

## Original Research Article

# Isolation of a pentacyclic triterpenoid from the antiplasmodial bioactive fraction of *Nauclea latifolia* (Sm) roots

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### Abstract

**Purpose:** To research the antiplasmodial property of aqueous extract, fractions, and residue of *Nauclea latifolia* roots and to isolate the components responsible for the antiplasmodial activity.

**Methods:** Roots of *N. latifolia* were macerated with distilled water; the extract was obtained, successively partitioned with ethyl acetate and butanol. The extract, fractions, and the residue obtained were evaluated for their *in vivo* antiplasmodial activity and compared with amodiaquine and artesunate. The residue (which exhibited the best therapeutic index) was subjected to column and thin layer chromatography to isolate its components. Purification led to the isolation of betulinic acid, which was characterized with the aid of spectroscopic techniques (<sup>1</sup>H, <sup>13</sup>C NMR, and EI-MS).

**Results:** The residue significantly inhibited parasite growth from 42.8 % (D2) to 77.6 % (D5). Therefore, residue exhibited the highest therapeutic index against *Plasmodium berghei* in the three *in vivo* antiplasmodial (prophylactic, suppressive, and curative) models and it compared favourably with amodiaquine (80.5 %) and artesunate (85.9 %). The major component of the residue was betulinic acid.

**Conclusion:** The results validate the antiplasmodial claims of the roots of *N. latifolia* in folkloric medicine, and demonstrated that the isolate has a high therapeutic index in this regard. Further investigations, however, are required to determine the clinical efficacy and safety of the compound/isolate.

**Keywords:** *Nauclea latifolia*, Malaria, Betulinic acid, *Plasmodium berghei*, Parasite density, Growth inhibition

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## INTRODUCTION

*Nauclea latifolia* (Rubiaceae) is also known as the Pincushion tree. It is highly distributed in the rainforest zone of Africa [1]. The stem, bark, root,

leaf, and fruit are traditionally used by the local population as ethnomedicine for the treatment of pain, diabetes, infections, and malaria. Indole alkaloids with tetracyclic, or pentacyclic rings seem to be common in this family [2]. Moreover,

compounds such as naucleafine, naucleatine, nauclechine, and naufoline [3,4], nauclefolinine [5], strictosamide, naucleamides A, naucleamide F [6] have been isolated from the root bark of *N. latifolia*. Furthermore, five monoterpene indole alkaloids, naucleamides A to E were isolated from the bark of the plant whereas the major indole alkaloid with amino acetal bridge and five cyclic ring structures was naucleamide E [7]. In addition, betulinic acid, a naturally occurring pentacyclic lupane-type triterpenoid, has been isolated from other plants and reported to have antiplasmodial activity [8,9].

A study involving thirty-three plants frequently used by traditional medical practitioners for the management of malaria in West Africa reported high antiplasmodial potentials of *N. latifolia* [10]. Consequently, *in vitro* antiplasmodial evaluation against *P. falciparum* revealed that the root extracts of *N. latifolia* were more potent than the stem extracts [1]. Preliminary studies on the plant showed that the aqueous extracts of *N. latifolia* roots exhibited a better antiplasmodial activity against strains of *P. berghei* as compared with the ethanol extract [11].

This prompted further work to isolate compounds from the bioactive fraction of the aqueous extract of *N. latifolia* roots.

## EXPERIMENTAL

### Plant material

The *N. latifolia* roots were obtained from Ikot Andem Ididep, Ibiono Ibom L.G.A., Nigeria. Dr. Udo, a plant, a taxonomist in the Department of Pharmacognosy and Natural medicine, University of Uyo, Nigeria identified the roots whereas herbarium number UUH67G was documented. Thereafter, the roots were cut into smaller pieces, washed with running tap water, drained, dried, and pulverized. The aqueous extract was prepared by the maceration of the pulverized roots (5.2 kg) in boiled distilled water (5 L), kept for 72 h with occasional shaking, filtration was carried out using filter paper thereafter, the filtrate underwent concentration in a rotary evaporator at 40 °C. The repetition of the procedure yielded a more aqueous extract.

### Extraction and isolation

The weight and % yield of aqueous extract was 246.9 g and 4.75 % respectively. Successive partitioning of the extract (150 g) with ethyl acetate (28x250 ml) and butanol (42 x 250 ml) using a separating funnel (1000 ml) yielded ethyl acetate fraction (13.66 g), butanol fraction (52.83

g), and residue (83.51 g) after the evaporation of the solvent. The extract, fractions, and residue were screened for acute toxicity and antiplasmodial activity. The residue was the most active against *Plasmodium berghei* in the three *in vivo* antiplasmodial models. The residue (10 g) was subjected to column chromatography (CC) using silica gel and eluted with the appropriate solvent systems (n-hexane, dichloromethane, ethyl acetate, and methanol) and yielded 358 fractions. These fractions were subjected to different phases of TLC analysis (pooling and bulking processes) and yielded twenty-four (24) and six (6) fractions (A-G) based on similarity in  $R_f$  values respectively. Bulked fraction C (3428 mg) was further subjected to CC using silica gel and eluted using appropriate solvent systems to yield 47 sub-fractions. These sub-fractions were identified with TLC to give six bulked sub-fractions (C<sub>1</sub>-C<sub>6</sub>). The sub-fraction C<sub>2</sub> showed one spot on the TLC plate (using varying solvent systems) and was identified as betulinic acid (2205 mg). The sub-fraction C<sub>2</sub> showed a single spot on TLC (using varying solvent systems) and was identified as betulinic acid (2205 mg).

### General procedures

<sup>1</sup>H and <sup>13</sup>C NMR spectra were run on an Avance AV-400 MHz spectrometer, DMSO-*d*<sub>6</sub> enhanced the dissolution of sub-fraction C<sub>2</sub> whereas trimethyl silane was utilized as referential ( $\delta_H = \delta_C = 0$ ), with coupling constants in Hertz. EI-MS (positive mode) enhanced the quantification of mass spectrometry. Thin-layer chromatography was carried out by using Sigma-Aldrich's analytical precoated TLC silica gel 60 and visualized under both long (366 nm) and short (254 nm) wavelength UV light. Moreover, Sigma-Aldrich's silica gel Kiesel gel 60 (200–400 mesh, Merck) and Sephadex LH-20 enhanced column chromatography (CC) analysis.

### Phytochemical evaluation of *Nauclea latifolia*

Standard procedures as described by Trease and Evans [12] were used to evaluate the qualitative phytochemical analysis of the extract, fractions, and residue of *N. latifolia* roots.

### Animals

The Animal House in the Faculty of Pharmacy, University of Uyo, Nigeria provided one hundred and seventy-four (174) albino mice (13 – 27 g) used for the antiplasmodial experiment. The NIH protocols for the use, handling, and care of laboratory animals were judiciously followed [13]. The mice were housed at ambient temperature, they enjoyed access to water and feed (standard pellet diet) throughout the experiment. The

Animal Ethics Committee of the faculty approved the animal studies (approval no. UUFPHARM/0317) which followed international guidelines for animal studies.

### Parasites

National Institute of Medical Research (NIMR), Nigeria provided the chloroquine-sensitive *P. berghei* (NK-65) infected donor mice that were used for the study.

### Inoculum preparation

A stock of *Plasmodium berghei*-infected red blood cells with 20 % minimal peripheral parasitemia was obtained by cardiac puncture of the parasitized mice and emptied into a tube containing anticoagulant. Evaluation of the number of *Plasmodium berghei*-infected erythrocytes against that of leucocytes enhanced the calculation of the percentage parasitemia. Normal saline was used for serial dilution and the final inoculum (0.2 ml) contained about  $1 \times 10^7$  *Plasmodium berghei*-infected erythrocytes known as the standard inoculum for a mouse [14].

### Drugs

Artesunate tablets (50 mg) from (Mekophar Chemical Pharmaceutical Joint-stock Company, Ho Chi Minh City-Vietnam) and amodiaquine tablets (200 mg) from (Pfizer Afrique de l's Ouest Dakar R. P. Senegal) were dissolved in distilled water (100 ml) and given at dosages of 5 mg/kg and 30 mg/kg as positive controls in the antiplasmodial study.

### LD<sub>50</sub> determination of the plant extract

The LD<sub>50</sub> and effective dosages of the plant's root extracts were evaluated using Lorke's method [15]. The aqueous extract, fractions, and residue (50 – 2000 mg/kg) were given through the peritoneum then, signs of toxicity signs such as gasping decreased respiratory rate, palpitation, and death were observed within 42 h (loss of consciousness, asphyxia, and death) were observed within forty-two (42) h post-administration.

### Determination of *in vivo* antimalarial activity

The antiplasmodial potentials of the extract, fractions, and residue were determined by using curative, prophylactic, and suppressive models although with slight modifications of the experimental protocol as reported by Okokon *et al* [14].

### Slide staining and examination

Blood from each mouse was collected and used in the preparation of two smears (thin and thick); nevertheless, there were slight modifications to the experimental protocol as earlier reported by Owusu-Agyei *et al* [16]. The Parasite densities/ $\mu$ L of the blood were counted against 200 or 500 leucocytes.

### Statistical analysis

GraphPad Prism version 8.0 (GraphPad Software, Incorporated, San Diego, CA, USA) was used for data analysis, mean  $\pm$  SEM is the format of result presentation whereas One-way ANOVA statistically analyzed the significant differences within and between the groups. Afterward, Turkey's *post hoc* test enhanced multiple comparisons of means, and differences were adjudged statistically significant at a 95 % confidence level.

## RESULTS

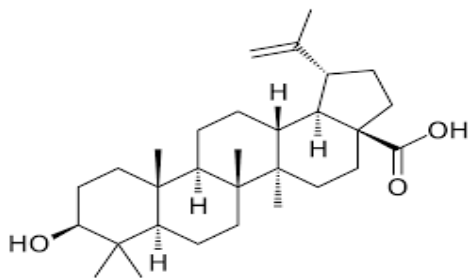
### Spectral characteristics

The spectral data of the isolated compound is presented as follows: the pure compound was observed as needle-like colorless substance; molecular weight and formula are given was 456 g/mol and C<sub>30</sub>H<sub>48</sub>O<sub>3</sub> respectively; based on EI-MS, base peak and molecular ion peak at m/z as 189.1 and 456.4 respectively.

**<sup>1</sup>H-NMR (DMSO, ppm):** The details of proton spectrum (DMSO,  $\delta$  ppm) are given as follows: the  $\delta$ H at 0.77 (s, 3-H, H-28), 0.88 (s, 3-H, H-29), 0.97 (s, 6-H, H-25 & 26), 1.02 (s, 3-H, H-30), 0.93 (s, 1-H, H-4), 1.06 (s, 1-H, H-12), 1.42 (m, 8-H, H-6, 8, 15 & 20), 1.58 (m, 4-H, H-7 & 14), 1.35 (m, 2-H, H-19), 1.71 (s, 3-H, H-24), 1.94 (m, 2H, H-16), 1.56 (m, 4-H, H1 & 13), 2.33 (m, 1H, H-21), 2.25 (m, 1H, H-18), 3.03 (m, 1H, H-2), 4.61 (s, 1-OH, H-27), 3.15 (dd, 1-H, H-23), and 3.03 (t, 1-H, H-23), representing different location and environment of proton present.

**<sup>13</sup>C-NMR (DMSO, ppm):** The detail <sup>13</sup>C-NMR (DMSO,  $\delta$  ppm) is as follows; 15.12 (C-30), 16.10 (C-28), 16.67 (C-7), 16.73 (C-29), 19.46 (C-14), 19.57 (C-24), 22.11 (C-25,26) 26.93 (C-13), 28.07 (C-1), 28.62 (C-15), 30.86 (C-20), 31.75 (C-16), 33.39 (C-8), 35.63 (C-19), 38.16 (C-6). 39.70 (C-9), 39.96 (C-11), 48.58 (C-21), 49.29 (C-18), 50.51 (C-10), 56.91 (C-4), 57.53 (C-17), 79.70 (C-2), 110.3 (C-23), 152.03 (C-22), and 180.20 (C-31). For final confirmations, HMBC, HSQC, NOESY, DEPT-90, DEPT-135 and COSY techniques were used and the

structure of compound (betulinic acid) is presented in Figure 1.



**Figure 1:** Chemical structure of betulinic acid

### The prophylactic activity of extracts, fractions, and residue

The repository antimalarial potentials of the extract, fractions, and residue of *Nauclea latifolia* roots were investigated and compared with amodiaquine, artesunate, and negative control groups for a test of significance at a 95% confidence level. Table 1 showed that the parasite densities in the entire group significantly reduced as compared with the negative control group. In ascending order of activity, *Plasmodium berghei*'s % growth inhibition was calculated as follows: butanol fraction (57.3 %) < residue (82.3 %) < artesunate (87.2 %) < ethyl acetate fraction (97.6 %) < amodiaquine (98.3 %) < aqueous extract (99.2 %). Moreover, the result of mean survival time (MST) revealed a significant elevation in all the groups except the butanol fraction that showed significant reduction as compared with the treatment group of amodiaquine.

### Suppressive activity of extracts, fractions, drugs, and residue

The investigation evaluated mean survival time, parasite densities, and % chemosuppressive potentials of the extract, fractions, and residue of *Nauclea latifolia* roots, artesunate, and amodiaquine. Also, Table 2 indicated a significant reduction in parasite densities at a 95 % confidence level in the entire group as compared with the negative control. In descending order of activity, the % growth inhibition of *P. berghei* in this study was calculated as follows: amodiaquine (99.1 %) > artesunate (98.9 %) > aqueous extract (98.1 %) > residue (91.4 %) > butanol fraction (70.3 %) > ethyl acetate fraction (52.4 %). Moreover, the significant elevation in MST at a 95 % confidence level was recorded in the entire group as compared with the negative control group.

### Schizonticidal activity of extracts, fractions, and residue

This study evaluated parasite densities, mean survival time, % growth inhibition of *Plasmodium berghei* to unravel the schizonticidal potentials of the infused extract, fractions, and residues of *Nauclea latifolia* roots at dosages (150, 300, and 450 mg/kg) and compared with the groups of negative control, amodiaquine, and artesunate. Table 3 indicated that there was a significant reduction in parasite densities at a 95 % confidence level in all the groups on day two (D<sub>2</sub>) and day five (D<sub>5</sub>) as compared with that in their respective negative control group. Also, significant elevation in MST at a 95 % confidence level was observed in all the treatment groups except ethyl acetate fraction as compared with the group of the negative control.

**Table 1:** Prophylactic activity of extracts, fractions, and residue

Treatment group	Mean weight (g)	Parasite density/ $\mu$ L	Mean survival time (days)
Negative control (distilled water)	17.5 $\pm$ 0.3	49960.0 $\pm$ 6491.6	14.5 $\pm$ 0.3
Amodiaquine (30 mg/kg)	19.8 $\pm$ 0.8	782.0 $\pm$ 17.7 <sup>a*</sup> (98.3%)	24.5 $\pm$ 1.1 <sup>a**</sup>
Artesunate (5 mg/kg)	18.5 $\pm$ 1.3	6434.9 $\pm$ 1167.5 <sup>a*</sup> (87.2%)	19.8 $\pm$ 1.3 <sup>a**</sup>
Aqueous extract (150 mg/kg)	19.3 $\pm$ 1.7	438.4 $\pm$ 33.5 <sup>a*</sup> (92.2%)	25.1 $\pm$ 0.5 <sup>a**</sup>
Ethyl acetate fraction (150 mg/kg)	19.3 $\pm$ 1.1	1276.3 $\pm$ 59.1 <sup>a*</sup> (97.6%)	22.4 $\pm$ 1.7 <sup>a**</sup>
Butanol fraction (150 mg/kg)	19.0 $\pm$ 1.3	21398.0 $\pm$ 86.7 <sup>a*,b**,c**</sup> (57.3%)	16.7 $\pm$ 0.6 <sup>b*</sup>
Residue (150 mg/kg)	18.3 $\pm$ 0.5	8781.0 $\pm$ 406.2 <sup>a*</sup> (82.3%)	19.4 $\pm$ 1.6 <sup>a**</sup>

a\* implies significant reduction at 95 % confidence level, the comparison was done with negative control whereas n = 6. b\*\* implies significant elevation at 95 % confidence level, the comparison was done with amodiaquine whereas n = 6. c\*\* implies significant elevation at 95 % confidence level, the comparison was done with artesunate whereas n = 6

**Table 2:** Suppressive activity of extract, fractions, and residue

Treatment groups	Mean weight (g)	Parasite densities/ $\mu$ L	Mean survival time (days)
Negative control (distilled water)	15.5 $\pm$ 0.7	47584.1 $\pm$ 12361.0	12.2 $\pm$ 1.5
Amodiaquine (30 mg/kg)	18.3 $\pm$ 0.9	390.7 $\pm$ 93.4 <sup>a*</sup> (99.1%)	25.7 $\pm$ 1.8 <sup>a**</sup>
Artesunate (5 mg/kg)	16.3 $\pm$ 0.8	560.1 $\pm$ 105.6 <sup>a*</sup> (98.9%)	24.4 $\pm$ 0.7 <sup>a**</sup>
Aqueous extract (150 mg/kg)	18.0 $\pm$ 1.5	978.1 $\pm$ 239.7 <sup>a*</sup> (98.1%)	23.8 $\pm$ 1.8 <sup>a**</sup>
Ethyl acetate fraction (150 mg/kg)	16.8 $\pm$ 1.3	22681.2 $\pm$ 3647.1 <sup>a* b**, c**</sup> (52.4%)	15.2 $\pm$ 0.4 <sup>b*, c*</sup>
Butanol fraction (150 mg/kg)	18.3 $\pm$ 0.8	14108.1 $\pm$ 4198.2 <sup>a*</sup> (70.3%)	17.1 $\pm$ 1.2
Residue (150 mg/kg)	13.8 $\pm$ 4.1	4119.1 $\pm$ 383.2 <sup>a*</sup> (91.4%)	21.1 $\pm$ 2.1 <sup>a**</sup>

a\* implies significant reduction at 95 % confidence level, the comparison was done with negative control whereas n = 6. b\*\* implies significant elevation at 95 % confidence level, the comparison was done with amodiaquine whereas n = 6. b\* implies significant reduction at 95 % confidence level, the comparison was done with amodiaquine whereas n = 6. c\* implies significant reduction at 95 % confidence level, the comparison was done with artesunate whereas n = 6. c\*\* implies significant elevation at 95 % confidence level, the comparison was done with artesunate whereas n = 6

**Table 3:** Schizonticidal activity of extracts, fractions, and residue

Treatment group	Parasite density/ $\mu$ L on D <sub>0</sub> & % growth inhibition	Parasite density/ $\mu$ L on D <sub>2</sub> & % growth inhibition	Parasite density/ $\mu$ L on D <sub>5</sub> & % growth inhibition	Mean survival time (days)
Negative control (distilled water)	134021.0 $\pm$ 15652.0	177052.0 $\pm$ 28312.0 (-32.2%)	222876.1 $\pm$ 30656.1 (-66.4%) <sup>*</sup>	9.6 $\pm$ 0.24
Amodiaquine (30 mg/kg)	130995.1 $\pm$ 16454.1	62746.0 $\pm$ 6998.0 <sup>a*</sup> (52.2%) <sup>**</sup>	25516.0 $\pm$ 2292.0 <sup>a*</sup> (80.8%) <sup>**</sup>	22.6 $\pm$ 0.23 <sup>a**</sup>
Artesunate (5 mg/kg)	169896.1 $\pm$ 12742.1	90092.0 $\pm$ 3098.0 <sup>a*</sup> (47.1%)	24004.1 $\pm$ 3745.1 <sup>a*</sup> (85.7%) <sup>**</sup>	24.2 $\pm$ 1.33 <sup>a**</sup>
Aqueous extract (150 mg/kg)	204132.1 $\pm$ 10805.1 (3.4%)	99256.0 $\pm$ 2273.0 <sup>a*</sup> (51.4%) <sup>**</sup>	57679.1 $\pm$ 494.6 <sup>a*</sup> (71.7%) <sup>**</sup>	19.66 $\pm$ 0.57 <sup>a**</sup>
Aqueous extract (300 mg/kg)	241429.1 $\pm$ 40596	88112.0 $\pm$ 14842.0 <sup>a*</sup> (63.4%) <sup>**</sup>	54017.2 $\pm$ 17178.1 <sup>a*</sup> (77.8%) <sup>**</sup>	22.2 $\pm$ 0.43 <sup>a**</sup>
Aqueous extract (450 mg/kg)	236963.1 $\pm$ 51136.1	118903.0 $\pm$ 13298.0 (49.9%)	60522.0 $\pm$ 14058.1 <sup>a*</sup> (74.6%) <sup>**</sup>	20.4 $\pm$ 0.36 <sup>a**</sup>
Ethyl acetate fraction (300 mg/kg)	168675.1 $\pm$ 18613.1	145654.0 $\pm$ 24429.0 <sup>b</sup> (13.7%) <sup>**</sup>	89443.2 $\pm$ 9388.1 <sup>a*</sup> (47.2%)	15.3 $\pm$ 0.36 <sup>b*, c*</sup>
Butanol fraction (300 mg/kg)	171646.1 $\pm$ 8238.6	120106.0 $\pm$ 21748.0 (30.3%)	61678.1 $\pm$ 11616.1 <sup>a*</sup> (64.2%) <sup>**</sup>	17.1 $\pm$ 0.42 <sup>a**, c*</sup>
Residue (300 mg/kg)	206217.1 $\pm$ 43858.1	117922.0 $\pm$ 16843.0 (42.9%)	46138.1 $\pm$ 5267.0 <sup>a*</sup> (77.8%) <sup>**</sup>	22.2 $\pm$ 0.46 <sup>a**</sup>

\*\* implies elevation at 95 % confidence level, the comparison was done with baseline parasite density on day 1 (D<sub>0</sub>). a\* implies significant reduction at 95 % confidence level, the comparison was done with negative control whereas n = 6. a\*\* implies significant elevation at 95 % confidence level, the comparison was done with negative control whereas n = 6. b\*\* implies significant elevation at 95 % confidence level, the comparison was done with amodiaquine whereas n = 6. b\* implies significant reduction at 95 % confidence level, the comparison was done with amodiaquine whereas n = 6. c\* implies significant reduction at 95 % confidence level, the comparison was done with artesunate whereas n = 6

**Table 4:** Phytochemical screening of the extract, fractions, and residues of *Nauclea latifolia* roots

S/no.	Test	Infused aqueous extract	Ethyl acetate fraction	Butanol fraction	Residue
1.	Alkaloids				
	Dragendorff's reagent	+++	+	++	+++
	Hagger's reagent	+++	+	++	+++
2.	Saponins				
	Froth test	+++	++	+++	++
	Emulsion test	+++	++	++	++
	Fehling's test	+++	++	++	++
3.	Tannins				
	Ferric chloride	+	+++	+++	+
4.	Flavonoids				
	Magnesium metal	++	++	++	++
	Ethyl acetate	++	++	++	++
5.	Glycoside				
	Hydrolysis	++	+	++	++
6.	Cardiac glycoside				
	Salkowski test	+++	+++	++	++
7.	Terpenes & steroids				
	Lieberman-Burchard	++	+	+	++
8.	Deoxysugar				
	Keller-Killiani test	++	+	+++	++
9.	Anthraquinone	-	-	-	-
10.	Carbohydrate	++	++	+++	+++

+++ implies high relative abundance, ++ stands for moderate relative abundance, + connotes low relative abundance, and - implies absence

The qualitative phytochemical evaluation of extract, fractions, and residue of plant roots is presented below.

## DISCUSSION

Some secondary metabolites like alkaloids, terpenoids have been reported to have antiplasmodial activity [17] therefore, the abundance of alkaloids, saponins, carbohydrates, terpenes, cardiac glycosides, and flavonoids in the extracts, fractions, and residues of *N. latifolia* roots corroborated the assertion by these researchers hence, its traditional usage in the treatment of malaria in Nigeria.

Also worthy of note is the median LD<sub>50</sub> (1500.19 mg/kg body weight of mice) of the plant extract deduced as the geometrical mean of the highest dosage that produced zero % death in mice. The value revealed medial lethality of the plant extract with the possibility of inducing minimal multi-organ toxicity when consumed as a concoction or as an herbal formulation.

Moreover, the significant reduction in the parasite density in the entire groups as compared with the group of negative control revealed the varying degrees of repository activity of the plant's extract, fractions and residue hence, the consumption of the root *N. latifolia* infusion may avert malaria incidence as well as its symptoms through the destruction of the pre-erythrocytic stage of the parasite. Furthermore, percentage growth inhibition of parasites is one of the indices when evaluating drug efficacy in anti-plasmodial studies so, the higher the value the better the therapeutic index of the drug. The result of the repository activity of the plant's extract, fractions, and the residue was in tandem with the reports by Boampong *et al* [17] on the prophylactic efficacy of the extract of *Haematostaphis barteri* stem bark and 49.58% for ethanol extract of *N. latifolia* stem bark at the dosages of 300 mg/kg [18,19]. The corroboration of parasite density reduction, increased percentage growth inhibition of parasite with increased mean survival time of the mice suggests that the root *N. latifolia* has good repository activity.

The study further showed that the plant roots had good chemosuppressive potentials due to their

improved parasite density reduction, increased mean survival time, and percentage growth inhibition of *Plasmodium berghei*. Whereas some researchers reported the inhibition of protein synthesis in parasites [20, 21] and the possibility of alkaloids blocking protein synthesis in *P. falciparum* [22] as the possible mechanism of actions of some plant extracts. So, the plant roots may have acted through a similar mechanism of action due to the abundance of alkaloids in them. In addition, the study showed higher percentage growth inhibition of the parasite when compared with 55.61% of aqueous extract of *Parkia biglobosa* administered at a dosage of 600 mg/kg [23] as well as 82.18% and 61.87% for *Clerodendrum myricoides* leaves and roots respectively [24]. Interestingly, the chemosuppressive activity of the residue group compared favorably with the artesunate and amodiaquine drugs, due to the presence of betulinic acid, a compound already reported to have anti-plasmodial activity. Therefore, the similarity in therapeutic index of this plant's roots with the conventional drugs showed that the plant has good chemosuppressive potentials against *P. berghei* [18], it might use a similar mechanism of action as artesunate and amodiaquine drugs as well as being a candidate in the quest of profiling and designing drugs to avert high mortality rate from malaria, aggravated by the prevalence of drug-resistant strains of *Plasmodium falciparum*.

Since anti-plasmodial microscopic investigation targets the schizont stage of the parasite as the stage that causes clinical symptoms in patients; so, its evaluation gives clue on the therapeutic efficacy of a drug. Therefore, the schizonticidal activity of the extracts, fractions, and residue investigated in this study showed that the significant reduction in their parasite densities as compared with the negative control group was consistent with similar studies like 75.4% for the aqueous extract of *N. latifolia* stem bark [25] and 81-91% for the extract of *N. pobeguini* [26]. Hence, the validated schizonticidal activity of these plant roots may arise due to betulinic acid alone or its interactions with other bioactive compounds by directly inducing cytotoxicity on *P. berghei*. In addition, the spectral data from <sup>13</sup>C-NMR (DEPT-135, BB, and DEPT-90) revealed the compound has 30 carbons atoms constituting six methines, six methyls, seven quaternary carbon, and eleven methylenes atoms. Also detected were five rings, one double bond, and one acidic carbonyl in the molecule. <sup>1</sup>H-NMR revealed six singlet methyls, a carbinolic proton, a pair of olefinic protons with an exocyclic methylene group which was characteristic for lupane triterpenes [27]. Furthermore, the

fragmentation pattern from EI-MS was typical of lupane triterpenes through the presence of m/z 438 [M-H<sub>2</sub>O], 411 [M-COOH], 248 [C<sub>16</sub>H<sub>24</sub>O<sub>2</sub>], 220 [C<sub>14</sub>H<sub>20</sub>O<sub>2</sub>], 220 [C<sub>15</sub>H<sub>24</sub>O], 220 [C<sub>14</sub>H<sub>20</sub>O<sub>2</sub>], 207 [C<sub>14</sub>H<sub>23</sub>O], 205 and 207 [M - C<sub>16</sub>H<sub>27</sub>], 203 [248 - COOH], 203 [220 - OH], 189 [207 - H<sub>2</sub>O], 175 [220 - COOH]. Based on the foregoing spectral data, the compound was similar to those earlier reported for betulinic acid [27].

## CONCLUSION

The plant root extract of *N. latifolia* and its fractions have been bioactively screened via *in vivo* anti-plasmodial studies while the residue with the best therapeutic indices have been subjected to column chromatography, and the eluent NLA1 characterized with the aid of spectroscopic techniques. The isolation of betulinic acid from the *Nauclea* genus is reported here for the first time although the pentacyclic triterpenoid had previously been reported to possess anti-plasmodial activities in other plants. Hence, the high therapeutic indices observed in this study may solely be due to betulinic acid or its synergistic interaction with other compounds. Further investigations, however, are required to determine the clinical efficacy and safety of the compound/isolate.

## DECLARATIONS

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### Conflict of interest

No conflict of interest is associated with this work.

### Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities about claims relating to the content of this article will be borne by the authors. Asanga, Edet Effiong conceived, designed the study; collected and analyzed the data as well as wrote the manuscript. Igile, Godwin O., Ebong, Patrick E., and Eseyin, Olorunfemi A. read and approved the manuscript for publication. Emmanuel E. Essien and Thomas Paul Sunday collected and analyzed the data.

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