In vitro antiplasmodial activity, cytotoxicity, antioxidant action and GC-FID analysis of *Allanblackia floribunda* Oliv

Francis Irabor¹ · Osamudiamen Ebohon¹ · Nekpen Erhunse^{2,4} · Osariemen T. Okugbo³ ·
Ehimwenma S. Omoregie⁴

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Abstract

This study evaluated the in vitro antiplasmodial efficacy and cytotoxicity of *Allanblackia floribunda* stem bark extract, leaf extract and oil. It also assessed the phytochemical compositions and antioxidant action of the stem bark fractions as well as the phytochemical fingerprint of the most active fraction (dichloromethane). Trager and Jensen method was used to culture *Plasmodium falciparum*, Mark III test developed by WHO was used to assess the antiplasmodial activity of the plant's crude extract and fractions against the ring stage of *P. falciparum* strain, Pf3D7. Cytotoxicity was determined against Vero cell line using microculture tetrazolium (MTT) test. Gas chromatography with flame ionization detection (GC-FID) was employed to identify phytochemical fingerprint of the most active fraction. The stem bark extract had better antiplasmodial activity ($IC_{50Pf3D7}$ of $4.3 \pm 0.17 \mu\text{g/mL}$) compared with the leaf extract ($IC_{50Pf3D7}$, $8.0 \pm 0.28 \mu\text{g/mL}$) and oil ($IC_{50Pf3D7} > 100 \mu\text{g/mL}$). Both the leaf and stem bark extracts were found to be non-cytotoxic compared with the standard cytotoxic drug, doxorubicin. The selectivity indices (S.I.) of the extracts against the parasite were 20.06 and 8.85 for the stem bark and leaf respectively. Dichloromethane fraction had the highest inhibition against the *P. falciparum* parasite with $IC_{50Pf3D7}$ of $1.51 \mu\text{g/mL}$. GC-FID analysis showed high presence antiplasmodial flavonoids and terpenes. This investigation confirmed that *A. floribunda* stem bark has potent activity against *P. falciparum*, and it is relatively safe to normal cell.

Article Highlights

- *Allanblackia floribunda* methanol stem bark and leaf extracts could inhibit the growth of chloroquine sensitive *Plasmodium falciparum* (Pf3D7) in vitro.
- The stem bark infusion of *Allanblackia floribunda* was found to be nontoxic and safe at moderate doses to normal cell line (Vero cell line).
- Dichloromethane fraction of the stem bark showed excellent inhibition against chloroquine sensitive malaria parasite.

Keywords Antiplasmodial activity · Cytotoxicity · *Plasmodium falciparum* · *Allanblackia floribunda* · Phytochemical fingerprint

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✉ Francis Irabor, francis.irabor@yahoo.com | ¹Department of Biological and Chemical Sciences, Faculty of Natural and Applied Sciences, Michael and Cecilia Ibru University Agbarha-Otor, P.M.B 100, Ughelli, Delta State, Nigeria. ²International Centre for Genetic Engineering and Biotechnology, New Delhi, India. ³Department of Basic Sciences, Faculty of Basic and Applied Sciences, Benson Idahosa University, Benin City, Nigeria. ⁴Department of Biochemistry, Faculty of Life Sciences, University of Benin, P.M.B 1154, Benin City, Nigeria.

1 Introduction

Malaria is a disease that is caused by *Plasmodium* parasite. The symptoms and clinical manifestations of this protozoan infection is severe when the infection is caused by *Plasmodium falciparum* in children, pregnant women and individuals from malaria free regions [1]. However, the negative impact of *P. falciparum* is less complicated when previously exposed to the parasite infection [1]. Though Artemisinin-based combination therapy (ACT) recommended by WHO for the treatment of uncomplicated malaria has been effective in combating this malady. but the scourge of the disease is still fierce in sub-Saharan Africa because of the mortality rate on a yearly basis [2]. For example, there were approximately 216,000,000 cases of malaria infection worldwide resulting in 445, 000 death with 91% of the mortality in Africa [2]. It has been impossible to eliminate malaria from Africa due to the existence of hard-to- reach communities, inaccessibility of antimalarial drugs, exposure to fake antimalarial drugs, drug resistance, lack of information flow on effective antiparasmodial agents and individuals' low-income status. In addition, recent reports in eastern [3], northern and southern [4, 5] regions of Nigeria suggest possible cases of parasite resistance and recrudescence to the currently used combined antimalarial therapies. The present circumstances necessitate the need for alternative antimalarial drug and adjuvant therapy to curtail the challenges of parasite elimination and curb possible future resistance.

Despite the death toll resulting from parasite plaque in Nigeria, the tribals of southern Nigeria are able to control the ravaging effect to some extent by using plant infusion from *Allanblackia floribunda* commonly known as elephant rice [6, 7]. *A. floribunda* is rich in bioactive substances with varied pharmacological properties on living systems [6, 7]. The stem bark, leaf and fruit of *A. floribunda* infusions are used in African herbal medicine preparation to manage pain and inflammation. In Nigeria, Ghana, Cameroon and Ivory Coast the stem bark extract is used to heal aching tooth and asthma, the pounded bark is used traditionally to stop diarrhea, a decoction of the whole fruit is used to relieve scrotal elephantiasis [6, 7] and the stem bark infusion is used to maintain strong erection in aging men [8].

Preliminary phytochemical screening of the stem, leaf and oil showed that stem has more phenolics, flavonoids, terpenoids compared with the leaf extract and oil [Labor et al., unpublished]. The methanol extract of a close species, *Allanblackia parviflora* Chev. was reported to have a strong antimicrobial activity against *Entamoeba faecalis* [9]. In 2017, high performance liquid chromatography

analysis of methanol extract of the seed by Akpanika et al. [10] revealed the presence of important polyphenols (morelloflavone) and glucosides and their antioxidant activities were comparable to vitamin C.

Despite the relevance of *A. floribunda* in Africa folk medicine there exist a limited scientific data and documentation on its antimalarial activity, the relative abundance of its pharmacologically active substances, best solvent for its extraction and level of safety of its most active infusions. This investigation therefore examined the in vitro antiparasmodial activity, cytotoxicity, antioxidant action and GC-FID analysis of *Allanblackia floribunda* extracts.

2 Materials and methods

2.1 Plant collection and identification

The plant parts used in this study were selected based on ethnopharmacological information as antimalarial agents used in southern part of Nigeria. *Allanblackia floribunda* leaf, stem bark and seed were collected during the period of January and July 2016, from a forest area at Ohogua community, Ovia East Local Government, Benin City, Edo State, Nigeria. The plant was authenticated by Dr. Henry A. Akinnibosun at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Nigeria, and voucher specimen of the sample (UBHA₃₆₁) was deposited at the herbarium of the same department. The plant scientific name was checked with <http://www.theplantlist.org> on 26th October, 2020.

2.2 Preparation of extract

A 100g of the *A. floribunda* stem bark and leaf were macerated in 1000 mL of absolute methanol for 72 h with occasional stirring. The extracts were then filtered using double layered muslin cloth and the filtrate concentrated to dryness by using a rotary evaporator at reduced pressure while the oil was obtained from about 200 g of the powdered seed using hot water floatation method. The extracts obtained were stored at 4°C until used.

2.3 Cultivation of parasites

Trager and Jensen procedure was used to culture *P. falciparum* [11, 12]. The *Plasmodium* strain (Pf3D7) used was chloroquine sensitive isolate obtained from the Malaria Research and Reference Reagent Resource Center (MR4) at the American Type Culture Collection (ATCC) in Manassas, Virginia, US. The parasites were cultured in O + RBC as host cells and maintained in RPMI 1640 medium supplemented with gentamicin solution 0.01 mg/mL, 25 mM

HEPES buffer, 25 mM NaHCO₃ and 1% Albumax II maintained in 5% CO₂ and incubated at 37 °C. Parasitaemia was determined using light microscopy (Giemsa stain).

2.4 In vitro antiplasmodial activity

The growth inhibition of chloroquine-sensitive *Plasmodium falciparum* strain (Pf3D7) by the plant extracts was evaluated by means of the Mark III test, as developed by WHO [13]. The stock solutions were filter-sterilised through a 0.2 µm Millipore filters. Two-fold serial dilutions of drug/extracts were performed to generate required concentrations (refer to supplementary file S2) for treatment of parasitized cells in vitro. One hundred microliters (100 µL) of 0.5% *P. falciparum* culture that had been synchronized (refer to supplementary file S1) was aliquoted into the wells of the pre-treated 96-well microtitre plate to a final hematocrit of 3%. Wells with parasitized red blood cells without plant extract serve as negative controls whereas wells containing cultures with chloroquine diphosphate serve as positive control. Parasitaemia was evaluated after 48 h by Giemsa stain and the average suppression of parasitaemia was calculated by using the formula below:

$$\frac{\text{Average \% parasitaemia in control} - \text{Average \% parasitaemia in test}}{\text{Average \% parasitaemia in control}} \times 100$$

The antiplasmodial activities of the extracts were expressed using IC₅₀. Each sample was tested in triplicate and the IC₅₀ obtained were pooled and expressed as geometric means and standard deviations. The independent sample t-test was used to compare mean IC₅₀ of antimalarial activity between plant extracts. Finally, the in vitro antiplasmodial activity was rated in accordance with the system of antiplasmodial activity of Rasoanaivo et al. [14]. According to this system, an extract is regarded to be very active if IC₅₀ is less than 5 µg/mL, active if IC₅₀ is greater than 5 µg/mL but less than 50 µg/mL, weakly active if IC₅₀ is greater than 50 but less than 100 µg/mL and inactive if IC₅₀ is greater than 100 µg/mL.

2.5 Cytotoxicity test (cell viability assay)

Vero cells were provided by the National Veterinary Research Institute, Vom Jos, Plateau State, Nigeria to assess the cytotoxicity of the most active plant extracts. Vero cells were maintained in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), glutamine (2 mM), penicillin (100 units/mL) and streptomycin (100 µg/mL). The cells were seeded in 96 well plates at 10,000 cells per well in 100 µL culture medium. The cells were incubated at 37°C in a

humidified 5% CO₂ atmosphere. After 48 h, the medium was discarded by inversion of the microtitre plate and thereafter replaced with 100 µL of fresh culture medium followed by 100 µL of crude plant extract at a concentration of 2000 µg/mL in row H of the 96 well plate and serially diluted twofold to give concentrations ranging from 1000 to 1.95 µg/mL. Row A of the 96 well plate serve as control wells. Doxorubicin was used as standard cytotoxic drug and concentration range tested for doxorubicin was 5- 0.01ug/ mL. The cells were maintained at 37°C in a CO₂ incubator before determining their viability by using MTT assay as described by Mosmann [15]. The absorbance for each well was measured between 490 nm in a micro-titre plate reader and the percentage cell viability (CV) was calculated using the formula below. A dose-response curve was plotted to calculate the concentration that resulted in 50% death of viable cells (CC₅₀).

$$CV = \frac{\text{Average Absorbance of duplicate drug wells}}{\text{Average absorbance of control well}} \times 100$$

2.6 Fractionation of *A. floribunda* stem bark extract

The stem bark extract was most active against *Plasmodium falciparum* (Pf3D7) compared with the leaf extract and oil of *A. floribunda* hence it was fractionated. Fractionation of the stem bark extract was done using solvent of increasing polarity as described by Hassan et al. [16]. The methanol stem bark extract of *A. floribunda* was partitioned into fractions of n-hexane, dichloromethane, ethyl acetate and hydromethanol. The fractions obtained were concentrated using a rotary evaporator (RE 300, Bibby Scientific, UK) with reduced pressure at 45°C.

2.7 Qualitative phytochemical screening and antioxidant activity of *A. floribunda* stem bark fractions

The qualitative test for phytochemicals present in fractions were carried out using standard procedures [17, 18]. The free radical scavenging capacity of the fractions against 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined by the method of Brand-Williams et al. [19] and phosphomolybdate reduction capacity was estimated using the method described by Prieto et al. [20].

2.8 Gas chromatography (GC) analysis of most active fraction (dichloromethane fraction)

The phytochemical fingerprint for flavonoids, terpenes, alkaloids, terpenoids, and volatile organic constituents of the most active fraction was determined using gas chromatography with flame ionization detector. Gas chromatography analysis was carried out on a HP 6890 Powered with HP ChemStation Rev. A 09 01 [1206] Software. Active constituents were identified based on comparison of the retention times of the peaks with those of corresponding pure standard mixtures used. The flavonoids extraction was carried out by the method described by Millogo-Kone et al. [21]. The extraction of terpenes was carried out by method described by Ortan et al. [22]. The alkaloids extraction was carried out by the method described by Ngounou et al. [23]. The composition of volatile organic compounds was carried out on a HP 6890 powered with HP ChemStation Rev. Bioactive metabolites were identified based on comparison of the retention times of the peaks with those of the corresponding reference standards (Supelco Inc.) mixtures used (refer to supplementary file for the detailed methods of extraction and GC-FID conditions).

2.9 Statistical analysis

The various results obtained from this study were expressed as mean $\pm$ SEM. One way analysis of variance (ANOVA) followed by Tukey's HSD (honest significant difference) test was used to determine significance differences between the groups. Statistical significance was declared when P value was less than 0.05. The statistical analysis was performed using the statistical package for social science (SPSS) for windows, version 16.0.

3 Results

3.1 In vitro antiplasmodial activity of *A. floribunda* stem bark extract, leaf extract and oil

The inhibition of parasite growth by the plant extracts was dose dependent from 0.78 to 25 $\mu\text{g/mL}$. At about 5 $\mu\text{g/mL}$ and above, both leaf and stem bark extracts had over 45% parasite inhibition. However, *A. floribunda* oil extract recorded less than 40% of *P. falciparum* inhibition even at the highest dose (see Fig. 1a). The IC_{50} values of the tested medicinal plant against *P. falciparum* are listed in Table 1. The stem bark extract of *A. floribunda* showed excellent antiplasmodial activity ($4.3 \pm 0.17 \mu\text{g/mL}$) followed by the leaf extract ($8 \pm 0.28 \mu\text{g/mL}$). However, the oil extract showed IC_{50} value that was over 100 $\mu\text{g/mL}$.

Fig. 1 Effects of *A. floribunda* extracts on: *Plasmodium falciparum* (PF3D7) growth using Mark III test and cell viability in Vero cell using MTT assay

3.2 Cytotoxic activity of *A. floribunda* crude stem and leaf extract

Results of the cytotoxicity of *A. floribunda* stem bark extract, leaf extract and doxorubicin against Vero cell line are shown in Fig. 1b and 1c. The stem bark extract at 1.95 to 1000 $\mu\text{g/mL}$ had cell viability that was majorly over 50%. This same trend was observed for the leaf extract at 1.95 to 250 $\mu\text{g/mL}$. However, we observed less than 50 percent cell viability at concentration greater than 250 $\mu\text{g/mL}$ for the leaf extract. The methanol extracts of *A. floribunda* exhibited non significant activity against the Vero cell line with CC_{50} values of $88.26 \pm 0.15 \mu\text{g/mL}$ (stem bark) and $70.81 \pm 0.06 \mu\text{g/mL}$ (leaf) as shown in Table 1. The standard cytotoxic drug, doxorubicin was found to be highly cytotoxic having CC_{50} of $0.05 \pm 0.00 \mu\text{g/mL}$; the selectivity

Table 1 IC₅₀, CC₅₀ values and selectivity index of *A. floribunda* extracts

Plant/Drug	In vitro antiplasmodia activity IC ₅₀ Pf3D7 (μg/mL)	Cytotoxicity of Plant Extract CC ₅₀ (μg/ mL)	Selectivity Index CC ₅₀ /IC ₅₀
<i>A. floribunda</i> stem	4.3 ± 0.17 ^a	86.26 ± 0.15 ^c	20.06
<i>A. floribunda</i> leaf	8 ± 0.28 ^b	70.81 ± 0.06 ^b	8.85
<i>A. floribunda</i> oil	> 100 ± 1.73 ^c	NA	NA
Chloroquine	1.76 ± 0.01 ^a	NA	NA
Doxorubicin	NA	0.05 ± 0.00 ^a	NA

Data represent mean ± SEM (n=3/group). Values in the same column with different lowercase letters represent significant difference between means at $p < 0.05$; NA = Not applicable

index for *A. floribunda* leaf was lower (8.85) when compared with the stem bark extract (20.06) Table 1.

3.3 Phytochemical composition, in vitro antiplasmodial and antioxidant activity of the partitioned fractions of *A. floribunda* methanol stem bark extract

Table 2 depicts the phytochemical screening results of the partition fractions of *A. floribunda* most active infusion, stem bark extract. The result revealed the presence of quinones, flavonoids, cardiac glycosides, alkaloids, terpenoids, tannins and phenols in all the fractions. Majority of the phytochemicals were highly detected in the dichloromethane, ethylacetate and hydromethanol fractions. However, some of these bioactive compounds were less detected in the hexane fraction. Quinones were undetected in the hydromethanol fraction. Figure 2a represents the total antioxidant capacity obtained for the fractions of the stem bark extract; these results have similar pattern to the data obtained for the phytochemical screening of *A. floribunda* stem bark fractions. The reduction of molybdenum (VI) to molybdenum (V) by the fractions was highest in the hydromethanol (205 μg/mL) followed by ethylacetate fraction (101.4 μg/mL) and then the dichloromethane

(93.2 μg/mL). The hexane fraction had the lowest antioxidant activity (82.4 μg/mL). DPPH radical scavenging activity of the stem bark fractions of *A. floribunda* (Fig. 2b) was over 70% at a concentration range of 20 to 100 μg/ mL. An exception to this was the activity recorded for hexane fraction in which less than 60% DPPH radicals were inhibited at 100 μg/ mL. Table 3 shows the IC₅₀DPPH of the various fractions as follow: ethylacetate (IC₅₀DPPH = 14.62 ± 0.41), hydromethanol (IC₅₀DPPH = 5.26 ± 0.55 μg/ mL) and dichloromethane (IC₅₀DPPH = 18.42 ± 0.65). Ethylacetate, hydromethanol and dichloromethane fractions had better DPPH inhibiting ability compared to hexane fraction (IC₅₀ DPPH = 37.55 ± 0.79 μg/ mL) which was less active against free radical generated by DPPH.

The activity of the various fractions of *A. floribunda* stem bark with respect to inhibition of parasite growth is shown in Table 3 while Fig. 3 shows the dose response curve. Among the fractions, dichloromethane (DCM) had the best inhibitory potential against the *P. falciparum* parasite (IC₅₀ Pf3D7 1.51 μg/ mL). DCM was closely followed by the hydromethanol (HMet) fraction (IC₅₀ Pf3D7 5.26 ± 0.55) while that of ethylacetate (EAct) and hexane (Hxn) fractions were almost similar (IC₅₀ Pf3D7 = 6 and 6.25 μg/ mL, respectively). In general, the in vitro antiplasmodial activity of the fractions followed the order: DCM > HMet > EAct > Hxn.

Table 2 Qualitative analysis of phytochemicals in *A. floribunda* fractions

Phytochemicals	Hexane	Dichloromethane	Ethylacetate	Hydromethanol
Phenolics	–	+	+	+
Terpenoids	+	++	+++	+++
Tannins	–	+	+	+
Alkaloids	–	+	+	+
Flavonoids	+	+	++	–
Quinones	–	+	++	+++
Cardiac Glycosides	++	++	++	++

KEY: +++ = Very highly detected; ++ = Highly detected; + = Less detected; – = Not detected

Fig. 2 Antioxidants capacity and free radical inhibiting activity of *A. floribunda* stem bark fractions **a** Total antioxidant capacity of *A. floribunda* stem bark fractions; **b** DPPH radical scavenging ability of *A. floribunda* stem bark fractions. Data are expressed as mean $\pm$ SEM of results from three independent experiments. Bars with different lowercase letters represent significant difference between mean at $p < 0.05$

Table 3 IC_{50} -DPPH and IC_{50} Pf3D7 of *A. floribunda* stem bark fractions

Fraction/Standard	IC_{50} DPPH (μ g/mL)	IC_{50} Pf3D7 (μ g/ mL)
Vitamin C	5.62 ± 0.35^a	ND
<i>A. floribunda</i> Hexane	37.55 ± 0.79^d	6 ± 0.05^b
<i>A. floribunda</i> Dichloromethane	18.42 ± 0.65^c	$1.51 \pm 0.04^{b,a}$
<i>A. floribunda</i> Ethylacetate	14.62 ± 0.41^b	6.25 ± 0.05^b
<i>A. floribunda</i> Hydro-methanol	5.26 ± 0.55^a	5 ± 0.06^b

Data represent ability of extracts to inhibit DPPH's radical and parasite growth by 50%. Values represent mean $\pm$ SEM of triplicate analyses; different lower-case letters in the same column indicates significant difference at $p < 0.05$; ND = Not detectable

Fig. 3 Dose response curve showing percentage parasite growth inhibition after 48 h exposure to different doses of *A. floribunda* fractions using Mark III test

3.4 Gas chromatography with flame ionization detection (GC-FID) analysis on dichloromethane fraction (most active fraction)

Flavonoids profile of dichloromethane fraction showed the presence of higher level of kaempferol; other flavonoids found in high amount are catechin, naringenin, luteolin, epigallocatechin, quercetin and myricetin (Table 4). Table 5 shows the relatively abundance terpenes, they are azulene, α -pinene, pinene-2-ol, γ -terpinene, camphor, 1,8-cineole, borneol, neryl acetate, β -caryophyllene and humulene. Humulene was significantly higher in the terpenes profile compared to others. Terpenoids profile showed the presence of low α -amyrin, and β -amyrin in the DCM fraction but their concentrations were still much higher than others like taraxerol, lupeol, bauerenol acetate (Table 6). The analyzed *A. floribunda* dichloromethane fraction was generally low in alkaloids and volatile organic constituents (Tables 7 and 8) compared with the flavonoids fingerprint. However, alkaloids such as coniine, coniceine, cassine, spectraline and volatile organic constituents like butanoic acid, 2-methyl butanoic acid were detected in relatively high amount compared to other alkaloids and volatile organic compounds in DCM fraction. See the chromatograms for all the phytochemical fingerprint analyzed in supplementary Fig. S1-S6.

(a) Dose response curve showing percentage parasite growth inhibition after 48 h exposure to different doses of *A. floribunda* leaf extract, stem bark extract and oil; (b) Dose response curve showing percentage cell viability after 48 h exposure to different doses of *A. floribunda* leaf and stem bark extracts in Vero cells; (c) Dose response curve showing percentage cell viability after 48 h exposure to different doses of doxorubicin in Vero cells. Data are expressed as mean $\pm$ SEM of results from three independent experiments.

Table 4 Names and amount of the various flavonoids present in dichloromethane fraction

Name	Amount (mg/100 g)	Area	Amount/Area	Retention time (min)
(+) – Catechin	1.42	14.56	9.77×10^{-2}	13.75
Resveratrol	6.61×10^{-8}	0.47	1.41×10^{-7}	15.16
Genistein	9.76×10^{-7}	6.28	1.55×10^{-7}	15.50
Daidzein	5.81×10^{-7}	3.75	1.55×10^{-7}	15.63
Apigenin	2.02×10^{-1}	0.25	8.12×10^{-1}	15.75
Daidzein	3.51×10^{-7}	2.25	1.55×10^{-7}	16.31
Butein	3.21×10^{-7}	2.01	1.55×10^{-7}	16.61
Biochanin	7.2×10^{-7}	4.50	1.61×10^{-7}	17.16
Naringenin	1.40	0.10	13.74	17.29
Luteolin	1.78	0.15	12.21	17.67
Kaempferol	12.05	52.27	2.31×10^{-1}	18.06
(-) – Epicatechin	8.70×10^{-1}	53.37	1.62×10^{-5}	19.52
(-) – Epigallocatechin	6.27	5.37	1.17×10^{-5}	20.60
Gallocatechin	1.34×10^{-5}	27.94	4.80×10^{-7}	21.82
Quercetin	3.38	55.59	6.07×10^{-2}	22.60
(-) – Epicatechin-3-gallate	3.06×10^{-5}	7.45	4.11×10^{-6}	22.85
(-) – Epigallocatechin-3-gallate	2.41×10^{-5}	8.48	2.84×10^{-6}	23.47
Isorhamnetin	9.55×10^{-7}	18.95	5.04×10^{-8}	23.97
Robinetin	3.14×10^{-7}	1.99	1.57×10^{-7}	24.19
Myricetin	2.64	3.34	7.91×10^{-1}	24.79
Baicalein	2.65×10^{-7}	1.72	1.55×10^{-7}	25.17
Nobiletin	1.21×10^{-7}	7.83	1.55×10^{-7}	25.48
Baicalin	432×10^{-7}	2.78	1.56×10^{-7}	25.86
Isoquercetin	2.09×10^{-3}	3.51	5.95×10^{-4}	26.01
Tageretin	2.02×10^{-7}	1.30	1.56×10^{-7}	26.51
Artemetin	1.71×10^{-7}	1.12	1.55×10^{-7}	26.83
Silymarin	4.67×10^{-8}	0.31	1.55×10^{-7}	27.00
Hesperidin	1.49×10^{-7}	0.21	6.93×10^{-7}	28.41

4 Discussion

Allanblackia floribunda stem bark extract, leaf extract and oil were examined for their in vitro antiparasmodial activity, the stem bark and leaf infusions were thereafter assessed for their cytotoxic activity; fractionation of the most active extract (stem bark extract) was done with solvents of increasing polarity and phytochemical screening, in vitro antioxidant and antiparasmodial assays were further investigated on the various fractions, finally, phytochemical fingerprint analysis of the most active fraction (dichloromethane) was carried out using gas chromatography with flame ionization detector.

The result of this investigation showed that the inhibition of parasite growth by the plant extracts in vitro was dose dependent, the stem bark and leaf extracts had better antiparasmodial activities compared with the oil which had low activity against the strain of *Plasmodium* parasite used (*Pf3D7*). The stem bark extract of *A. floribunda* showed excellent antiparasmodial activity

followed by the leaf extract. However, the oil extract had weak activity against *Pf3D7* because the $IC_{50 \text{ Pf3D7}}$ was over 100 $\mu\text{g/mL}$, hence inactive against the parasite in vitro. IC_{50} is the inhibitory concentrations of the drug/extracts that can cause 50% reduction in parasitaemia level. According to the classification of Rasoanaivo et al. [14], the stem extract is considered very active while the leaf extract, is active. This investigation confirms the report of Ayoola et al. [6] and Azebaze et al. [7] that *A. floribunda* has antiparasmodial activity [24, 25]. From this investigation, we could establish that the most active part of this plant against chloroquine sensitive *P. falciparum* is the stem bark extract.

The result of the cytotoxicity study showed that the stem and leaf extracts of *A. floribunda* were non-toxic compared with the standard cytotoxic drug, doxorubicin (an anthracycline antibiotic), which was found to be highly cytotoxic. The selectivity indices, a ratio of CC_{50} to $IC_{50 \text{ Pf3D7}}$ revealed that the two extracts were selectively active against the parasite at the concentrations used but

Table 5 Names and amount of various terpenes present in dichloromethane fraction

Name	Amount (mg/100 g)	Area	Amount/Area	Retention time (min)
Azulene	11.15	2.34	4.76	9.46
α -Pinene	28.35	1.44	19.69	9.83
β -Pinene	5.01×10^{-4}	2.63	1.90×10^{-4}	10.80
cis-Ocimene	2.93×10^{-5}	4.48	6.53×10^{-6}	12.16
Myrcene	8.77×10^{-5}	3.64	2.41×10^{-5}	12.99
Allo Ocimene	1.03×10^{-4}	2.88	3.59×10^{-5}	13.20
Pinene-2-ol	1.56	0.58	2.70	13.78
α -Thujene	1.40×10^{-4}	21.63	6.47×10^{-6}	14.16
γ -Terpinene	2.08	2.37	8.80×10^{-1}	14.94
Citral	1.54×10^{-4}	1.44	1.07×10^{-4}	15.11
Camphor	4.45	1.93	4.38	15.25
Neral	2.19×10^{-4}	13.45	1.62×10^{-5}	15.39
1,8-Cineole	3.82	8.69	4.439×10^{-1}	16.61
Borneol	6.70	5.97	1.14	17.51
Linalool	8.25×10^{-5}	6.60	1.25×10^{-5}	17.70
Citronellal	8.25×10^{-5}	4.44	1.86×10^{-5}	18.19
Nerol	8.25×10^{-5}	4.61	1.79×10^{-4}	18.45
α -Terpineol	1.14×10^{-4}	9.38	1.22×10^{-5}	18.68
Terpinen-4-ol	3.97×10^{-1}	12.38	3.20×10^{-1}	19.02
Citronellol	1.51×10^{-4}	13.91	1.09×10^{-5}	19.49
α -Terpinenyl acetate	4.24×10^{-1}	9.84	4.31×10^{-2}	21.09
Ethyl cinnamate	2.45×10^{-4}	7.59	3.20×10^{-2}	21.46
Borneol acetate	3.15×10^{-2}	14.02	2.25×10^{-3}	21.61
Neryl acetate	2.01	8.65	2.32×10^{-1}	21.71
Geranyl acetate	7.67×10^{-2}	17.92	4.28×10^{-3}	21.85
Germacrene B	3.61×10^{-4}	18.91	1.91×10^{-5}	22.15
β -Caryophyllene	3.75	11.12	3.38×10^{-1}	22.39
Cyperene	6.04×10^{-5}	11.08	5.44×10^{-6}	23.34
[6] – Shogaol	6.81×10^{-3}	14.82	4.60×10^{-6}	24.02
α -Copane	8.86×10^{-5}	13.25	6.69×10^{-6}	24.79
Humulene	18.82	8.01	2.35	27.76
Valencene	1.30×10^{-4}	10.52	1.23×10^{-5}	28.02
β -Selinene	1.04×10^{-4}	16.27	6.40×10^{-6}	28.45
Aromadendrene	1.37×10^{-4}	11.19	1.22×10^{-5}	28.75
γ -Murolene	1.32×10^{-4}	1.24	1.06×10^{-4}	29.30

Table 6 Names and amount of the various terpenoids present in dichloromethane fraction

Name	Amount (mg/100 g)	Area	Amount/Area	Retention time (min)
Taraxerol	5.89×10^{-4}	189.40	3.11×10^{-6}	19.52
α -amyr in	1.66×10^{-1}	114.17	1.45×10^{-3}	20.80
β -amyrin	5.56×10^{-1}	156.96	3.55×10^{-3}	21.82
Lupeol	7.82×10^{-2}	95.13	8.22×10^{-4}	23.12
Baurenol acetate	2.45×10^{-3}	76.36	3.27×10^{-5}	24.61

Table 7 Names and amount of the various alkaloids present in dichloromethane fraction

Name	Amount (mg/100 g)	Area	Amount/Area	Retention time (min)
Dopamine	5.35×10^{-5}	259.04	2.07×10^{-7}	7.92
3 – methoxytyrarnine	1.18×10^{-5}	139.20	6.12×10^{-8}	9.72
Thiarubrine A	2.23×10^{-5}	57.30	3.90×10^{-7}	11.06
Aporphine	2.73×10^{-4}	118.13	2.31×10^{-6}	11.35
Crinine	5.49×10^{-5}	140.61	3.91×10^{-7}	13.42
Cherylline	5.25×10^{-5}	136.34	3.81×10^{-7}	13.74
Lycorine	1.17×10^{-4}	298.53	3.90×10^{-7}	14.70
Gelanthamine	1.92×10^{-4}	502.72	3.81×10^{-7}	15.51
Coniine	1.53×10^{-1}	241.28	6.34×10^{-4}	16.04
Crotsparine	2.42×10^{-3}	342.84	7.06×10^{-6}	16.46
Glaziovine	3.62×10^{-4}	443.74	8.15×10^{-7}	16.78
1,2-dithiin polyne	5.05×10^{-5}	311.84	1.61×10^{-7}	17.77
Coniceine	1.52×10^{-1}	428.22	3.54×10^{-4}	18.05
Lycopsamine	1.54×10^{-5}	334.30	4.62×10^{-8}	18.59
Echinatine	3.85×10^{-2}	511.24	7.53×10^{-5}	18.93
Montanine	1.38×10^{-4}	476.94	2.89×10^{-7}	19.10
Cassine	3.77×10^{-1}	1134.34	3.32×10^{-4}	19.52
Proaporphine	4.47×10^{-3}	551.28	8.10×10^{-6}	20.47
Hornolycorine	1.17×10^{-4}	345.21	3.38×10^{-7}	21.82
Tazettine	5.41×10^{-5}	170.80	3.16×10^{-7}	22.08
Spectaline	2.44×10^{-1}	561.44	4.35×10^{-4}	22.60
Dicentrine	2.15×10^{-3}	299.77	7.18×10^{-6}	22.85
Albomaculine	1.23×10^{-4}	171.66	7.30×10^{-7}	23.97
2-Descarboxyl-betanidin	2.00×10^{-4}	272.22	7.53×10^{-7}	24.20
2-Descarboxyl-betanin	1.76×10^{-4}	234.07	7.53×10^{-7}	24.61
Isoamaranthine	1.17×10^{-4}	165.37	7.10×10^{-7}	25.69
Amaranthin	3.02×10^{-5}	42.800	7.06×10^{-7}	27.29

the stem bark infusion was more selectively toxic to the parasite.

Among the four different fractions obtained from the stem bark extract, hydromethanol, ethylacetate and dichloromethane fractions showed the presence of more bioactive compounds including phenolics, terpenoids, tannins, flavonoids and quinones cardiac glycosides compared with hexane fraction which had the list phytochemicals. In vitro antiplasmodial activity showed that the dichloromethane fraction was highly active against the parasite compared with the ethylacetate, hydromethanol and hexane fractions. Literature report on the antimalaria activity of *Allanblackia gabonensis*, and *Allanblackia monticola* had parasite inhibition close to the data obtained for dichloromethane fraction against chloroquine sensitive *Plasmodium falciparum* [7]. Dichloromethane fraction significantly suppressed the growth of *P. falciparum* compared with ethylacetate, hydromethanol and hexane fractions. The better antimalarial activity of the dichloromethane fraction may be credited to its ability to extract highly active

antiplasmodial compounds of flavonoids and terpenes's origin that are intrinsic to *A. floribunda* stem bark compared with other solvents used for extraction.

GC-FID analysis of *A. floribunda* dichloromethane fractions showed significant levels of flavonoids (kaempferol, catechin, naringenin, luteolin, epigallocatechin, quercetin and myricetin) and terpenes (α -pinene, pinene-2-ol, γ -terpinene, camphor, 1,8-cineole, borneol, neryl acetate, β -Caryophyllene, and humulene). However, there appeared to be low levels of terpenoids, alkaloids and volatile organic compounds in the dichloromethane fraction. Some of the phytochemicals present in the dichloromethane fraction have been reported to be potent against *P. falciparum* [26–28]. The better antimalarial activity observed in dichloromethane fraction of *A. floribunda* agrees with the result obtained by Lekana-Douki et al. [29] who used different solvents to extract *Tetrapleura tetraptera* and *Copaifera religiosa*. These authors reported that the dichloromethane extract of the aforementioned plants had significant inhibition against parasite growth compared with other solvent used for extraction.

Table 8 Names and amount of the various volatile organic constituents present in dichloromethane fraction

Name	Amount (mg/100 g)	Area	Amount/Area	Retention time (min)
Acetic acids	1.34×10^{-4}	6.75	1.96×10^{-5}	6.79
Butanol	1.05×10^{-3}	6.51	1.61×10^{-4}	6.94
Chloromethane	2.74×10^{-7}	5.60	4.90×10^{-8}	7.37
Acrylonitrile	3.16×10^{-6}	16.05	1.97×10^{-7}	7.65
2-Propenal	3.63×10^{-7}	2.64	1.38×10^{-7}	8.50
Chloroethane	1.51×10^{-8}	1.26	1.20×10^{-7}	9.14
Bromomethane	1.82×10^{-8}	2.48	7.52×10^{-8}	9.33
Benzene	7.36×10^{-8}	4.56	1.61×10^{-7}	9.46
Butanoic acid	8.49×10^{-2}	41.43	2.05×10^{-2}	9.69
Toluene	6.73×10^{-8}	9.75	6.91×10^{-8}	10.00
Ethylbenzene	1.18×10^{-7}	1.45	8.13×10^{-8}	10.28
p-xylene	1.45×10^{-7}	1.79	8.11×10^{-8}	10.40
m-xylene	2.31×10^{-7}	1.17	1.97×10^{-7}	10.80
o-xylene	9.25×10^{-8}	3.36	2.75×10^{-8}	11.17
2-Methyl Butanoic acid	1.09	94.28	1.15×10^{-2}	11.37
Chloroethyl vinyl ether	3.85×10^{-7}	2.48	1.55×10^{-7}	11.58
cis-1,3-Dichloropropene	2.09×10^{-6}	5.30	3.95×10^{-7}	12.01
trans-1,3-Dichloropropene	2.54×10^{-7}	1.58	1.61×10^{-7}	12.44
1,1-Dichloropropene	1.34×10^{-7}	27.85	4.80×10^{-7}	12.83
2,2-Dichloropropane	7.39×10^{-8}	9.11	8.12×10^{-8}	13.08
1,3-Dichloropropane	5.64×10^{-8}	9.33	6.04×10^{-8}	13.21
Dichlorodifluoromethane	1.14×10^{-7}	1.89	6.05×10^{-8}	13.62
2-Methyl Butanoic acid	1.53×10^{-7}	1.31	1.17	13.78
o-Chlorotoluene	1.65×10^{-5}	27.31	6.06×10^{-7}	14.16
2-Methyl Butanoic acid ethyl ester	9.61×10^{-7}	1.00	9.60×10^{-1}	14.51
Bromochloromethane	3.72×10^{-7}	4.59	8.12×10^{-8}	14.77
(1-Methylpropyl) benzene	1.78×10^{-7}	2.19	8.11×10^{-8}	15.04
(1,1-Diethylethyl) benzene	3.12×10^{-7}	2.83	1.10×10^{-7}	15.50
1,4-Dichloro benzene	5.25×10^{-8}	1.28	4.11×10^{-8}	15.80
1,3-Dichloro benzene	5.44×10^{-7}	6.62	8.22×10^{-8}	16.13
1,2,3-Trichloropropane	4.75×10^{-6}	15.97	2.98×10^{-7}	16.55
1,2-Dichloroben zene	6.16×10^{-7}	3.90	1.58×10^{-7}	17.09
Bromobenzene	1.60×10^{-7}	1.04	1.55×10^{-7}	17.33
2-Methyl Butanoic acid ethyl ester	1.05×10^{-1}	13.41	7.81×10^{-3}	17.64
1,1,1,2-Tetrachloroethane	6.85×10^{-8}	1.13	6.06×10^{-8}	18.01
1,2,3-Trichlorobenzene	3.20×10^{-7}	2.07	1.55×10^{-7}	19.01
Hexachloro-1,3-butadiene	6.38×10^{-8}	0.41	1.55×10^{-7}	20.74

Literature has earlier reported that some of these compounds detected in dichloromethane fraction have strong inhibitory activity against chloroquine sensitive and chloroquine resistant strains of *P. falciparum* [30, 31]. Although the mechanism by which *A. floribunda* inhibited *Pf3D7* growth has not been identified, however some malaria researchers have suggested possible ways by which the pharmacologically active compounds (flavonoids and terpenes) which were detected in *A. floribunda* can exhibit schizonticidal activity against *Plasmodium* parasite. Herbert et al. [32] reported that terpenes

are capable of inhibiting *Plasmodium* parasite growth because of the structural similarity it has to the substrate needed for isoprenoids biosynthesis in *Plasmodium* parasite. Isoprenoids' biosynthesis depends on the DOXP/2-C-methyl-d-erythritol-4-phosphate (MEP) pathway in *Plasmodium falciparum* [33] while in humans, isoprenoids are synthesized via the mevalonate pathway [34]. This made isoprenoid biosynthesis a potential target for *P. falciparum* especially in the presence of phytochemicals such as terpenes. Terpenes inhibit dolichol biosynthesis in the trophozoite and schizont stages and it may

be acting through inhibition of the isoprenyl diphosphate synthases [32]. This family of enzymes catalyzes consecutive 1'-4 condensations of isopentenyl-pyrophosphate with the allylic substrate to form the linear backbone for all isoprenoid compounds, including prenylated proteins, prenylated quinones, and dolichol [35]. Due to the structural similarity terpenes have with the substrates required for dolichol synthesis, it might interfere with the parasite's biosynthesis of polyisoprenoids by competing with original substrates in enzyme-substrate reactions or by interfering with the mechanisms of elongation of isoprenic chains [32]. This metabolic interference may have accelerated parasite death when chloroquine-sensitive *P. falciparum* culture at ring stage were treated with *A. floribunda* stem bark infusion. Other possible mechanisms of parasite inhibition by the plant extracts could be through parasite enzyme inhibition (1, 5-lipoxygenase, carbonic anhydrase, and aspartic protease) by phenolics [31], prevention of merozoites invasion of erythrocytes by flavonoids, inhibition of haemozoin biocrystallization by alkaloids, inhibition of *P. falciparum* fatty acid biosynthesis and protein synthesis disruption by triterpenoids [36–39].

5 Conclusions

Findings from this study show that *A. floribunda* methanol stem bark and leaf extracts have some levels of activity against *Plasmodium falciparum* parasite and are relatively non-toxic to normal cells. Dichloromethane fraction was the most active fraction from the stem bark extract. Based on the GC-FID results, we are suggesting that flavonoids and terpenes may have been responsible for the excellent antimalarial activity of *A. floribunda* stem bark extract. This therefore supports the use of *A. floribunda* in the treatment of malaria in ethno-medicinal practices. The next phase of this study is to conduct activity guided isolation on the dichloromethane fraction to obtain the active antimalarial compound in *A. floribunda* stem bark.

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Declarations

Conflict of interests The authors declare that they have no competing interests.

Ethics approval The study was approved by the Institutional Ethics Review Committee, University of Benin (LS19114).

Data Availability All data generated and analyzed are included in this research article.

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