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Cytotoxicity and antiplasmodial activity of alkaloid extracts prepared from eight African medicinal plants used in Nigeria

U. E. Uzuegbu, O. A. Opajobi, J. E. Utalor, C. O. Elu, I. Onyesom

Department of Medical Biochemistry, Delta State University, Abraka, Delta State, Nigeria

Corresponding Author:
I. Onyesom, Department of Medical Biochemistry, Delta State University, Abraka, Delta State, Nigeria.
Tel: +234 8030 5280 16.
E-mail: onyesominno@yahoo.co.uk

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ABSTRACT

Objectives: Malaria morbidity and mortality rates are worsening, despite the dedicated commitment to eradicate the infestation, principally because of failing chemotherapy, the mainstay of treatment, and lack of registered vaccine. Therefore, the search for more potent antimalarial agents has become urgent and imperative. Hence, in this study, the cytotoxicity and antiplasmodial activity of the ethanolic leaf extracts and alkaloid fractions of eight 8African medicinal plants used in Nigerian traditional medicine were evaluated using standard methods and documented procedures. **Materials and Methods:** *In vitro* antiplasmodial activity 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 microassay technique using L929 animal cell fibroblasts by the lactate dehydrogenase method. **Results:** Results indicate that the ethanolic leaf extracts of six out of the eight plants (*Tridax procumbens*, *Ipomoea purpurea, Sida acuta, Senna alata, Phyllanthus amarus*, *Azadirachta indica, Nauclea latifolia,* and *Polyalthia longifolia*) studied were non-toxic (CC_{50} range: 33.88–954.99 µg/ml; and Chloroquine, CQ: 79.43µg/ml), but *T. procumbens* and *S. acuta* showed mild toxicity (CC_{50}=23.99µg/ml for each plants) as judged by the standard reference (CC_{50}: 30µg/ml= non-toxic). However, all leaf extracts of the eight plants demonstrated significant ($P < 0.05$) growth inhibition of *P. falciparum* (IC_{50} range: 0.05–0.28µg/ml, CQ: 0.03 µg/ml, standard reference: ≤5 µg/ml=highly active) with very highly rated selectivity index, (SI), values (range: 343–3990, CQ: 2648, reference: >10=selectable). In addition, the alkaloid fractions of the eight plants showed no toxicity (CC_{50}:44.67–954.99 µg/ml) and had very active antiplasmodial activity (IC_{50}: 0.23–2.04µg/ml) with great SI figures (22–2942). Alkaloids from *P. amarus, A. indica, N. latifolia,* and *P. longifolia* were particularly reputable (SI=2942, 1361, 2081, and 1516, respectively) for selection. **Conclusion:** This *in vitro* study confirms the antiplasmodial activity of these plants and justifies their use in the Nigerian tradomedicine, but further identified the alkaloid constituent as the medicinal phytochemical responsible for the observed antiplasmodial activity. Alkaloids from especially the four notable plants with high SI should, therefore, be characterized with the hope of discovering novel agent(s) against malaria.

Keywords: Alkaloid, Chloroquine, Malaria, *Plasmodium falciparum*, Selectivity index

INTRODUCTION

Malaria, now known as a tropical disease, is an ancient disease that has affected humankind for about 500,000 years. It has been spread throughout the world killing billions of people and has had enormous impact on human colonization.[1] The WHO African Region accounted for 93% of all malaria deaths in 2017. Although the WHO African Region was home to the highest number of malaria deaths in 2017, it also accounted for 88% of the 172,000 fewer global malaria deaths reported in 2017 compared with 2010.[2] Malaria remains an important public health concern in countries where transmission occurs regularly, as well as in areas where transmission has been largely controlled or eliminated. The disease is characterized by fever, headache, chills, tiredness, nausea, and general malaise.[3] The disease is caused by intracellular parasites of the

genus *Plasmodium* spp. To date, five *Plasmodium* spp have been linked to human infection (*vivax, ovale, malariae, knowlesi,* and *falciparum*), where *Plasmodium falciparum* is known to cause the most severe forms of the disease, killing upward of 600,000 people per year.[1]

Globally, the economic impact of malaria is estimated to cost Africa $12 billion every year.[4] This figure includes costs of health care, absenteeism, days lost in education, decreased productivity due to brain damage from cerebral malaria, and loss of investment and tourism. Malaria control is increasingly recognized as an important element of national poverty reduction strategies for malaria-endemic countries because of the social and economic impacts of the disease.[4]

Malaria parasites are genetically very diverse and their genomes are changing all the time. Control measures are, however, hampered by the development of resistant strains of the disease parasite. There are reported cases of *P. falciparum* resistance to artemisinin based combination therapy (WHO's recommended first line treatment for malaria) in South East Asia and in at least a dozen African countries.[5,6] These resistance problems underlie the need to continually search and develop new, low cost anti-malarial drugs to sustain the gains made in the fight against malaria.

This search is predominantly in plants, since local plants preparations contribute to the traditional treatment of malaria in most malaria-endemic areas. The use of plants for therapeutic purposes dates back to human history.[7] Several medicinal plants have been used locally to treat malaria infection. Several rural dwellers depend on traditional herbal medicine for the treatment of many infectious diseases including malaria.[8] The reputed efficacies of these plants have been recognized and passed on from one generation to the other. These eight African medicinal plants, *A. indica*, *Ipomoea purpurea, Nauclea latifolia, Phyllanthus amarus, Polyalthia longifolia, Senna alata, Sida acuta,* and *Tridax procumbens*, selected for this study are used for the traditional treatment of malaria in Nigeria.

The antiplasmodial activity of the crude extracts of these eight herbs, *A. indica,*[9] *I. purpurea*,[10] *N. latifolia,*[11,12] *P. amarus*,[13,14] *Polyalthia longifolia*,[15] *S. alata*, *S. acuta,*[16] and *T. procumbens,*[13] has been reported, validating the rationales for their use in the Nigerian traditional system to treat malaria. However, the phytochemical constituent(s) responsible for this observed biological activity (antiplasmodial potency) is yet to be fully documented. The alkaloids' phytochemical has always sustained interest due to its potential as a drug.[17]

This study, therefore, attempts to investigate the *in vitro* activity of the alkaloid and ethanolic leaf extracts of *A. indica, I. purpurea, N. latifolia, P. amarus, P. longifolia, S. alata, S. acuta,* and *T. procumbens* against *P. falciparum* parasite in culture all with the hope of establishing the antiplasmodial selectivity indices of the plants' extracts for further characterization and possible development into antimalarial agents.

MATERIALS AND METHODS

Collection of Plant Material

Fresh plants of *T. procumbens, I. purpurea, S. acuta*, *S. alata*, *A. indica, P. longifolia,* and *P. amarus* were collected in Abraka community located in Ethiope East Local Government Area of Delta State, Nigeria. The plants taxonomic identification was made by Dr. A.H Anherini, Department of Botany, Delta State University, Abraka, by matching them with existing Voucher specimens deposited at same institution. The plants were collected carefully to avoid contamination with other plants and strange materials.

Preparation of ethanolic extracts

The leaves of each plant were plucked, washed, and air-dried for 2 weeks at laboratory room temperature (28–32°C). The dried leaves were then powdered using laboratory blender (Kenwood, Japan). The powdered plant materials (100 g each) were separately macerated for 3 days at room temperature in ethanol. The resulting mixtures were filtered using Whatman paper and were concentrated using the Soxhlet apparatus (Corning, USA). The concentrated filtrate was then re-suspended in distilled water to obtain the ethanolic leaf extract for use.

Preparation of crude alkaloid extracts

To obtain the crude alkaloid extracts, 100 g of the dried, powdered leaves of each plant were mixed with 100 ml of ammonia at room temperature for an hour and were percolated with 250 ml of dichloromethane for 24 h. The extracting solvent was filtered and evaporated under vacuum. The residue was then dissolved in H_2O/HCl and filtered. Thereafter, the filtrate was treated several times with dichloromethane (6 ml × 150 ml). The mixture was evaporated and concentrated to dryness under residual pressure. The crude alkaloid extract was then confirmed with Dragendorff reagent. Dilutions were prepared from the ethanolic and crude alkaloid plant extract and the final concentrations were prepared as required for the study.

Determination of the in vitro antiplasmodial activity of extracts

Chloroquine sensitive *P. falciparum* 3D7 was maintained at 5% hematocrit (human type O-positive red blood cells) in complete RPMI 1640 medium by the candle jar method[18] with some modifications. *In vitro,* antiplasmodial activity was determined by parasite growth inhibition assay with some modifications.

The *in vitro* antiplasmodial assay was performed in triplicates. Different concentrations of the standard drug-chloroquine (prepared by dissolving in distilled water with purity of 98%) and extracts were dissolved in sterilized water. Concentration of extracts used was between the ranges 6.25–0.05 µg/ml. The synchronized culture with parasitemia of 1.5% and 3% hematocrit was incubated in 96-well microtiter plates predisposed with multiple concentrations of extracts for 48 h at 37°C in CO_2 condition. 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 for 10 min in 10% Giemsa stain (pH 7.3). The smear was then viewed under the microscope at ×100. Parasite growth was counted in 10 microscopic fields and the mean calculated. The percentage parasite suppression was calculated using the formula of the World Health Organization[19] and Ngemenya *et al*.[20] as stated below:

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

The concentration at which parasite growth was inhibited (suppressed) by 50% (IC_{50}) was determined from linear interpolation from the parasite growth inhibition curves, that is, parasite inhibition versus log of concentration generated from each parasite extract interaction.

In vitro cytotoxicity test

Cytotoxicity of plant extracts was assessed against animal cell fibroblast L929 cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 0.21% sodium bicarbonate, and 5 mg/ml gentamicin at 37°C. Assay was performed in 96-well microplates using lactate dehydrogenase assay. Concentration ranges of extracts tested were between 50 and 1.5 µg/ml and all cultures were performed in triplicates.

About 100 µL of cell suspension were seeded unto each well of the 96-well plate in complete medium. Fibroblasts were maintained in medium, for 24 h under 5% CO_2 atmosphere at 37°C. About 100 µL of extracts were added at concentrations ranging from 50 to 1.5 µg/ml and were incubated for 48 h under a humidified atmosphere at 37°C 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, 20 µL of working solution of lactate dehydragenase (LDH) were added to each well and were incubated for 4 h at room temperature. The supernatant was then removed from each well and 100 µL 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 570 nm. 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 cytotoxicity and analyzed by ANOVA using SSPS software (version 23). Results were regarded as significant at $P \leq 0.05$.

RESULTS

The results of the *in vitro* antiplasmodial activity of the crude alkaloid and ethanolic leaf extracts of the different plants on the growth of *P. falciparum* were determined. Figures 1a and 2a, respectively, show the mean percentage suppression of parasites

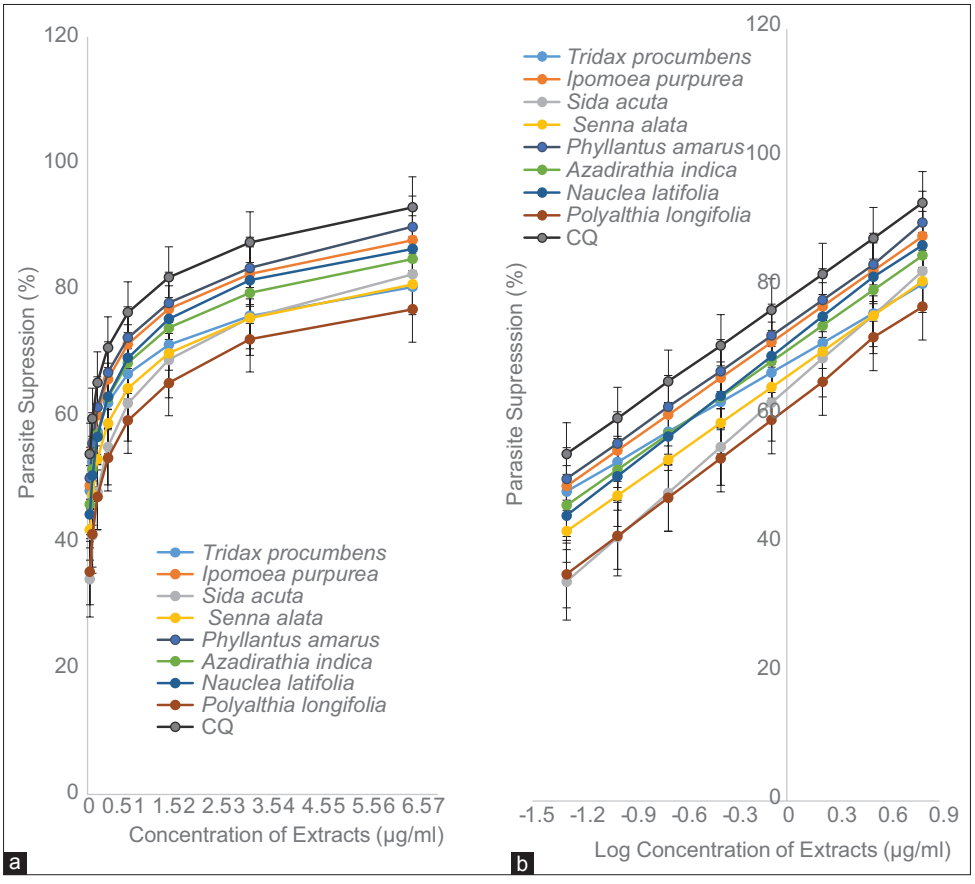

Figure 1: (a) Growth inhibition curves of the ethanolic extracts of the studied plants (b) parasite suppression of ethanolic extracts of the studied plants against log concentration of extracts

by the plants ethanolic leaf extracts and alkaloid fractions at various concentrations. The administration of varying doses (6.5, 3.25, 1.63, 0.51, 0.41, 0.20, 0.1, and 0.05 μg/ml) of the alkaloid and ethanolic leaf extract of the different plants to cultured *P. falciparum* produced parasite suppressive effects against the chloroquine-sensitive 3D7 strain of *P. falciparum* in a dose-dependent manner which compared well with the standard chloroquine treatment. The ethanolic leaf extracts suppressed more at each dose, but not significantly different (P>0.05) when compared with those of the alkaloid extracts. This implies that the alkaloid extracts of all the plants possess significant antiplasmodial activity, although, at varying degrees.

Figures 1b and 2b show the plots of percentage suppression versus log concentration used for the ethanolic leaf extracts and alkaloid fractions, respectively, and these lines were used to determine IC_{50}.

The estimated values of IC_{50} for both alkaloid and ethanolic leaf extracts of all the eight plants [Table 1] showed very active antiplasmodial activity against the chloroquine-sensitive, 3D7 strain of *P. falciparum* in culture.

In vitro Cytotoxicity

The cytotoxic activity of the crude alkaloid and ethanolic leaf extracts prepared from the selected eight plants was tested on L292 animal cell fibroblasts at varying doses (50.00, 25.00 12.50, 6.25, 3.13, and 1.57 μg/ml).

Figures 3a and 4a show the percentage cytotoxicity curves for the ethanolic leaf extracts [Figure 3a] and alkaloid fractions [Figure 4a]. However, Figures 3b and 4b indicate the percentage cytotoxicity plots against log concentrations of the ethanolic leaf extracts [Figure 3b] and alkaloid fractions [Figure 4b] used to estimate CC_{50}, which is the extrapolated concentration at 50% cytotoxicity. The CC_{50} values show that the ethanolic leaf extracts of *T. procumbens* and *S. acuta* demonstrated mild toxicity, while others, including the alkaloid extracts, were non-toxic [Table 1].

The alkaloid extracts are selectable (SI >10) in terms of their antiplasmodial activity, but only those from four plants (*P. amarus, A. indica, N. latifolia,* and *P. longifolia*) are highly reputable [Table 1] and should be selected for further study.

DISCUSSION

The present study examined the *in vitro* cytotoxicity and antiplasmodial activity of alkaloid extracts of eight different plant species used traditionally, to treat malaria, in Nigeria. The study also analyzed the ethanolic leaf extracts of the herbs for antiplasmodial activity and cytotoxicity to compare the previous reports which support their use in the Nigerian traditions to treat malaria.

All the plants ethanolic leaf extracts were found to be highly reputable in their activities against the 3D7 strain of *P. falciparum* parasites with IC_{50} ranging from 0.05 μg/ml to 0.28 μg/ml (alkaloid:0.23–2.04μg/ml) and SI values from 343 to 3990 (alkaloid:22–2942). The antiplasmodial activity and hence, the SI, were highest for the ethanolic leaf extract of *P. amarus,* followed by alkaloid extract of *P. amarus*, alkaloid extract of *N. latifolia*, alkaloid extract of *P. longifolia,*

Table 1: IC_{50}, CC_{50}, and SI values obtained with alkaloid and ethanolic extracts of the eight studied plants

Plant	Extracts	IC_{50} (μg/ml)	CC_{50} (μg/ml)	SI
Tridax procumbens	Ethanolic	0.07±0.02	23.99±4.61	343±9
	Alkaloid	0.50±0.08	50.12±6.72	100±11
Ipomoea purpurea	Ethanolic	0.06±0.01	33.88±3.89	565±16
	Alkaloid	0.37±0.07	45.71±6.97	124±13
Sida acuta	Ethanolic	0.25±0.04	23.99±3.88	96±6
	Alkaloid	2.04±0.11	44.67±3.76	22±4
Senna alata	Ethanolic	0.14±0.06	70.79±7.12	506±12
	Alkaloid	0.47±0.10	204.17±11.31	434±8
Phyllanthus amarus	Ethanolic	0.05±0.03	199.52±8.19	3990±26
	Alkaloid	0.27±0.07	794.33±13.02	2942±28
Azadirachta indica	Ethanolic	0.08±0.01	95.50±6.85	1194±31
	Alkaloid	0.36±0.05	489.78±9.92	1361±25
Nauclea latifolia	Ethanolic	0.10±0.03	40.74±6.30	407±21
	Alkaloid	0.23±0.07	478.63±11.13	2081±27
Polyalthia longifolia	Ethanolic	0.28±0.12	158.49±7.84	566±13
	Alkaloid	0.63±0.16	954.99±12.67	1516±18
Chloroquine		0.03±0.01	79.43±2.43	2648±11

Values are expressed as mean of triplicate determinations: IC_{50}=Inhibition concentration at 50% *P. falciparum* suppression, CC_{50}=Cellular cytotoxic concentration at 50% cytotoxicity, SI=Selectivity index, IC_{50}>10μg/ml is inactive; >5–10 μg/ml is moderately active; ≤5 μg/ml is active[21], IC_{50}>10μg/ml is inactive; <10 μg/ml with SI <4 is marginally active; 10 μg/ml with SI 4-10 is partially active and >10 μg/ml and SI>10 is active[22], CC_{50}<1 μg/ml is highly toxic; 1–10 μg/ml is moderately toxic; 10–30 μg/ml is mildly toxic and >30 μg/ml is non-toxic[23]

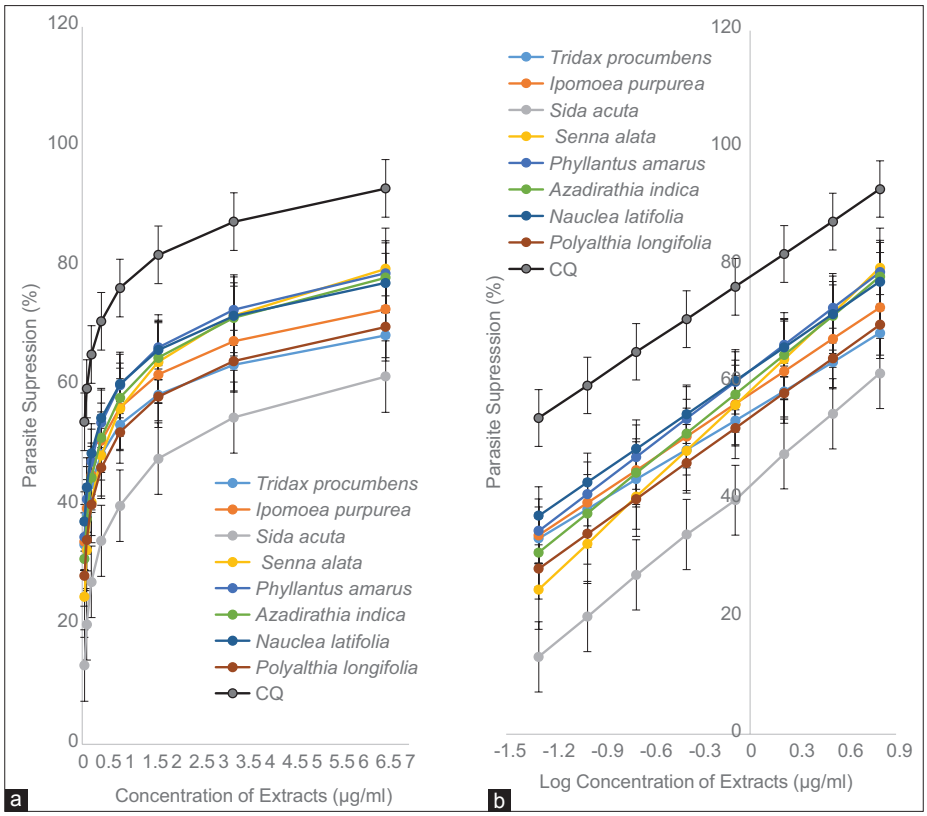

Figure 2: (a) Growth inhibition curves of alkaloid extracts of the studied plants (b) parasite suppression of alkaloid extracts of the studied plants against log concentrations of extracts

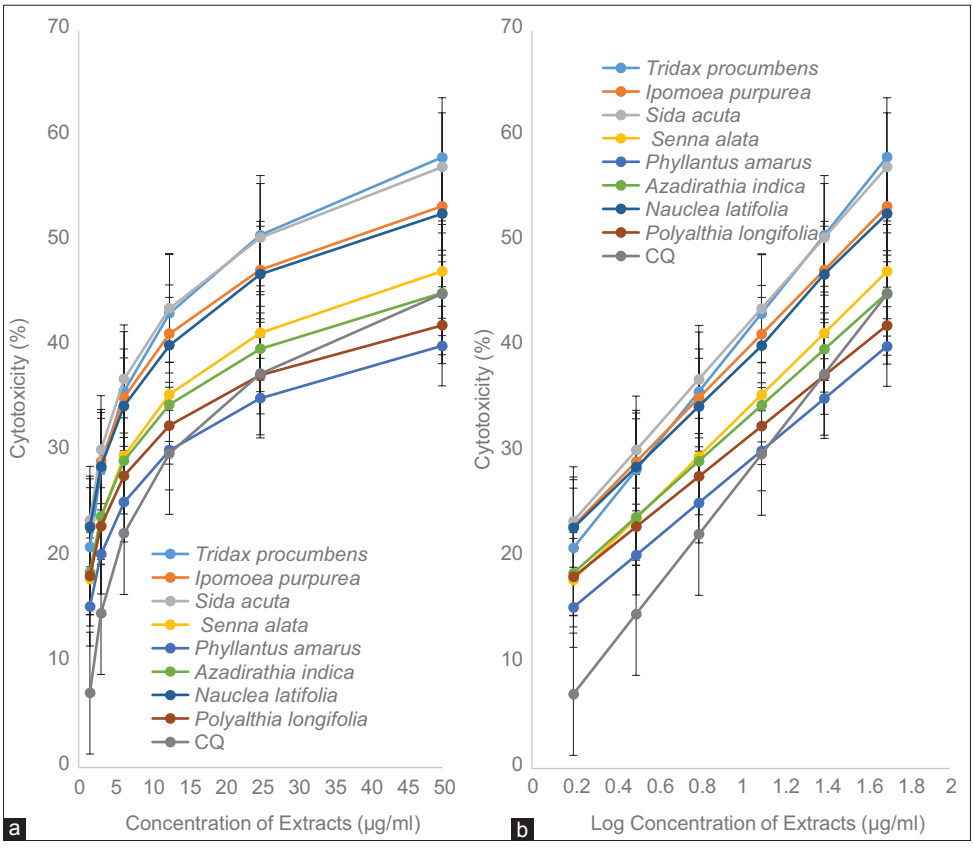

Figure 3: (a) Comparative cytotoxic activity of the ethanolic leaf extract of the studied plants (b) cytotoxicity of ethanolic leaf extracts of the studied plants against log concentrations of extracts

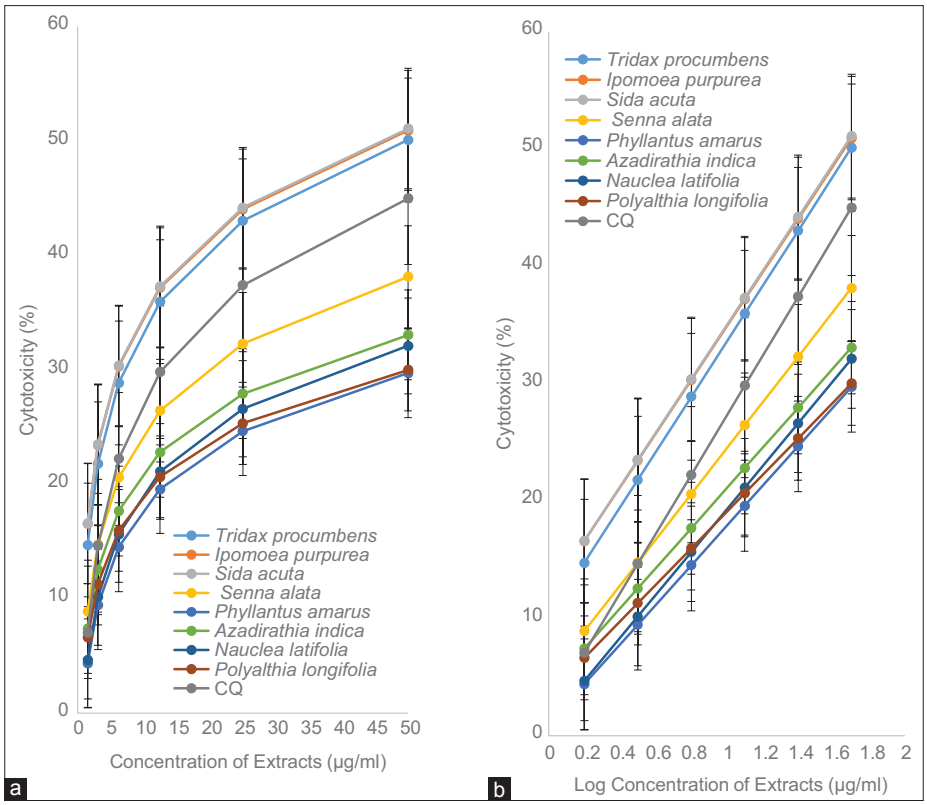

Figure 4: (a) Comparative cytotoxic activity of alkaloid fraction of the studied plants (b) cytotoxicity of alkaloid fraction of the studied plants against log concentration of extracts IC_{50} values obtained with the 3D7 strain of Plasmodium falciparum and CC50 figures obtained with the L929 strain of animal cell fibroblasts are given in Table 1, along with the calculated selectivity indices. Extracts that display high selectivity should offer safer therapy

alkaloid extract of *A. indica*, ethanolic leaf extract of *A. indica*, ethanolic extract of *P. longifolia,* ethanolic leaf extract of *I. purpurea*, ethanolic extract of *S. alata,* alkaloid extract of *S. alata*, ethanolic extract of *N. latifolia*, ethanolic extract of *T. procumbens*, alkaloid extract of *I. purpurea,* and alkaloid extract of *T. procumbens*. These compared well with the chloroquine values.

To estimate the therapeutic value of the extracts, the crude alkaloid and ethanolic leaf extracts of plants selected for this study were analyzed for cytotoxicity against L929 animal cell fibroblasts. Low SI indicates that the antiplasmodial activity is probably due to cytotoxicity rather than activity against the *Plasmodium* parasite themselves. In contrast, high SI should offer the potential of safer therapy.[24] Valdes *et al.*[22] defined four as the minimal SI value to validate a safe antimalarial for use, and a SI value >10 and IC_{50} value lower than 10 µg/ml should be promising sources of antimalarial molecules. The alkaloid extracts of all the plants in this study were found to be non-toxic on L929 animal cell fibroblasts with CC_{50}s ranging from 44.67 to 954.99µg/ml so also the ethanolic leaf extracts (33.88–954.99 µg/ml), except those of *T. procumbens* and *S. acuta*, which were mildly toxic (23.99 µg/ml: non-toxic >30 µg/ml). All plant extracts, including the alkaloids, showed great selectivity, being >10.

The antiplasmodial activity of *I. purpurea* has not been previously reported; however, the pigment and aqueous extracts of the flower of *I. purpurea* have been reported to possess antibacterial activities against *Staphylococcus aureus* and *Escherichia coli.* The antibacterial activity of *I. purpurea* may be due to the presence of flavonoids and alkaloids present.[25] Conventionally, infusions of the *I. purpurea* leaf extract are used as diuretic, to stop hemorrhage, as purgative agent and in the treatment of syphilis.[26]

Extracts of *A. indica* have been previously reported to possess antimalarial activities. Alshawsh *et al.*[27] revealed an active antiplasmodial activity of the aqueous extract of *A. indica* with an IC_{50} of 2 µg/ml and the moderate activity of the methanolic extract possessing an IC_{50} of 16.9 µg/ml, which is greater than the IC_{50} obtained in this study for the ethanolic extracts (IC_{50}=0.08 µg/ml). This may indicate ethanol a better solvent for extracting the active compounds. However, Ravikumar *et al.*[28] reported less antiplasmodial activity of the ethanolic extract of the leaf of *A. indica*, with an IC_{50} 47.20 µg/ml.

The ethanolic and alkaloid extracts of *S. acuta* demonstrated significant activity with IC_{50} of 0.25µg/ml and 2.04 µg/ml, respectively. The results indicate that the antiplasmodial activity of the plant resides more in the ethanolic fraction, but Karou *et al.*[16] reported higher antiplasmodial activity of the alkaloid extract (IC_{50}: 0.05 µg/ml) than the ethanolic fraction (IC_{50}:4.37 µg/ml) of the leaf extract of *S. acuta*. In addition, they demonstrated the antiplasmodial activity of the ether, chloroformic, and aqueous fraction of the extract of *S. acuta*, all having great antiplasmodial activity (IC_{50} <1 µg/ml) with

exception of the ether fraction, demonstrating no activity, IC_{50}=57.04 µg/ml.

The antiplasmodial activity of *P. longifolia* is revealed by this study presents very active activity of the ethanolic and alkaloid extracts. A study into the antiplasmodial activity of the ethanolic, aqueous and ethyl acetate extracts of *P. longifolia* by Kwansa- Bentum *et al.*[29] presented IC_{50} values ranging from 9.50 to 24.00 µg/ml and CC_{50} values all >100 µg/ml. These results suggest the moderate activity of the plant extracts. The antiplasmodial activity of the extracts has been attributed to saponin, tannin, and flavonoid phytochemical constituents,[21] but this study further adds alkaloids.

In this investigation, the ethanolic extracts of *P. amarus* presented greater activity than the alkaloid extract with each having an IC_{50} of 0.05 and 0.27 µg/ml, respectively. The present results demonstrated greater activity in comparison to results obtained in other reports. Donkor *et al.*[3] revealed IC_{50} of the ethanolic extract of the whole plant to be 10.10 µg/ml, while that of the aqueous extract of same whole plant was 115.43 µg/ml, suggesting ethanol as a better solvent for extracting the active ingredient. Komlaga *et al.*[30] reported the active antiplasmodial activity of the aqueous extract (IC_{50}=9.93 µg/ml, CC_{50}>100 µg/ml and SI >10.1). Appiah-Opong *et al.*[13] reported no activity of the aqueous, ethanolic, ethyl acetate, and chloroformic extract of *P. amarus* with IC_{50} values ranging from 31.2 to 368.4µg/ml and CC_{50} all >300 µg/ml.

In general, the IC_{50}s obtained for the antiplasmodial activity of *S. alata* were less than those reported by other researchers. The IC_{50} of 0.14 µg/ml for the ethanolic leaf extract and 0.47 µg/ml for the alkaloid, of *S. alata* were less in comparison to the one (12.5 µg/ml) reported by Kayembe *et al*.[31] when they tested the antiplasmodial effects of the ethanolic seed extract. Jouse *et al*.[32] obtained an IC_{50} of 0.48 µg/ml and 0.67 µg/ml for the methanolic and aqueous extracts, respectively. Kaushik *et al.*[33] reported an IC_{50} of 18 µg/ml with very low toxicity (CC_{50}:100 µg/ml) producing a SI of 5. However, Zirihi *et al*.[34] reported no antiplasmodial activity with IC_{50} >50 µg/ml.

The *in vitro* antiplasmodial activity of this *N. latifolia* as demonstrated by Zirihi *et al*.,[34] showed active antiplasmodial activity with IC_{50}; 8.9 µg/ml, CC_{50}>50 µg/ml and SI >5.6. However, Ajaiyeoba *et al.*[35] reported no antiplasmodial activity with IC_{50} >479.9 µg/ml. Present study, however, demonstrated active antiplasmodial activity for both alkaloid (IC_{50}:0.23 µg/ml, CC_{50}: 478.63 µg/ml, SI: 2081) and ethanolic leaf extracts (IC_{50}: 0.10 µg/ml, CC_{50}: 40.74 µg/ml, SI: 407).

Komlaga *et al.*[30] previously reported the antiplasmodial activity of the aqueous extract of *T. procumbens* with an IC_{50} of 10.15 µg/ml, CC_{50} 24.89 µg/ml, and SI of 2.45. The antiplasmodial activity of aqueous, chloroform and ethyl acetate extracts of *T. procumbens* studied by Appiah-Opong *et al*. (2011) produced no antiplasmodial activity, showing IC_{50} of extracts tested to be, >500 µg/ml with the exception of the ethanolic extract which produced an IC_{50} of 121.3 µg/ml. Cytotoxic activity of the extracts studied by Appiah-Opong *et al.*[13] showed no cytotoxic activity with up to 500 µg/ml. The IC_{50} values are greater in comparison to the values obtained in this study.

The ethanolic extracts of the plants studied, demonstrated higher antiplasmodial activity when compared with the alkaloid fractions. The active antiplasmodial constituents which have been identified[21,23] in these plants include: Flavonoids, tannins, saponins, and alkaloids. It could be that these active chemicals synergize in the ethanolic extract to enhance its biological (antiplasmodial) activity. Although, the alkaloid fraction alone is still, relatively reputable in its antiplasmodial action.

In several instances, the data of this present study differ from the previous reports and this may be due to plant location and enrichment, extracting solvent system, parasite strain and culture environment, and cell line. Overall, alkaloid and ethanolic extracts of the eight plants grown in Abraka, Delta State, Nigeria, possess significant antiplasmodial activity. Since alkaloids have drug potential, and it has been identified as one of the main antiplasmodial phytochemical from this present investigation, then, it warrants further study.

CONCLUSION

The results of this study indicate that the ethanolic leaf extracts of the eight African medicinal plants evaluated, showed significant antiplasmodial activity, very low cytotoxicity and high selectivity indices as antimalarial agents. This present observation confirms previous reports and justifies the use of these plants in the Nigerian traditional medicine system to treat malaria fever. In addition, this study further identifies the alkaloid phytochemical constituent as the principal antiplasmodial component of these plants; with those from *P. amarus, A. indica, N. latifolia,* and *P. longifolia* judged to be highly reputable. Therefore, alkaloid compounds from particularly these four plants should be characterized with the hope of developing potent antimalarial agents.

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