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Original Research Article

Antiplasmodial, Antimalarial and Hemozoin Inhibitory Potential of *Nauclea pobeguinii* Rootbark Methanol Extract

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Abstract

Purpose*:* *Nauclea pobeguinii* is one of the medicinal plants commonly used to manage malaria in Nigeria. This study sought to assess its antimalarial mechanism of action by evaluating its phytochemical content, antiplasmodial activity, cytotoxicity, and heme detoxification potential. Furthermore, we conducted an *in silico* ADME of the β-carboline alkaloids reported to be responsible for it's ~~their~~ antimalarial activity. Phytochemical screening revealed the presence of several phytometabolites, including alkaloids. The extract was non-toxic to HUH cells and inactive against the malaria parasite in culture. Given its *in vitro* inactivity, we assessed its antimalarial activity and probed for inhibition of heme biocrystallization *in vivo*.
Methods*:* Blood was taken from the hearts of 3 mice each in the control and study groups, and the blood was lysed to release the parasites, whose parasitophorous vacuole and digestive vacuole (DV) membranes were disrupted, releasing DV contents from which free heme was quantified.
Results*:* There was a dose-dependent decrease in parasitemia and hemozoin concentration, suggesting that higher doses of the extract led to greater inhibition of heme biocrystallization. However, on day 10, parasitemia levels in *N. pobeguinii*-treated mice reached levels comparable to those of untreated controls, negatively impacting mouse survival. Our *in-silico* study revealed that the *N. pobeguinii*-derived β-carboline alkaloids are not only substrates of Permeability glycoprotein but also of several Cytochrome P450 enzymes..
Conclusion: The result of our study thus suggests that while *N. pobeguinii* may kill the malaria parasite by inhibiting heme biocrystallization; its antimalarial activity may be impacted by poor oral pharmacokinetics.

Keywords: *Nauclea pobeguinii*, β-carboline alkaloids, Antiplasmodial, Antimalarial, Heme biocrystallization, Oral pharmacokinetics

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INTRODUCTION

Malaria is a deadly parasitic infectious disease responsible for millions of cases and hundreds of thousands of deaths annually, mostly in sub-Saharan Africa. According to the World Health Organisation (WHO), an estimated 282 million people worldwide contracted malaria in 2024, with 610,000 deaths recorded, the majority of which occurred in sub-Saharan Africa.[1] The disease is caused by *Plasmodium* parasites and spread by female *Anopheles* mosquitoes. The continued prevalence of the disease is exacerbated by factors such as inadequate healthcare infrastructure, poverty, and the increasing spread of drug-resistant malaria parasite strains.[1] Additionally, the rise of insecticide resistance by *Anopheles* mosquitoes further complicates malaria control efforts, necessitating the development of new strategies, such as genetically modified mosquitoes or novel insecticides.[2]

Aside from the rise of drug-resistant *Plasmodium* parasites, several other challenges, including unwanted side effects, economic barriers, and limited mechanisms of action, continue to undermine the effectiveness of current antimalarial drugs.[1] This necessitates the continuous search for novel antimalarial treatments.

Medicinal plants have provided several antimalarial drugs, such as Artemisinin and Quinine which were isolated from *Artemisia annua* and *Cinchona succirubra* respectively. The accumulation of resistance mutations by the malaria parasite has resulted in resistance to these antimalarial agents.[3] As a result, scientists have continually sought new plant-derived agents that can combat drug resistance effectively.[4]



Figure 1: *Nauclea pobeguinii* plant parts; Root bark (A) Stem branch (B) Front of leaf (C) Back of leaf (D)

Nauclea pobeguinii (Figure 1) is one of the seven species of the *Nauclea* genus found in Africa. Others include *N. latifolia, N. diderrichii, N. vanderguchtii, N. gilletii, N. nyasica* and *N. xanthoxylon*.[5] Members of the *Nauclea* genus are useful for managing several ailments, including viral diseases, respiratory infections and malaria.[5] Preparations of *N. pobeguinii* stembark (80% ethanolic extract containing 5.6% strictosamide, denoted as PR259CT1) have been clinically developed by the Democratic Republic of Congo for the management of malaria.[6,7,8] This has made ~~the~~ *N. pobeguinii* plant one of the natural products to be listed in the Malaria drug development pipeline.[9] Although *N. pobeguinii* (PR259CT1) has reached Phase IIb/III in the malaria drug development pipeline, its mechanism of antimalarial action remains unknown. Given the relevance of mechanistic studies in the fight against malaria,[3] we set out to explore this in the current study.

MATERIALS AND METHODS

Materials

The malaria parasite {*Pf*3D7 (MRA 102)} and human hepatoma (HUH) cell line used for this study were sourced from Malaria Research and Reference Reagent Resource Centre (MR4) and the American Type Culture Collection (ATCC), respectively. The chloroquine-sensitive parasite, *P. berghei* NK65, was obtained from the Nigerian Institute of Medical Research (NIMR), Lagos. Chloroquine diphosphate salt and strictosamide were gotten from Sigma, USA. Other chemicals and reagents used were of research grade.

Methanol extract preparation

N. pobeguinii root bark was collected from its natural habitat close to Benin City, Nigeria, in September 2020. It was thereafter authenticated at the Department of Plant Biology and Biotechnology of the University of Benin, with voucher specimen number UBH^{N180} allotted. The methanol extract was prepared by macerating 40 g of the pulverised root bark of *N. pobeguinii* in 400 ml of methanol, which was then placed in a rotary shaker (120 rpm, 25 °C) for 72 hr. Thereafter, it was concentrated in a rotary evaporator and lyophilised.

Phytochemical screening

Phytochemicals were screened by first preparing a 1 mg/mL stock of the extract in methanol. Thereafter, established protocols[10,11,12] were followed to qualitatively determine the phytochemicals present in the sample.

In vitro antiplasmodial activity testing

Briefly, *Pf*3D7 parasites were grown in human red blood cells.[13] For activity testing, culture was synchronised using 5% sorbitol[14] after which it was diluted (1% P and 2% H) and exposed to various test concentrations of the extract (0, 0.78, 1.56, 3.3, 6.25, 12.5, 25, 50, 100 µg/ml) and strictosamide (0, 0.78, 1.56, 3.3, 6.25, 12.5, 25 µg/ml). These were incubated at 37 °C under reduced oxygen (5% CO_2, 5% O_2 and 90% N_2). At the end of the 48-hr incubation period, smears were prepared from triplicate wells, and Giemsa-stained microscopic images were captured to determine % parasitemia.

Cytotoxicity testing

Human Hepatoma (HUH) cells, upon reaching full confluence, were trypsinised and transferred into a tube and washed with complete media, i.e. Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 5% fetal bovine serum (FBS) (cDMEM). It was thereafter suspended in 2 ml of complete media. From this, a 2 µL suspension was taken, and a 2-fold dilution was made with trypan blue to enable counting with a hemocytometer. Following a hemocytometer count, the cells were diluted to 1×10^4 HUH cells per well in a 96-well plate, and incubation was performed for 20 hr. Prior to drug addition, 90 µl of spent media was replaced with 86 µl of fresh media and 4 µl of drug (i.e., 0 to100 µg/ml of the extract or strictosamide). Plates were thereafter placed in a CO_2 incubator at 37 °C and incubated for 24 hr. At the end of the 24-hr. incubation period, 20 µl of 3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (2 mg/mL) was added to each well, and incubation was done for 3 hr. After 3 hr. of incubation, 120 µl of MTT, media, and drugs were removed from each well, and 200 µl DMSO was added for 10 minutes to convert MTT to formazan, which absorbs maximally at 570 nm. Wells containing 0.4% DMSO in cDMEM served as 100% growth control, while wells treated with 10% DMSO served as 0% growth control.[15]

Acute toxicity

The *in vivo* studies were conducted in accordance with standard animal care guidelines (REC Approval No: CMS/REC/2025/876). Animals were placed in plastic cages and housed in a room with proper ventilation and 12 hr. light/dark cycle in the animal house facility of Biochemistry Department, University of Benin, Nigeria. The acute toxicity of *N. pobeguinii* rootbark methanol extract was assessed using the Organisation for Economic Co-operation and Development guideline 423 method.[16] Six male albino mice (~8 weeks old; ~25 g) were randomly placed in 2 groups of 3 mice each and fasted for 6 hr. with water *ad libitum*. Group 1 received normal saline (and thus served as the control), while members of group 2 were administered 2 g/kg b.wt. of *N. pobeguinii* rootbark methanol extract, and toxicity signs monitored for 14 days.[16]

***In vivo* antimalarial assessment using Peter's 4-day suppressive test**

Passage mice infected with chloroquine-sensitive (NK65) *P. berghei* were obtained from NIMR, Lagos, Nigeria. Peter's 4-day suppressive method was used for the *in vivo* antimalarial testing of *N. pobeguinii* rootbark methanol extract. Test extracts (100, 250 and 500 mg/kg b.wt.) were prepared in normal saline. Forty male mice (Wistar strain) (6–8 weeks old; ~25 g) were randomly distributed into five groups of eight mice, intraperitoneally inoculated with 1×10^5 *P. berghei* NK65 infected RBCs, and orally fed 100, 250 or 500 mg/kg b.wt. of *N. pobeguinii* 3 hr. after infection. While the positive control group received 25 mg/kg b. wt. of the standard antimalarial drug, chloroquine, the negative control group received normal saline. Administration of the drug/extract was performed daily for 4 consecutive days (i.e., on day 0, the day of infection, through day 3). On day 4 after infection, 3 animals from each group were sacrificed, and blood was collected via cardiac puncture for the hemozoin inhibition assay (see section below). Blood smears were made using a drop of blood from the lateral tail vein of mice on days 4 and 10. These were fixed in absolute methanol and stained with 10% Giemsa. Smears were thereafter subjected to microscopic determination of % parasitemia. Mortality was monitored within a 30-day period, and data derived therefrom were used to compute the mean survival time (MST).[17]

***In vivo* hemozoin biocrystallization assay**

A modified method of Men et al.,[18] was adopted for the assessment of inhibition of heme detoxification by the *N. pobeguinii* rootbark methanol extract. Briefly, on day 4 of the suppressive antimalarial study, blood (500 µl) was drawn via cardiac puncture from 3 infected mice each from *N. pobeguinii* treated groups (100, 250 and 500 mg/kg b.wt. treatment) as well as the positive (25 mg/kg b.wt. chloroquine treated) and the negative control (administered normal saline) groups. These were placed in Eppendorf tubes and treated with 1 ml of 2.5% sodium dodecyl sulphate (SDS) in 0.1 M sodium bicarbonate (pH 8.8). The solutions were thereafter thoroughly mixed at room temperature for 15 min. After incubation, samples were centrifuged (13,000 rpm, 10 min) and the supernatant removed. The pellet was then washed

twice with 800 µl of 2.5% SDS in 0.1 M sodium bicarbonate (pH 8.8). Thereafter, 200 µl of 5% SDS in 50 mM NaOH was added to convert the hemozoin to heme. After incubation at 37°C for 30 min, absorbance was measured at 405/750 nm using a spectrophotometer (Thecmel and Thecmel) to quantify monomeric heme. While heme absorbs at 415 nm (Soret region) and 750 nm (Q region), hemozoin shows a strong band at 700 nm. So, by monitoring the absorbance at 750 and subtracting from that of 415, the absorbance of only free heme was obtained.

***In silico* ADME**

Structures of the *N. pobeguinii*-derived β-carboline alkaloids[19] were drawn using ChemAxon and submitted for absorption, distribution, metabolism and excretion (ADME) prediction using the SwissADME web tool, which is freely available at http://www.swissadme.ch.

Statistical analysis

Results are presented as mean ± standard deviation. One-way analysis of variance was used to compare the differences between means. SPSS version 25 software was used for analysis.

RESULTS AND DISCUSSIONS

Yield of extraction

A % yield of 4.15 (Table 1) was obtained following the extraction of the rootbark of *N. pobeguinii* with methanol and its concentration.

Table 1: Extraction Yield

Sample	Amount of dried sample extracted (g)	Extraction yield (g)	% yield
N. pobeguinii root bark	40	1.66	4.15

Phytochemical screening

Table 2: Phytochemical screening of *N. pobeguinii* methanol rootbark extract

Phytochemicals	Abundance
Alkaloids	+
Tannins	-
Flavonoid	+
Steroid	-
Phenolics	+
Saponin	-
Cardiac glycosides	-
Quinones	+++
Terpenoids	-
Coumarins	++

Key: = Highly detected^{+++}, Moderately detected^{++}, Lowly detected^{+}, Absent $^{-}$

N. pobeguinii is documented to have good antimalarial effects.[6,7,8] A preparation of its stembark (PR 259 CT1) [6,7,8] has been developed by the Democratic Republic of the Congo for the management of malaria.[5,9] In the present study, several phytochemicals, including alkaloids, flavonoids, phenolics, quinones and coumarins, were observed to be present in *N. pobeguinii* rootbark with coumarins and quinones being moderately and highly abundant, respectively (Table 2).

TLC-based metabolite profile of *N. pobeguinii* rootbark methanol extract

Alkaloids of the β-carboline class have been reported to be responsible for *N. pobeguinii*'s antimalarial activity.[19] To visualise, these alkaloids, the TLC plate was stained with Dragendorff's stain. Strictosamide served as a marker (Figure 2).

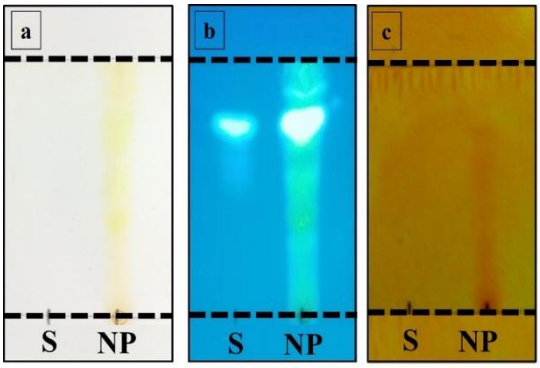


Figure 2: TLC metabolite fingerprint of *N. pobeguinii* rootbark methanol extract (NP) and strictosamide (S) viewed under visible light (a), long UV (b) as well as following staining of separately run TLC plates with dragendorff's reagent (c) {to reveal the presence of alkaloid(s)}. Solvent system used: Chloroform: Ethylacetate: Formic acid (10:08:02 v/v/v), Amount loaded/spot = 50 µg crude extract, 5 µg pure compound. The dotted lines at the top and bottom represent the solvent front and sample loading sites respectively.

Antiplasmodial activity and cytotoxicity of *Nauclea pobeguinii* methanol rootbark extract

Antiplasmodial activity testing of the extract revealed that it was inactive against the malaria parasite in culture, with 100 µg/ml of the extract suppressing parasite growth by 43% (i.e. IC_{50} >100 µg/ml).[20] Further, it was non-toxic to the mammalian cell line used for this study (CC_{50} HUH >100 µg/ml) (Figure 3). Whereas the 80% ethanol and 80% methanol extracts of *N. pobeguinii* stembark were reported by [19,21] to be moderately potent against the malaria parasite in culture (IC_{50}:

22 – 44 µg/ml), the absolute methanol extract of the rootbark displayed no antiplasmodial activity *in vitro* (IC_{50}: > 100 µg/ml) and was non-toxic to HUH cells (Figure 3). The inactivity may be due to the plant part investigated by us. Several factors including plant part and extraction solvent can impact on the antiplasmodial activity of medicinal plants.[22]

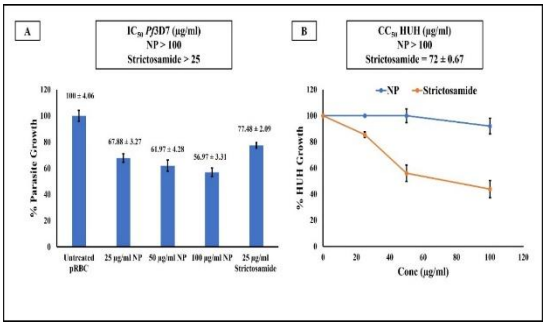


Figure 3: *In vitro* antiplasmodial activity and cytotoxicity of *N. pobeguinii* methanol root bark extract.
Key: NP = *N. pobeguinii* methanol rootbark extract

Acute toxicity study

Acute toxicity testing was done using the OECD[16] method. We started testing with the highest dose suggested (i.e. 2 g/kg b.wt.). After administering a single dose of 2 g/kg b.wt. to the 3 animals, no sign of toxicity, including mortality, was observed up to the 14 days recommended for the study. As a result, the extract was deemed safe at that dose, and as such, testing at lower doses wasn't necessary.

4-day Suppressive Antimalarial Study

On day 4, the parasitemia in the groups administered 250 and 500 mg/kg b.wt. of *N. pobeguinii* was comparable to that of mice treated with the standard drug (25 mg/kg b.wt. chloroquine) and significantly lower than the negative control (untreated group). However, on day 10, parasitemia in mice from all groups administered different doses of the extract was not significantly different from that of the negative control group (Figure 4).

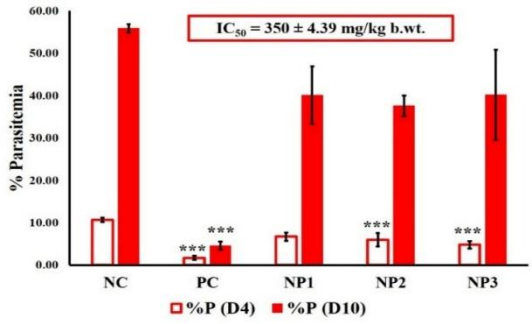


Figure 4: *P. berghei* infected mice treated with either chloroquine (25 mg/kg b.wt.) or *N. pobeguinii* (100, 250 or 500 mg/kg b.wt.). Different superscripts denote significant difference (***p < 0.001) between groups. Key: %P = % Parasitemia; D4 = Day 4 and D10 = Day 10.

Although *N. pobeguinii* rootbark methanol extract did not suppress parasite growth *in vivo* to a degree comparable to chloroquine, the extract showed a dose-dependent decrease in parasitemia, which was significantly lower than in the untreated group (Figure 4). The decrease in parasitaemia indicates that the treatment suppressed the growth of *P. berghei* parasites in mice. While chloroquine suppressed parasite growth by 84.82% on day 4, *N. pobeguinii* rootbark methanol extract administered at 100, 250 and 500 mg/kg b.wt. suppressed parasite growth by 39.39, 46.31 and 56.98%, respectively.

***In vivo* Determination of Hemozoin Concentration**

While *N. pobeguinii*'s antimalarial activity has been validated,[19] its mechanism of antimalarial action is unknown. This study thus assessed its hemozoin inhibitory potential as a possible mechanism of its antimalarial action. On day 4 of the study, mice treated with graded doses of the extract showed a dose-dependent decrease in hemozoin, quantified as monomeric heme in the parasite's DV (Figure 5).

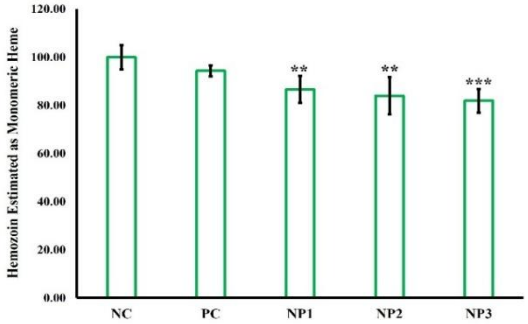


Figure 5: Hemozoin inhibition in mice administered graded doses of *N. pobeguinii* root bark methanol extract. Hemozoin concentration was quantified by calculating % free heme in digestive vacuole of *P. berghei* from parasitized mice that were administered vehicle (NC), 25 mg/kg b.wt. chloroquine (PC) and graded doses 100, 250 and 500 mg/kg b.wt. (NP1, NP2 and NP3 respectively) of *N. pobeguinii*. (**p < 0.01, ***p < 0.001)

The pathogenesis of malaria involves the lysis of parasite-laden erythrocytes following the multiplication of the *Plasmodium* parasites within the red cells, resulting in the release of free haemoglobin or its products; haem and globin. Free haem, if not detoxified, is toxic to the parasites since it generates free radicals {reactive oxygen species (ROS)} that can disrupt biological membranes.[23] *Plasmodium* parasites can detoxify

free haem by polymerising and converting it to a crystalline, insoluble, nontoxic pigment called hemozoin (β-hematin).[24] Quinolone antimalarials, such as chloroquine, act by binding to free haem, interfering with hemozoin formation, resulting in toxic haem accumulation and parasite death.[25] Inhibition of hemozoin formation leads to the accumulation of free haem, causing oxidative damage and parasite death.[22] However, more recently, Singh et al.[26] have shown that chloroquine's complexation with haem doesn't affect haem availability nor inhibit β-hematin formation. Instead, chloroquine kills the parasite by slowing the conversion of haem to hemozoin, a process that may perturb the nucleation of hemozoin crystals and/or the stability of their nuclei.[26] As a result, the hemozoin crystals that form within the parasite in the presence of chloroquine grow to a size that may compromise the DV's integrity and cause the parasite's death via the leakage of proteases into the parasite's cytosol, which may digest vital proteins and organelles.[26] Furthermore, chloroquine's mechanism of action extends beyond the hemozoin inhibition pathway. While the haem biocrystallization pathway is chloroquine's primary target, it also kills the parasite by targeting the host's autophagy and by modulating the host's innate immune response.[27] This may explain the better clearance seen by chloroquine than *N. pobeguinii*, even though *N. pobeguinii* appeared to inhibit haem detoxification more than chloroquine on day 4 of our study (Figures 4 & 5).

Mice Survival

The decrease in parasitemia observed for *N. pobeguinii* on day 4, however, did not translate into a significant increase in the mice's survival (Figure 6).

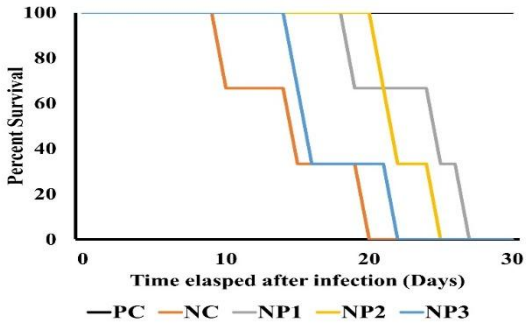


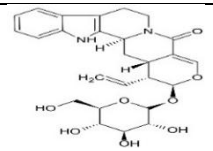
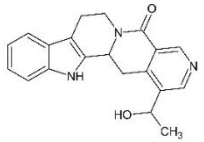
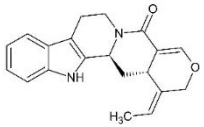
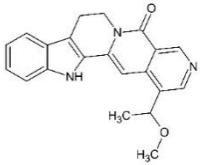
Figure 6: Kaplan Meier Survival curve of *P. berghei* infected mice treated with either chloroquine (25 mg/kg b.wt.) or *N. pobeguinii* {100 (NP1), 250 (NP2) or 500 (NP3) mg/kg b.wt.}

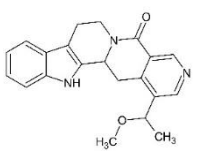
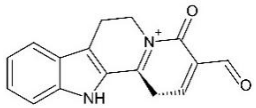
While mice treated with the standard drug (chloroquine) survived for the entire duration of the study, administration of *N. pobeguinii* at all doses did not significantly increase mice survival (MST: NP1 = 23.7 ± 41.6, NP2 = 22.3 ± 1.53, NP3 = 18.3 ± 4.93 days) in comparison with the negative control group (MST = 22.6 ± 5.81 days). Interestingly, when % parasitemia was computed on day 10, it was observed that % parasitemia of mice treated with *N. pobeguinii* had increased in a manner that was not significantly different from the untreated group (Figure 4). This suggests that the bioactive principles in the extract may have poor oral pharmacokinetic properties, limiting their bioavailability.[28] Several factors likely contributed to this reduced bioavailability. One of the key determinants of bioavailability is the metabolism of these compounds by cytochrome P450 (CYP) enzymes. Cytochrome P450 enzymes are a superfamily of heme-containing monooxygenases responsible for the metabolism of endogenous and exogenous compounds.[29] These enzymes play a significant role in the phase I metabolism of xenobiotics, converting them into more hydrophilic metabolites that can be further conjugated or excreted outright. Although strictosamide has previously been touted as an antiplasmodial agent ~~against~~ for *N. pobeguinii*,[7] the recent study by Peeters et al.[19] refutes this claim. Instead, several β-carboline alkaloids present in small quantities have been reported to be responsible for *N. pobeguinii*'s antimalarial activity.[19] These β-carboline alkaloids have been reported to be present as pro-drug forms in *N. pobeguinii* and thus display antimalarial activity *in vivo* despite *in vitro* inactivity.[19,30]

***In silico* ADME**

Given the inactivity of *N. pobeguinii* against the malaria parasite in culture and an *in vivo* antimalarial activity that wasn't sustained, we studied the pharmacokinetics of the putative (strictosamide) and confirmed (β-carboline alkaloids) antimalarial agents in *N. pobeguinii*[19] using *in silico* techniques. Strictosamide, previously thought to be responsible for *N. pobeguinii*'s antimalarial action,[7] was predicted by our *in-silico* model to be a Permeability glycoprotein (P-gp) substrate with limited ability to cross the intestine and blood-brain barriers (Figure 7; Table 3).

Table 3: Oral pharmacokinetics of some *N. pobeguinii*-derived β-carboline alkaloids

S/N	Compound	GI absorption	BBB permeant	Pgp-substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Bioavailability Score
1	 Strictosamide ($C_{26}H_{30}N_2O_8$)	Low	No	Yes	No	No	No	No	No	0.56
2	 Angustoline ($C_{20}H_{19}N_3O_2$)	High	Yes	Yes	Yes	Yes	No	Yes	No	0.55
3	 Nauclefilline ($C_{20}H_{20}N_2O_2$)	High	Yes	Yes	Yes	Yes	No	Yes	Yes	0.85
4	 19-O-methyl angustoline ($C_{21}H_{19}N_3O_2$)	High	Yes	Yes	Yes	Yes	Yes	Yes	Yes	0.55
5		High	Yes	Yes	Yes	Yes	No	Yes	Yes	0.55

	 19-O-methyl-3,14-dihydroangustoline ($C_{21}H_{21}N_3O_2$)										
6	 Nuclefidine ($C_{16}H_{13}N_2O_2$)	High	Yes	Yes	Yes	No	No	Yes	No	0.55	

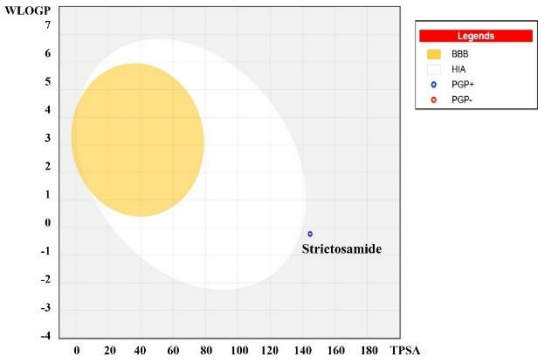


Figure 7: Boiled-egg of Strictosamide; *N. pobeguinii*'s putative antimalarial agent

Furthermore, it is predicted not to be a substrate for any CYP enzymes. This suggests that it does not undergo biotransformation *in vivo*. Thus, ruling it out as a bioactive component of the extract since the bioactive components of the extract are present in their pro-drug forms that are converted to their bioactive forms *in vivo*.[19] This corroborates the report of Peeters et al., [19] that strictosamide does not contribute to *N. pobeguinii*'s antimalarial activity.

The β-carboline alkaloids reported to be responsible for *N. pobeguinii*'s antimalarial action[19] were shown to be Pgp-substrates; their boiled-egg shape suggests that they have high gastrointestinal absorption and can cross the blood-brain barrier (Figure 8; Table 3). Further, they were identified as substrates of various CYP enzymes, suggesting that they can be converted to bioactive forms *in vivo* if they exist as prodrugs. Following biotransformation, compounds are converted into forms that are easily excretable and, as such, may be rapidly cleared from the circulation.

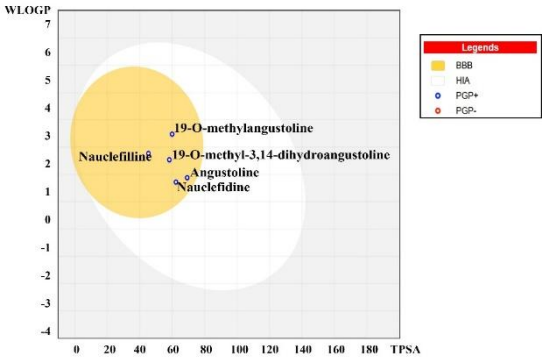


Figure 8: Boiled egg of some *N. pobeguinii*-derived β-carboline alkaloids.

Apart from our *in-silico* study predicting that these *N. pobeguinii*-derived β-carboline alkaloids are substrates of several CYP enzymes (Table 3), *N. latifolia*, a closely related species of *N. pobeguinii*, is reported to serve as a substrate for Cytochrome P450 3A4.[31] This may explain why, despite *N. pobeguinii* root extract reducing parasitaemia and inhibiting hemozoin formation on day 4, it showed no significant decrease in parasitaemia on day 10 and could not increase the survival of treated mice. This may be similar to the autoinduction observed with artemisinin, a key antimalarial drug that induces CYP3A4, reducing its own plasma concentration over time.[32] Recrudescence observed following artemisinin monotherapy is one of the reasons why the drug is partnered with other long-lasting antimalarial agents today~~,~~ as ~~in~~ artemisinin-based combination therapy.[33]

The result of our study thus suggests that~~,~~ while inhibition of hemozoin formation may contribute to malaria parasite killing by *N. pobeguinii*, its bioactive agents seem to be rapidly cleared from the circulation. Future studies focusing on improving its bioavailability through advanced formulations, e.g., by partnering with other plants such as *Cassia occidentalis* (Malaria®)[35,36] which is rich in CYP substrates,[35] may enhance its antimalarial activity and usefulness in combating malaria.

CONCLUSION

Our study has shown that, despite *N. pobeguinii* rootbark methanol extract being inactive *in vitro* (IC_{50} against *P. falciparum* > 100 µg/ml), the extract possesses potent antimalarial activity that is not sustained *in vivo*. A decreased hemozoin content within the DV of malaria parasites administered the extract suggests that the extract interferes with the parasite's heme-to-hemozoin biocrystallization process~~.~~.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS DECLARATION

The authors hereby declare that the works presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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