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Research Article

The Antiplasmodial Selectivity Index of the Alkaloid fractions of *Phyllanthus Amarus, Nuclea Latifolia* and *Polyalthia Longifolia*

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ABSTRACT

About 60-80% world population rely on plant-based medicines which are used in traditional health care systems. It is now known that, the medicinal values of these plants lies in their bioactive phytochemical constituents which produce definite physiological effects on human body. These natural compounds formed the base of modern drugs as used today. Therefore, this study seeks to evaluate the *in vitro* antiplasmodial activity of the phytochemicals in three herbal plants used to treat malaria and associated fever in Nigerian medicine. The three herbal plants (*Phyllantus amarus, Nuclea latifolia* and *Polyalthia longifolia*) were authenticated and their leaves were used to prepare the phytochemical extracts (alkaloid, tannin, flavonoid, saponin, glycosides and anthraquinone). Then, *In vitro* antiplasmodial activity of the extracts was assessed using 3D7 chloroquine sensitive strain of *Plasmodium falciparum* maintained at 5% hematocrit (human type O-positive red blood cells) in complete RPMI 1640 medium, while cytotoxicity was determined by the micro-assay technique using L929 animal cell fibroblasts and the lactate dehydrogenase method. Results indicate that the alkaloid fractions of *P. amarus* and *N. lafifolia* **h**ad the highest same activity (IC_{50}:3µg/ml, CC_{50}: 45µg/ml and SI: 15), and *P. longifolia* next (SI=13), while, the glycoside fraction of all three plants and the anthraquinone fraction of *P longifolia* and *N. latifolia* had least activity (SI=1 in all) when compared with the other phytochemical extracts from the three plants. All the phytochemical extracts showed no toxicity against the animal cell fibroblasts with CC_{50} values ranging from 20µg/ml to 63µg/ml.

Keywords: *Malaria, Plasmodium falciparum, Alkaloid, Chloroquine, Selectivity Index, Anthraquinone, Phytochemicals*

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INTRODUCTION

Malaria remains one of the most widespread human infections around the world. Above 40% of the world's population live in malaria-endemic areas (Suh *et al.,* 2004). Malaria in humans is caused by five species of *Plasmodium;* Plasmodium *falciparum, Plasmodium ovale, Plasmodium malariae, Plasmodium vivax and Plasmodium Knowleisi*. In Nigeria, malaria is mostly caused by *P. falciparum (*Odugbemi *et al.,* 2007). Emergence of *P. falciparum* resistance to the most commonly available antimalarial drugs hinders effective control of the disease, making malaria a leading cause of mortality in about 100 countries, half of which are sub-Saharan African countries (Muhammad *et al.,* 2017). Although, there are promising reports of declining malaria morbidity, still there were global estimate of 229 million cases of malaria and 409 thousand deaths in 2019 (WHO, 2020) with Nigeria having both the highest cases (27% or) and death (23% or).

At the moment, the COVID-19 pandemic and associated restrictions has caused disruptions in important malaria services in many countries, including Nigeria, and modelling analysis shoes that reduction in access to effective antimalarial treatment by 10%, 15%, 25% and 50% in sub-Saharan Africa (where Nigeria is located) in 2020 may cause additional 19,000, 28,000, 40,000 and 100,000 malaria deaths, respectively, in spite the completion of all prevention campaigns (WHO, 2020). It seems that COVID-19 may worsen the fight against malaria, whose mainstay of treatment is chemotherapy (Zofuo *et al.,* 2011). So, treatment by available and easily accessible local herbs could be encouraged in the phase of the COVID-19 pandemic. Therefore, research on local medicinal plants has become imperative and pivotal.

Medicinal plants are the "backbone" of traditional medicine, which means that more than 3.3 billion people in less developed countries utilize medicinal plants on a regular basis (Mansi and Vaghela, 2019). World health Organization (WHO, 2001) estimates that about 80% of these people rely almost exclusively on traditional medicine for their primary healthcare needs.

Medicinal plants are not only used for the treatment of diseases, but also, as a potential material for maintaining good health and conditions and the reasons for this is because of their perceived better compatibility and adaptability with the

human body which also, pose lesser side effects (Mansi and Vaghela, 2019). It is now known that the medicinal values of plants lie in their bioactive phytochemical constituents which produce definite physiological effects on the human body (Katiyar *et al*., 2015). These natural compounds formed the base of most modern drugs in use today.

The important bioactive compounds responsible for attributing the medicinal value to a plant are: alkaloids, flavonoids, tannins and phenolic compounds (Kukreja *et al*., 2015). Plant tannins and flavonoids have been reported to exhibit antibacterial properties (Kukreja *et al*., 2015), while phenolic compounds are known to alter microbial cellular permeability resulting in loss of macromolecules. They, also, interact with membrane proteins causing structural changes (Tana *et al*., 2015). In addition, risk of cancer can be significantly reduced by the antioxidant and anti-inflammatory activities of polyphenols (Kukreja *et al*., 2015). Alkaloids have been shown to possess significant *in vitro* antiplasmodial activity (Uzuegbu *et al.,* 2020). The biological action of plant alkaloids as antiplasmodial agent may be due to their inhibition of protein synthesis and haem degradation in *Plasmodium* species (Bapna *et al.,* 2014). The screening of plant extracts has been of great interest to scientists in the search for new drugs for greater effective treatment of several diseases. Therefore, plant extracts and phytochemicals with known antiplasmodial action can be of great significance in screening and development of more effective drugs for therapeutic treatment of malaria.

Therefore, in this present study, the *in vitro* antiplasmodial activities of several phytochemicals from three medicinal plants (*P. amarus, N. latifolia* and *P. longifolia*) used to treat malaria and associated fever in the Nigerian traditional medicine practice were undertaken.

MATERIALS AND METHODS

Collection of plant materials: Mature whole plants of *Phyllantus amarus, Nuclea latifolia* and *Polyalthia longifolia* were collected from their natural habitat in Abraka community located in Ethiope East Local Government Area of Delta State, Nigeria. The plants were collected carefully to avoid contamination with other plants and strange materials. The leaves of the plant were plucked, washed and air-dried for two weeks at laboratory room temperature (28-32ºC). The dried leaves were then powdered using laboratory blender (Kenwood, Japan).

Preparation of alkaloid extracts: About 200g of the dried powder was soaked in 100mL of ammonia for 1h and filtered with 500mL of dichloromethane for 24h for each plant. The filtrate was percolated and the dichloromethane extraction solvent was evaporated under vacuum. The residue was then dissolved in water/hydrochloric acid mixture at pH 2.5, and allowed to seep. The collected solution was adjusted to pH 8 with ammonia and washed (6 x 150 mL) with dichloromethane. Then, the dichloromethane was evaporated and the resulting residue was concentrated to dryness under reduced pressure to yield the solid alkaloid extract which was confirmed using Dragendorff reagent to give purple colour (Bapna *et al.,* 2014).

Preparation of tannin extracts: The extraction of tannins was done using the cold extraction process already described by Ukoha *et al*. (2011), although, with some modifications. About 100g of the dried, powdered leaves was extracted using subsequent mixture of 90% methanol and water (1:10v/v) at 80ºC for 2h to obtain crude extract. The resultant mixture was filtered and the residue was re-extracted. Then, the methanol and water were removed using a rotary evaporator to obtain the solid tannins.

Preparation of flavonoid extract: The flavonoid extract was prepared according to the procedure stated by Cia *et al*. (2010). The methanol extract was poured onto a column (400 × 2.5 cm i.d.) packed with pre-treated AB-8 resin. After complete absorption of the solution, the column was washed with enough distilled water to remove carbohydrates, and further washed with 65% ethanol to elute flavonoids. The eluate, abundant in flavonoids, was collected and then, concentrated at 40°C with a laborata 4000 rotary evaporator (Heiddph, Schwabach, Germany) until the formation
of sediment. Extracts were collected and vacuum-dried at 40°C to obtain flavonoid extracts.

Preparation of saponin extract: The filtered crude methanol extract was defatted with n-hexane and extracted four times with n-butanol saturated with water. The dried n-butanol extract was dissolved in methanol and dropped into excess EtOAc to give the saponin fraction (Klujanabhagavad and Wmk, 2009)

Preparation of anthraquinone extract: Filtered crude methanol extracts of the plants dried leaves were refluxed for 15min with 30ml of water to get the aqueous mixture. The aqueous mixture was allowed to cool and then, adjusted with water to its original weight. The supernatant was collected and refluxed with 20ml of 10% $FeCl_3 6H_2O$ for 20min. The mixture was refluxed again with 1ml of concentrated HCl for 20 minutes. The mixture was then extracted with ether and washed with water. The ether layer was evaporated to dryness and the remaining residue was dissolved with 10ml of 0.5% magnesium acetate in methanol to produce the anthraquinone extract (Bruneton, 1995).

Preparation of glycoside extracts: Glycoside extracts were obtained from the three plants as described by Sharma *et al*. (2014), but with some modifications. About 100g of the powdered plants' leaves were extracted with 80% methanol with stirring at room temperature and then, filtered to obtain the crude extracts. The filtrate was then distilled in methanol at 60ºC using vacuum. The remaining aqueous extract was then extracted with n-hexane. The n-hexane fraction was thereafter distilled out under vacuum and remaining aqueous phase rich in mixture of glycosides was exchanged with *n*-butanol for three (3) times. The remaining *n*-butanol phase was distilled out under vacuum at 80ºC. A brownish mass was obtained which was rich in glycosides. The glycoside-rich brownish mixture was further subjected to column chromatography and eluted with $CHCl_3$:CH_3OH:H_2O [65:40:12], and further. with methanol to obtain the glycoside fraction.

Percentage yield of extracts was calculated using the formula:

$$\text{Yield\%} = \frac{\text{wt. of dry extract}}{\text{wt of dry plant}} \times 100$$

Dilutions were prepared from each phytochemical extract as required for the study.

Determination of the *in vitro* antiplasmodial activity of extracts: *Plasmodium falciparum* culture was maintained according to the method described by Trager and Jensen (1976). Chloroquine sensitive *Plasmodium falciparum* 3D7 were maintained at 5% hematocrit (human type O-positive red blood cells) in complete RPMI 1640 (supplemented with 10% Albumax II, 50 µg mL^{-1} gentamycin, 1% L-glutamine) medium and kept in a 37°C incubator. *In vitro* antiplasmodial activity was determined by parasite growth inhibition assay as described by Uzuegbu *et al*. (2020), with some modifications. The *in vitro* antiplasmodial assay was performed in duplicates. The concentrations of standard drug (artesunate 98% purity) were prepared by dissolving in DMSO

Concentrations of extracts and artesunate used, were between the ranges 5.080.0µg/ml. then, 96-well microtiter plates already containing multiple concentrations of the extracts were used to incubate the culture (parasitaemia 1.5% and 3% hematocrit) for 48h at 37ºC in CO_2 condition. Positive control wells were also prepared by incubating culture with different concentrations of artesunate under the same conditions. Wells incubated, under the same conditions, without extracts or standard drug served as negative control. After incubation, the upper part of the suspension was removed and transferred to a clean microscopic slide to form a series of thick blood smears. The films were stained with 10% Giemsa stain (pH 7.3). The smear was then viewed under the microscope at 100× magnification. Parasites were counted in 10 microscopic fields and the mean calculated. The percentage parasite suppression was calculated using the formula of WHO (2001) and Ngemenya *et al.* (2006), as stated below:

$$\frac{\text{\% Parasitemia in negative control wells} - \text{\% Parasitemia in test wells}}{\text{\% Parasitemia in negative control wells}} \times 100$$

Parasite growth inhibition curves were plotted to determine parasite growth suppression at 50% (IC_{50}) i.e. parasite suppression versus log concentration graphs.

***In vitro* Cytotoxicity Test:** Cytotoxicity of plant extracts was assessed against animal cell fibroblast L929 cultured in RPMI-1640 medium supplemented with 10% foetal bovine serum, 0.21% sodium biocarbonate and 5mg/ml gentamicin at 37ºC. Assay was performed in 96-well microplates using lactate dehydrogenase assay as described by Uzuegbu *et al*., (2020). Concentration ranges of extracts tested were between 200-6.25 µg/ml and all cultures were performed in duplicates. In each well, about 100µg/ml of cell suspension were seeded in complete medium. Fibroblasts were maintained in medium for 24h under 5% CO_2 atmosphere at 37ºC. About 100µL of extracts was added at concentrations ranging from 2006.5µg/ml and was incubated for 48h under a humidified atmosphere at 37ºC and 5% CO_2. Untreated cultures and cultures in medium with 1% Triton X-100 were included as negative and positive controls, respectively. After incubation, 20µL of working solution of LDH was added to each well and was incubated for 4h at room temperature. The supernatant was then removed from each well and 100µL of DMSO was added to each well to stop the reaction. The amount of formazan formed was measured by scanning with a spectrophotometer (Spec 20D, Techmel and Techmel, USA) at 570nm. The percentage of inhibition was calculated using the assay manual formula:

$$\text{Cytotoxicity}(\%) = \frac{[\text{LDH}]_{\text{test sample}} - [\text{LDH}]_{\text{negative control}}}{[\text{LDH}]_{\text{positive control}} - [\text{LDH}]_{\text{negative control}}} \times 100$$

The 50% cellular cytotoxic concentration (CC_{50}) of the test extracts was calculated by linear interpolation from the curves of cytotoxicity against the log of concentration.
The selectivity index (SI) was calculated as the ratio of the CC_{50} to the IC_{50}.

Statistical Analysis: : The results were expressed as Mean ± Standard Deviation. Comparisons of the different inhibition rates on *P. falciparum* growth and extracts cytotoxicity were analyzed by ANOVA using SSPS software (version 23). Results were regarded as significant at $p \leq 0.05$.

Table 1:
Percentage yields, IC_{50}s, CC_{50}s and SI values of the phytochemical extracts of the three plants studied

		Alkaloid	Tannin	Saponins	Glycosides	Anthraquinone	Flavonoid	Whole extract	Artesunate
Phyllantus amarus	Yield (%)	5.3	4.2	3.2	0.8	2.4	0.9	18.9	
	IC_{50} (µg/ml)	3	10	13	22	14	6	2	1
	CC_{50}(µg/ml)	45	56	63	25	40	32	24	25
	SI	15	6	5	1	3	5	12	25
Polyalthia longifolia	Yield (%)	4.7	3.8	2.6	0.5	2.1	1.2	19.3	
	IC_{50} (µg/ml)	3	14	24	24	19	7	5	1
	CC_{50}(µg/ml)	38	40	46	20	21	40	20	25
	SI	13	3	2	1	1	6	4	25
Nuclea latifolia	Yield (%)	5.1	4.0	3.1	0.9	2.6	1.3	19.8	
	IC_{50} (µg/ml)	3	15	30	30	20	12	21	1
	CC_{50}(µg/ml)	45	48	52	31	27	40	3	25
	SI	15	3	2	1	1	3		25

IC_{50}=Extract concentration at 50% parasite suppression (potency); CC_{50}= Extract concentration at 50% cytotoxicity
SI= Selectivity index (ratio of CC50 to IC50)

RESULTS

The percentage yields of the phytochemicals (alkaloid, tannin, saponin, glycosidic, anthraquinone, and flavonoid) from the evaluated in this study, percentage yields are listed in Table 1. The *in vitro* antiplasmodial properties of all the phytochemical extracts, using chloroquine sensitive strain of *P. falciparum* (3D7) expressed as IC_{50} and CC_{50} values in µg/ml and selective indexes are also presented in Table 1.

Then, Figures 1a, 2a and 3a show the combined plots of the mean percentage suppression of parasites by the phytochemical extracts of *P. amarus*, (Fig. 1a) *N. latifolia* (Fig, 2a) and *P. longifolia* (Fig. 3a), respectively, at various concentrations (80, 40, 20, 10 and 5µg/ml).

Figures 1b, 2b and 3b show the combined plots of percentage suppression versus log concentrations of the seven fractions of the three plants and these lines were used to determine IC_{50} values shown in Table 1.

The cytotoxic activity of the seven extracts of the three plants were estimated against L292 animal cell fibroblasts at varying concentrations (6.25, 12.5, 25, 50, 100, 200 µg/ml). Figures 4b, 5b and 6b indicate the percentage cytotoxicity vs log concentration curves which were used to estimate the CC_{50} the concentration at 50% cytotoxicity.

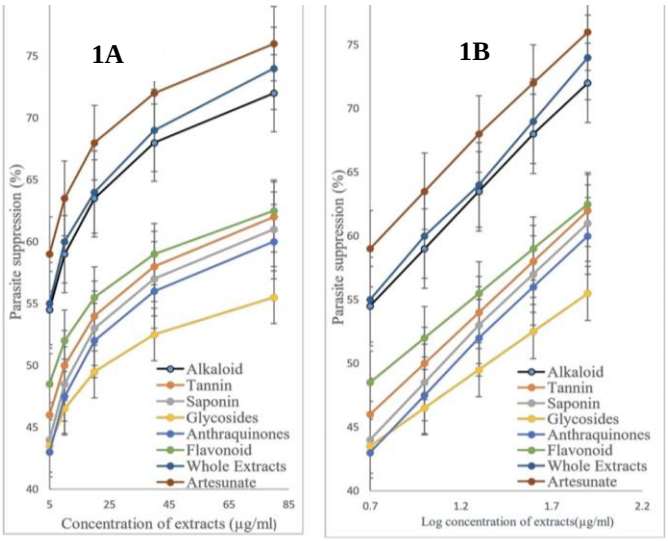


Figure 1
A. Comparative malarial parasite suppressive activities of *Phyllantus amarus* phytochemical extracts and reference drug.
B. Log concentration based comparative malarial parasite suppressive activities of *Phyllantus amarus* phytochemical extracts and reference drug.

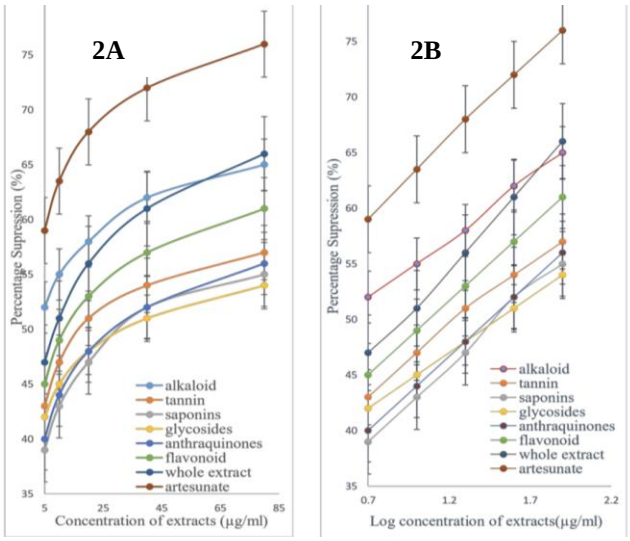


Figure 2
A. comparative malarial parasite suppressive activities of *Nuclea latifolia* phytochemical extracts and reference drug.
B. Log concentration based comparative malarial parasite suppressive activities of *Nuclea latifolia* phytochemical extracts and reference drug.

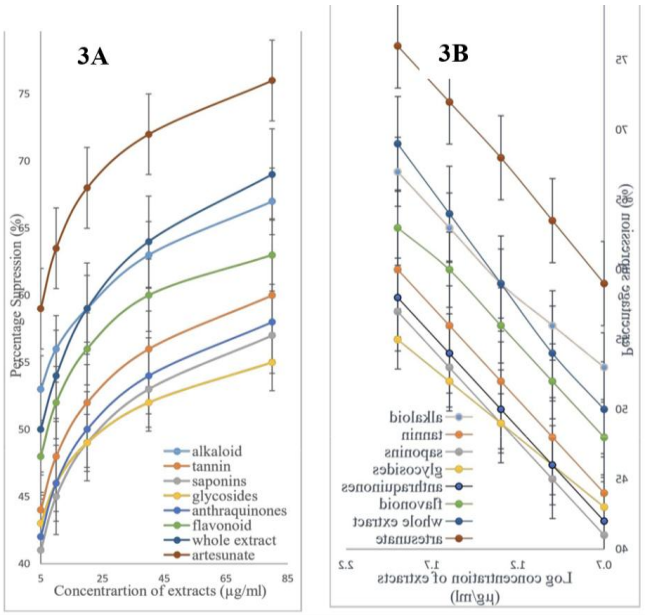


Figure 3
A. Concentration dependent comparative malarial Parasite suppressive activities of *Polyalthia longifolia* phytochemical extracts and reference drug.
B. Log concentration based comparative malarial parasite suppressive activities of Polyalthia longifolia phytochemical extracts and reference drug.

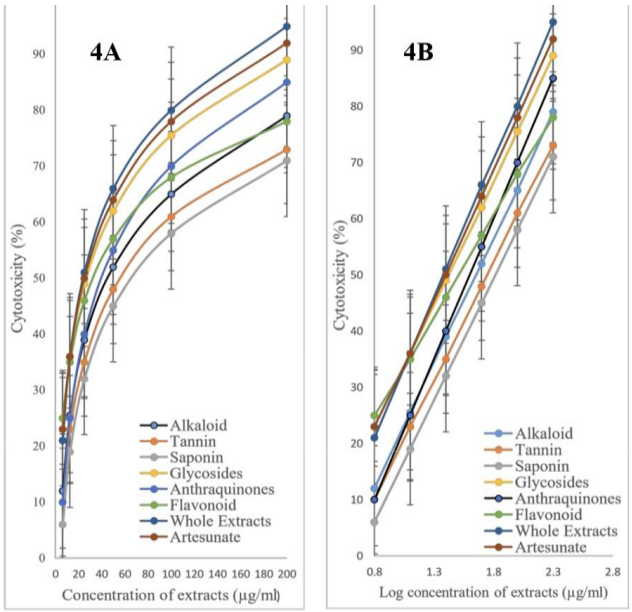
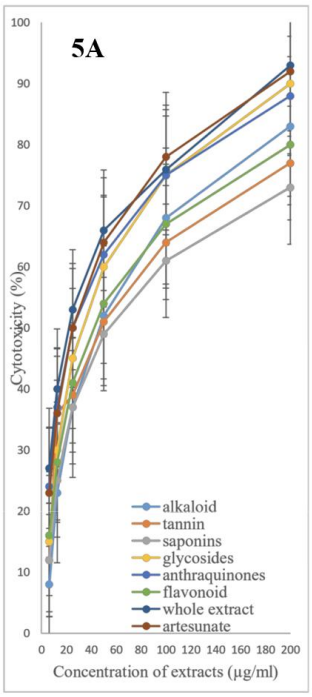


Figure 4
A. Concentration dependent comparative cytotoxic activity of Phyllantus amarus extracts and reference drug.
B. Log concentration based comparative cytotoxic activity of Phyllanthus amarus phytochemical extracts and reference drug.



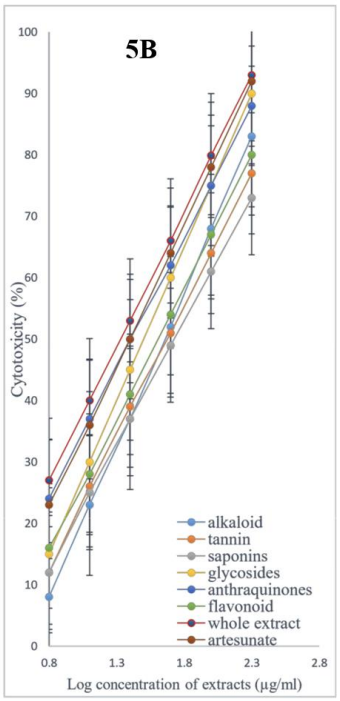
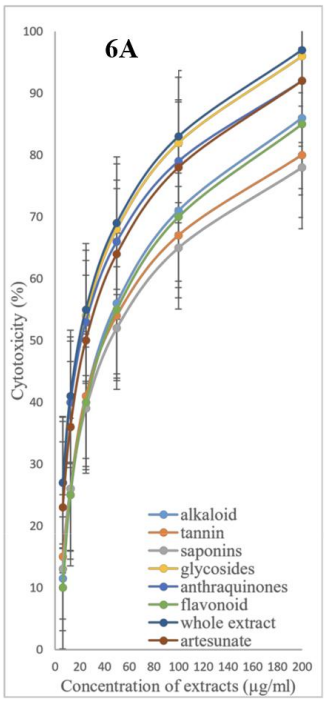


Figure 3
A. Concentration dependent comparative malarial Parasite suppressive activities of *Nuclea latifolia* phytochemical extracts and reference drug.
B. Log concentration based comparative malarial parasite suppressive activities of *Nuclea latifolia* phytochemical extracts and reference drug.



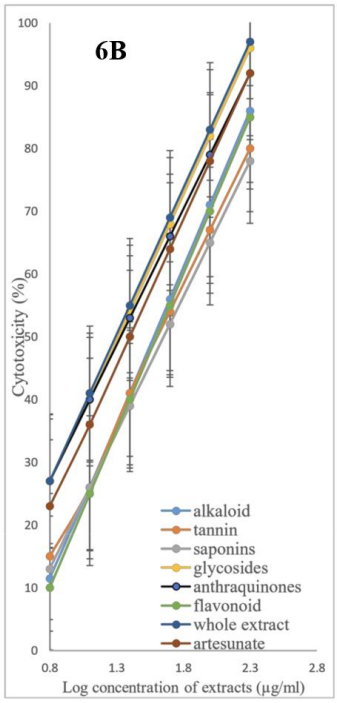


Figure 4
A. Concentration dependent comparative cytotoxic activity of *Polyalthia longifolia* extracts and reference drug.
B. Log concentration based comparative cytotoxic activity of *Polyalthia longifolia* phytochemical extracts and reference drug.

DISCUSSION

Traditional medicine is still considered as a remedy for various diseases plaguing the world at large. The leaf extracts of Phyllantus amarus, Nauclea latifolia and Polyalthia longifolia are used very often in traditional remedies for a vast number of illnesses and over the years have been accepted in traditional medicine as a cure for malaria infection. This present study assesses the antiplasmodial activity and cytotoxicity of various phytochemical extracts of P. amarus, N. latifolia and P. longifolia in order to determine the phytochemical component of the plants responsible for its antimalarial activity.

In this study, the alkaloid, tannin, saponin, glycoside, anthraquinone, flavonoid and whole extracts of the leaf of P. amarus, P. longifolia and Na. latifolia were studied. According to Rasoanaivo *et al*. (1992) the in vitro antiplasmodial activity of biologically active substances is categorized into four groups based on IC50 value < 5 µg/mL - very active, < 50 µg/mL - active, 50-100 µg/mL - weakly active, > 100 µg/mL inactive. Against this backdrop, the alkaloid extracts of all three plants exhibited very active antiplasmodial activity with IC50 values of 3µg/mL for each plant. Also, the whole extracts of P. amarus and P. longifolia demonstrated very active antiplasmodial activity with IC50 values of 2µg/ml and 5µg/ml, respectively. This present study recorded no toxicity for all the phytochemical extracts, as judged by their CC50 values (20-63µg/mL). Extracts are regarded as cytotoxic when CC50 is less than 10µg/mL (Zofou *et al*., 2011). Although in order to classify phytochemical fractions for further study, the selectivity index, SI of each extract was calculated. Valdes *et al*. (2010) defined SI value greater than 10 and IC50 value lower than 10µg/mL to be promising sources of antimalarial molecules. According to this classification, the alkaloid phytochemical extracts of all three plants have reputable antimalarial activity and could be reliable source for antimalarial drug development.

The in vitro antiplasmodial activity of P. amarus reported by other researchers have been inconsistent with our present study. Donkor *et al*. (2015), studied the antiplasmodial activity of aqueous and ethanolic extracts of different parts of P. amarus. They studied the aqueous extract of the whole plant, stem with roots, leaves with fruit and ethanolic extract of the whole plant which displayed IC50s of 115.43µg/mL, 21.14µg/mL, 13.96µg/mL, 10.10µg/mL, respectively.

Opong *et al*. (2011), studied the antiplasmodial activity of the whole plant of P. amarus against chloroquine-resistant strain of P. falciparum, Dd2 and reported no activity of the aqueous, ethanolic, chloroformic and ethyl acetate extracts with IC50s of 34.9µg/mL, 31.2µg/mL, 236.9µg/mL and 368.4µg/mL, respectively. They also reported no toxicity of the extracts (CC50 >100µg/mL). Adjobimey *et al*. (2004), however, reported high antiplasmodial activity of the methanolic (IC50: 5µg/mL) and moderate activity of the methylene chloride whole plant extract (IC50: 14.54µg/mL) on 3D7 chloroquine sensitive strain of P. falciparum. Uzuegbu *et al*. (2020) reported IC50s of 0.05 µg/ml; 0.27 µg/ml, CC50s of 199.52 µg/ml; 794.33 µg/ml and SI values of 3990; 2942 for the ethanolic and alkaloid fractions of the leaf extracts of P. amarus, indicating high activity.

The in vivo antiplasmodial activity of extracts of P. amarus has also been reported. Dapper *et al*. (2007), administered the aqueous extract of leaves and stem of P. amarus at doses of 108.33, 165 and 325 mg/kg to Swiss albino

mice and was found to cause a significant ($p<0.05$) dose-dependent suppression of P. berghei parasites, and comparable with standard drug artesunate. Ajala *et al*. (2011), administered aqueous and ethanolic extracts of the whole plant of P. amarus to Swiss albino mice at doses of 200, 400, 800 and 1600 mg/kg/day to investigate the prophylactic and chemotherapeutic effect of the extract against Plasmodium yoelii infection and compared with those of standard antimalarial drugs pyrimethamine, chloroquine and artesunate/amodiaquine, respectively. The aqueous extract showed slightly higher effect than the ethanolic extract. The antiplasmodial effects of extracts were comparable with the standard prophylactic and chemotherapeutic drugs used in chloroquine resistant Plasmodium infection. The extracts showed prophylactic effect by significant delay in the onset of infection with the suppression of 79% at a dose of 1600 mg/kg/day. The results indicate that the extracts of the whole plant of P. amarus possess repository and chemotherapeutic effects against resistant strains of P. yoelii in Swiss albino mice. Okoro *et al*. (2016) and Onyesom and Adu, (2015), have all demonstrated significant antiplasmodial activity of P. amarus leaf extract in murine model. In another study, however, Onyesom *et al*. (2019), administered varying doses of the alkaloid extracts of the leaves of P. amarus to swiss albino rats. Comparing the activity of the alkaloid extract with standard drug, chloroquine, they reported that at highest concentration (150 mg/kg/d), the alkaloid extract produced more biological activity. The cytotoxic activity of P. amarus has been studied by other researchers and has been consistent with this study. Lira *et al*. (2014), studied the cytotoxic effect of ethyl acetate crude extracts of P. amarus on shrimp nauplii. The crude extract exhibited cytotoxicity with the LC50 values of 9.15 µg/mL and 20.16 µg/mL of only leaves and the whole plant, respectively. Poompachee and Chudapongse (2012) who studied the cytotoxic effects of the hydromethanolic extracts of the whole plant on HepG2 cells, reported IC50 values of 500 (424–604) µg/mL and 372 (336–593) µg/mL calculated from trypan blue exclusion and MTT assay methods, respectively.

The in vitro antiplasmodial activity of N. latifolia as demonstrated by Uzuegbu *et al*. (2020), reported very active antiplasodial activity of the ethanolic and alkaloid leaf extracts (IC50: 0.1µg/ml; 0.23 µg/ml, CC50: 40.74 µg/ml; 478.63 µg/ml and SI: 407; 2081). Also, Zirihi *et al*. (2005) showed active antiplasmodial activity with IC50 of 8.9 µg/ml. However, Ajaiyeoba *et al*. (2004), reported no antiplasmodial activity with IC50 >479.9 µg/ml. Afolayan *et al*. (2020), investigated for the in vivo antiplasmodial activity of the n-hexane, aqueous, ethanolic and DCM-methanol fractions of the stem bark of N. latifolia against Plasmodium berghei infected mice at 250 mg/kg. At day 4 post infection after administration of extracts, the mice presented with percentage chemosupression of 8.81%, 61.47%, 79.04%, 74.66% of the n-hexane, ethanolic, aqueous and DCM-methanol fractions, respectively, and at day 6 post-infection, presented with percentage chemosupression of 28.8%, 36.55%, 65.55% and 45.31% in same order stated above. Alaribe *et al*. (2020), reported the percentage chemosupression of the aqueous extract and n-hexane and butanol fractions of the root of N. latifolia to be 85.22%, 84.52% and 91.32% in P. berghi infected mice at 100mg/kg. Asanga *et al*. (2017), studied the antiplasmodial activity of the ethanolic, dichloromethane, ethyl acetate, butanol and aqueous fraction of the roots of N. latifolia on Plasmodium berghei (NK-65 strain) in vivo by administering extracts at 150 mg/kg body weight of mice 72 h after infection. They reported percentage growth inhibition with the ethanolic extract having the greatest percentage growth inhibition (96.2%), followed closely by the aqueous extract (91.3%); dichloromethane fraction (88.9%); butanol fraction (70.4%) and ethyl acetate fraction (52.3%). Ettebong *et al*. (2014) also studied the in vivo antiplasmodial activities of the extract and fractions (n-hexane, chloroform, ethyl acetate, butanol, aqueous) of the stem bark in Plasmodium berghei berghei infected mice.

They reported percentage suppressions of 67.71%, 30.94%, 54.25%, 61.88% and 15.68% for aqueous, n-hexane, cholorform, ethyl acetate, and butanol fractions, respectively, at 200mg/kg of fractions. Adebajo *et al*. (2014), reported inferior activity of N. latifolia in vivo on the treatment of P. berghi infected mice with prophylactic, curative and chemosupressive ED_{50} values of 189.4, 174.5 and 279.3 mg/kg, respectively.

The antiplasmodial activity of Polyathia longifolia has also been reported severally by other researchers. Kwansa-Betum *et al*. (2019), reported very active antiplasmodial activity of the ethyl acetate extract (IC50: 9.5µg/ml) and moderately active antiplasmodial activity of the aqueous and ethanolic extracts (IC50:24µg/ml; 22.46µg/ml), presenting zero toxicity (CC50s>100µg/ml) and high selectivity for the ethyl acetate fraction (>10, the other fractions <10). Kumari *et al*. (2016), reported inferior activity when they researched on the methanol, ethyl acetate and chloroform extracts of the leaf, stem and roots of P. longifolia in comparison with this study. The methanol, ethyl acetate stem extracts and the methanol stem extracts presented moderately active antiplasmodial activity (IC50s: 44.73µg/ml, 33.00µg/ml and 18µg/ml, respectively), with the other extracts demonstrating no activity (IC50s: >50µg/ml). All the extracts presented no toxicity and no selectivity with indices less than 5. Uzuegbu *et al*. (2020), however, reported very active activity of the ethanolic and alkaloid leaf extracts of P. longifolia (IC50: 0.28µg/ml; 0.63 µg/ml, CC50: 158.49 µg/ml; 954.99 µg/ml and SI: 566; 1516). When reporting on the in vivo antiplasmodial activity, Bankole *et al*. (2016), reported minimal suppression (53%) at highest dose (800mg/kg) when they administered aqueous leaf extracts of P. longifolia on chloroquine-resistant Plasmodium berghei (ANKA) strain in Swiss albino rats.

In this study, the alkaloid fraction of all three plants presented remarkable activity in comparison with the activity of other extracts. Although the whole extracts of P. amarus also showed great activity. It is, therefore, obvious that the antiplasmodial activity is mostly due to the presence of alkaloids in the plants.

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