of the parasite at all concentrations. Ethanol crude extract (Nd-ET) and ethyl acetate fraction (Nd-EA) demonstrated the highest activity with percentage elimination of 75.50% and 72.65 at $625 \mu\text{g mL}^{-1}$, and 87.83% and 86.33% at $5000 \mu\text{g mL}^{-1}$ respectively. These results mentioned can be seen in Table 3.

Table 3. Percentage elimination on *Plasmodium falciparum*.

Fractions	Conc. ($\mu\text{g mL}^{-1}$)	Av. parasite before incubation	Av. Parasite after incubation	Percentage (%) Elimination
Nd-ET (crude)	625	52	15.78	75.56
	1250	52	14.56	79.06
	2500	52	10.00	85.90
	5000	52	6.33	87.83
Nd-F₁	625	52	16.11	68.02
	1250	52	16.22	68.81
	2500	52	12.67	75.63
	5000	52	08.00	84.61
Nd-F₂	625	52	14.45	72.21
	1250	52	12.11	76.71
	2500	52	10.67	79.48
	5000	52	10.33	80.13
Nd-F₃	625	52	12.67	72.65
	1250	52	10.87	75.21
	2500	52	7.33	76.29
	5000	52	6.33	86.33
Control (+ve)	2000	52	4.81	90.75
Control (-ve)	0	52	52	0

The anti-plasmodial activity of *Nauclea diderrichii* extract (Nd-ET) and fractions (Nd-F₁, Nd-F₂ and Nd-F₃) justifies part of the ethnomedicinal claims on the plant, as effective as conventional medicine in combating pathogenic microorganisms and it is a preliminary scientific validation for the use of the plant, *Nauclea diderrichii* as antimalaria to promote proper conservation and sustainable use.

The primitive use of the plant, in folk medicine in Madadistrict, Gusau local government, Zamfara State, Nigeria, represent a cheaper and safer alternatives in the treatment of infectious diseases.

Conclusion

The findings of present study virtually confirmed the phytochemical ingredients and anti-plasmodial potency of bioactive phytochemicals imbedded in *Nauclea diderrichii*, which validates the primitive use of the plant as a curative agent for malaria and other diseases of clinical concern.

Haudecoeur et al. [14] reported that the sustainability of the plant, *N. diderrichii*, should be kept in mind to adapt local uses and preparation modes of traditional remedies.

Further studies involving isolation, characterization and toxicological studies *in vivo* are needed with model animals to elucidate and validate the stability and dose dependent activity of the pure active compounds from the plant, *Nauclea diderrichi*.

References

- [1] C. Di Giorgio, M. Lamidi, F. Delmas, G. Balansard and E. Ollivier, Antileishmanial activity of quinovic acid glycosides and cadambine acid isolated from *Nauclea diderrichii*, *Planta Med.* 72 (2006), 1396-1402. <https://doi.org/10.1055/s-2006-951726>
- [2] H. V. Girish and S. Satish, Antibacterial activity of important medicinal plants on human pathogenic bacteria - a comparative analysis, *World Applied Science Journal* 5(3) (2008), 267-271.
- [3] Amol Gurav, D. B. Mondal and H. Vijayakumar, In vitro qualitative and quantitative phytochemical analysis of ethanolic and 50% ethanolic extract of *Tinospora cordifolia*, *Momordica charantia*, *Curbita maxima* and *Raphanus sativus*, *International Journal of Pharmaceutical Sciences and Research* 5(5) (2014), 1937-1941
- [4] A. J. Harborne, *Phytochemical Methods A Guide to Modern Techniques of Plant Analysis*, 3rd ed., Chapman and Hall, London, 1998.
- [5] M. Lamidi, E. Ollivier, M. Gasquet, R. Faure, L. Nzé-Ekekang and G. Balansard, Structural and antimalarial studies of saponins from *Nauclea diderrichii* bark, Saponins Used in Traditional and Modern Medicine, *Advances in Experimental Medicine and Biology* 404 (1996), 383-399. https://doi.org/10.1007/978-1-4899-1367-8_31

- [6] A. Valentin, Mustofa, F. Benoit-Vical, Y. Pélissier, D. Koné-Bamba and M. Mallié, Antiplasmodial activity of plant extracts used in West African traditional medicine, *Journal of Ethnopharmacology* 73 (2000), 145-151.
[https://doi.org/10.1016/S0378-8741\(00\)00296-8](https://doi.org/10.1016/S0378-8741(00)00296-8)
- [7] *Nauclea diderrichii* (PROTA) (2015): Plant Use English, ([https://uses.plantnet-project.org/e/index.php?title=Nauclea_diderrichii_\(PROTA\)&oldid=197774](https://uses.plantnet-project.org/e/index.php?title=Nauclea_diderrichii_(PROTA)&oldid=197774)).
- [8] N. J. Nwodo and M. O. Agbo, Antitrypanosomal effects of methanolic extracts of *Nauclea diderrichii* (Merr) and *Spathodea campanulata* stem bark, *J. Pharm. Allied Sci.* 7 (2010), 1219-1227. <https://doi.org/10.4314/jophas.v7i5.63466>
- [9] N. Y. Opuni-Frimpong and E. Opuni-Frimpong, *Nauclea diderrichii*: Plant Resources of Tropical Africa (PROTA), 2012.
- [10] C. Orwa, R. H. Jamnadass, R. Kindt, A. Mutua and A. Simons, Agroforestry database: a tree reference and selection guide version 4.0, 2009.
<http://www.worldagroforestry.org/sites/treedbs/treedatabases.asp>
- [11] C. Osigwe, P. Akah, C. Nworu, T. Okoye and M. Tchimene, Antihyperglycemic studies on the leaf extract and active fractions of *Newbouldia laevis* (Bignoniaceae), *Pharmacology and Pharmacy* 6 (2015), 518-532.
<https://doi.org/10.4236/pp.2015.611054>
- [12] Refaz Ahmad Dar, Mohd Shah Nawaz and Parvaiz Hassan Qazi, General overview of medicinal plants: a review, *Journal of Phytopharmacology* 6(6) (2017), 349-351.
- [13] Rohit Kumar Bargah, Preliminary test of phytochemical screening of crude ethanolic and aqueous extract of *Moringa pterygosperma* Gaertn, *Journal of Pharmacognosy and Phytochemistry* 4(1) (2015), 7-9.
- [14] Romain Haudecoeur, Marine Peuchmaur, Basile Pérès, Maxime Rome, Germain Sotoing Taïwe, Ahcène Boumendjel and Benjamin Boucherle, Traditional uses, phytochemistry and pharmacological properties of African *Nauclea* species: a review, *Journal of Ethnopharmacology* 212 (2017), 106-136. <https://doi.org/10.1016/j.jep.2017.10.011>
- [15] Shovon Lal Sarkar, Prianka Saha and Nigarin Sultana, *In vitro* evaluation of phytochemical components and antimicrobial activity of the methanolic extract of *Tridax procumbens* L. against pathogenic microorganisms, *Journal of Pharmacognosy and Phytochemistry* 5(5) (2016), 42-46.
- [16] R. Singh, Medicinal plants: a review, *Journal of Plant Sciences* 3(1-1) (2015), 50-55.

- [17] Theophine C. Akunne, Bonaventure C. Obi, Martha N. Ofokansi, Patience C. Nwonu and Charles O. Okoli, Antidiabetic activity and toxicological evaluation of the methanol-dichloromethane root bark extract of *Nauclea diderrichii* (De Wild) Merr, *International Journal of Pharmacy and Pharmaceutical Sciences* 9(9) (2017), 279-283.
<https://doi.org/10.22159/ijpps.2017v9i9.20556>
- [18] E. Ette, E. Ubulom, E. Ekpenyong, S. Ekong, E. Akpan and D. Tambari, In vivo antiplasmodial activities of *Nauclea latifolia*, *Asian Journal of Medical Sciences* 6(3) (2015), 6-11. <https://doi.org/10.3126/ajms.v6i3.11361>
- [19] H. Isa, U. A. Katsayal, A. Agunu, A. Nuhu and Z. Abdulhamid, Phytochemical screening and thin layer chromatographic profile of *Nauclea diderrichii* leaf extracts, *Bayero Journal of Pure and Applied Sciences* 10(1) (2017), 281-284.
<https://doi.org/10.4314/bajopas.v10i1.42>
- [20] M. D. Mukhtar, M. Bashir and A. H. Arzai, Comparative in vitro studies on Antiplasmodial activity of some Nigerian and foreign brands of Chloroquine oral formulations marketed in Kano, *African Journal of Biotechnology* 5(24) (2006), 2464-2468.
- [21] M. Khond, J. D. Bhosale, T. Arif, T. K. Mandal, M. M. Padhi and R. Dabur, Screening of some selected medicinal plants extracts for in-vitro antimicrobial activity, *Middle-East Journal of Scientific Research* 4(4) (2009), 271-278
- [22] T. Soderberg, *Organic Chemistry with a Biological Emphasis Volume II*, 2019. Chemistry Publications, 2. Available at: https://digitalcommons.morris.umn.edu/chem_facpubs/2/ [Accessed 12 Jul. 2018].
- [23] W. Trager and J. B. Jensen, Human malaria parasites in continuous culture, *Science* 193(4254) (1976), 673-675. <https://doi.org/10.1126/science.781840>