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In vitro Antiplasmodial Activity of Methanol Stem Extract of *Costus afer* Ker Gawl. (*Costaceae*) and its Residual Aqueous Fraction Against Some Drug-sensitive and Drug-resistant *Plasmodium falciparum* Strains

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ABSTRACT

Malaria remains a major public health problem in many countries in the tropical and sub-tropical regions of the world. The resistance of malaria parasites to commonly used antimalarial drugs including artemisinin-combination therapies in some Southeast Asian countries poses a great challenge to malaria control programmes. The aim of this study was to investigate the *in vitro* antiplasmodial activity of the methanol stem extract of *Costus afer* and its residual aqueous fraction against chloroquine-sensitive, chloroquine-resistant and artemether-resistant *Plasmodium falciparum* strains. Methanol stem extract of the plant was obtained by maceration of the powdered stem in 70% v/v methanol and residual aqueous fraction was obtained by successive solvent fractionation with dichloromethane, ethylacetate and finally n-butanol. *In-vitro* antiplasmodial activity was assessed using the candle jar method with some slight modifications. The methanol stem extract of *Costus afer* and its residual aqueous fraction produced significant and dose-dependent inhibitions of schizont growth in all the three *Plasmodium* strains with IC_{50} values of 8.86 and 10.51, 11.27 and 15.05, and 10.30 and 11.23 µg/mL against chloroquine-sensitive, chloroquine-resistant and artemether-resistant strains, respectively. Chloroquine phosphate was the most potent against chloroquine-sensitive strain (IC_{50} = 0.81 µg/mL) and Quinine sulphate was the most active against both chloroquine-resistant and artemether-resistant strains (IC_{50} values of 8.6 and 1.45 µg/mL, respectively). This study has shown that *Costus afer* stem extracts possess *in-vitro* antiplasmodial activity and it may be a good remedy for both chloroquine-sensitive, chloroquine-resistant and artemether-resistant *falciparum* malaria or a source of lead compounds for new antimalarial drugs.

Keywords: Antiplasmodial, *Costus afer*, *In vitro*, *Plasmodium falciparum.*

Introduction

Malaria is one of the most common infectious diseases in tropical and subtropical countries of the world. The disease is caused by a protozoan parasite of the genus *Plasmodium* and it is transmitted from the blood of an infected person to a healthy human by the bites of a female anopheles mosquito, through blood transmission, organ transplant or via shared use of needles or syringes that are contaminated with blood or congenitally from a mother to her foetus before or during delivery.[1,2] Human malaria is caused by five species of *Plasmodium* namely: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae* and recently in some parts of Southern Asia by *Plasmodium knowlesi*.[2,3] The world Health Organization in its 2018 World Malaria Report revealed that there were a total of 219 million cases of malaria in 2017, an increase of 3 million cases over the previous year, with a total death of 435,000.[4] Although the malaria case incidence has fallen globally since 2010, the rate of decline and mortality has stalled and even reversed in some regions since 2014.[4] The public health system in sub-Saharan Africa where malaria is endemic is very poor and in 2016, the total global investment in malaria control programmes was only about US$2.7 billion which was less than half of about US$6.5 billion required annually by 2020 to meet the 2030 targets of the WHO Global Malaria Strategy and there has been decline in investments in malaria control in many high-burden countries since 2014.[5] Although a large number of antimalarial drugs are available for the prevention and treatment of malaria, the quick emergence and spread of resistance to these drugs, including the reported resistance to artemisinins, the core compound of ACTs in four countries of Southeast Asia : Cambodia, Myanmar, Thailand and Vietnam has greatly limited their usefulness.[6,7] In addition to this problem of parasite resistance to antimalarial drugs, resistance of the mosquito vector to insecticides such as DDT (Dichloro-Diphenyl-Trichloroethane), HCH(Hexachloro-Cyclo-Hexane), Dieldrin and Pyrethroids (the insecticide used in Insecticide Treated Nets, ITNs and in Indoor Residual Spraying, IRS) has been reported in 27 countries in Africa and 41 countries worldwide and unless these

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problems are properly managed, they potentially threaten future progress in malaria control.[6]

Currently, no effective vaccine is available to fight human malaria; however, various antigen formulations are undergoing field trials. In particular, RTS, S/AS01E (Developed by GlaxoSmithKline in partnership with the PATH Malaria Vaccine Initiative) a vaccine based on the *P. falciparum* circumsporozoite protein and blood stage parasite proteins, has demonstrated promising results.[8-10] The vaccine has been found to reduce episodes of malaria in babies aged 6-12 weeks by 27 percent and by around 46 percent in children aged 5-17 months. The use of this vaccine has been associated with a few cases of meningitis and seizures,[11] and also a reduction in vaccine efficacy over time has been reported.[12] Vaccines based on the anti-merozoite surface protein of blood stages,[13] and a transmission-blocking vaccine are being tested.[14-16]

In spite of the progress made in the development of a malaria vaccine, lack of an effective and safe vaccine for the prevention of malaria constitute a major challenge in the long fight against malaria and for now drugs remain the only treatment option.[17]

From time immemorial, medicinal plants have been used as a source of medicine[18] and in Africa and other continents where malaria is endemic, plant based medicines often taken in the form of concoctions and decoctions and sometimes as steam baths have been the main source of treatment of malaria.[19-21] Many plant species having antimalarial properties have been identified in many cultural settings worldwide and many antimalarial drugs were derived from plant sources,[22] the most important being quinine from cinchona bark[23] and artemisinins from *Artemisia annua*.[24,25] Some of these medicinal plants include: *Azadirachta indica, Aspilla africana, Anogeisus leiocarpus, Mormodica balsamia*,[26] *Morinda lucida*, *Senna occidentalis*, *Citrus arantifolia*, *Uvaria chemae*,[27-32] *Alstonia boonei*,[33] *Anthocleista djalonensis*,[34] *Costus afer.*[35]

In Nigeria and sub-Saharan Africa, an estimated 80% of the population rely on traditional medicines and healers as the primary source of healthcare.[36] This is mainly due to easy accessibility and affordability of consulting with traditional healers as well as the perception of traditional medicine being natural and therefore safer compared to conventional medicine. In addition, malaria is more prevalent in the rural communities where modern heathcare facilities are grossly lacking.[37]

Costus afer Ker Gawl, commonly known as ginger lily or bush cane belongs to the family *Costaceae*. It is one of the 150 species of tall, perennial, and rhizomatous herbs of the genus *Costus.*[38] It can attain a height of up to 4 m. It is commonly found in moist and shady forest belt of Cameroon, Ghana, Guinea, Niger, Nigeria, Senegal, Sierra Leone and South Africa.[38,39] In Nigeria, *Costus afer* is known as 'Ireke omode' in Yoruba, 'Kakizuwa' in Hausa, 'Okpete' or 'Okpoto' in Igboland, 'Mbritem' in Efik. In Ijaw it is called 'Ogbodou', Anglophone Cameroon calls it 'Monkey sugar cane.[40]

Costus afer is commonly used as a medicinal plant throughout tropical Africa. It is highly valued for its anti-diabetic, anti-inflammatory and anti-arthritic properties in South-East and South-West Nigeria.[41] It is also widely used in Nigeria for the treatment of cough, malaria, veneral diseases, skin eruption and inflammation.[35]

Some of the reported pharmacological activities of *Costus afer* plant include: *in vitro* antibacterial and amoebicidal,[42] anti-inflammatory,[43] *in vitro* antioxidant,[44] abortificient and antidiabetic,[40,45] and antipyretic[46] activities.

A number of studies have been conducted on the pharmacological activities of *Costus afer* stem, leaf and rhizome extracts, but from our search of the literature, there are no studies of the antimalarial activities of this plant.. The objective of this study therefore, was to investigate the *in-vitro* antiplasmodial activity of the methanol stem extract of *Costus afer* and its residual aqueous fraction with a view to establishing a scientific basis for its use in traditional medicine for the treatment of malaria and for the development of new, effective and safe antimalarial drug or the discovery of lead compounds for the organic synthesis of new antimalarial drugs.

Materials and Methods

Parasite

Plasmodium falciparum: Chloroquine-sensitive, chloroquine-resistant and artemether-resistant *Plasmodium falciparum* strains were obtained from infected blood samples of patients attending the Specialist Hospital, Bauchi, Bauchi State, Nigeria in October, 2018 and the samples were immediately transferred into K-EDTA disposable plastic sample bottles, corked, mixed thoroughly and then transported to the Microbiology laboratory of the Department of Pharmaceutics and Pharmaceutical Microbiology at Ahmadu Bello University, Zaria in a thermo flask containing water maintained at 4ºC as demonstrated by Dacie and Lewis.[47]

Ethical approval

Ethical approval for the conduct of this research was obtained from the Research Ethics Committee, Bauchi State Ministry of Health, Bauchi. Nigeria (Protocol Approval No : NREC/12/05/2013/2017/57).

In vitro culture and maintenance of *Plasmodium falciparum*

Malaria parasites were continuously cultured according to the method of Trager and Jensen[48] and maintained with type O^+ erythrocytes suspended in complete culture medium (pH 7.3) which consisted of filter-sterilized RPMI 1640 solution enriched with 20 mM HEPES buffer, 10% type O^+ human serum, 1 g/L sodium bicarbonate and 40 µg/mL gentamicin. Incubation was at 37ºC in a dessicator containing a lighted candle to ensure the supply of required quantity of CO_2 (about 5%), O_2 gas (2%) and about 93% nitrogen gas as demonstrated by Hanne *et al*.[49] Monitoring of parasite growth was performed every 24 h during the daily refreshing of culture medium. The level of parasitaemia in the culture was kept at between 2 and 10%, with 5% haematocrit. Synchronization of the parasites was done before the tests were carried out.

Plant collection and identification

The plant was collected from Kagoro Hills in Kagoro, Kaura Local Government Area of Kaduna State, Nigeria in September, 2017 and it was identified and authenticated by Mallam Shehu Umar Gallah, a taxonomist in the Department of Biological Sciences, Kaduna State University, Kaduna. A voucher specimen (Voucher number 01/1087) was prepared and deposited at the Herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria. Nigeria for future references.

Preparation of crude extract

The leaves were plucked off from the stem and stem was then debarked. The pitch was cut into small pieces, air-dried in the laboratory at room temperature until constant weight was achieved. The dried stem was size-reduced using a wooden mortar and pestle. The pulverized stem was then ground into a fine powder using an electric grinder (Binatone). The resulting powder was stored in a dry air-tight container until it was ready for use. Three hundred grams (300 g) of the powdered stem was macerated with 1.8 L of 70%v/v methanol for 72 h with occasional shaking. The mixture was filtered using muslin cloth, followed by Whatman No. 1 filter paper. The filtrate obtained was then concentrated at 45ºC using a Rotary evaporator (Searchtech Instruments, RE 52-3) under reduced pressure. The residue was dried in an oven at 45ºC. The dried extract was weighed and the percentage yield obtained.

Fractionation of methanol extract

Ten grammes (10 g) of the methanol extract was dissolved in 15 mL sterile distilled water and filtered. The filtrate was successively partitioned with equal volumes of dichloromethane, ethyl acetate and n-butanol in a separating funnel in increasing order of polarity. The mixture was gently shaken and allowed to separate. The required layer was then decanted into a measuring cylinder. This process was repeated 4 times. The dichloromethane and ethyl acetate fractions were concentrated at 50ºC using a rotary evaporator under reduced pressure. The concentrates were then dried in an oven at 50ºC. The n-butanol fraction and the residual aqueous fraction were concentrated to dryness in a water bath at 50ºC.

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Preparation of extract/fraction/drugs solutions
Methanol extract/residual aqueous fraction: 10 mg each of the methanol stem extract of *Costus afer* and its residual aqueous fraction was dissolved in 10 mL of sterile distilled water to give a solution of 1 mg/mL crude extract/fraction.

Chloroquine phosphate: 5 mg chloroquine phosphate (Sigma-Aldrich) was dissolved in 25 mL 70% v/v ethanol to give a molar concentration of 625.2 µmol/L. To 5.8 mL of stock solution, 4.2 mL absolute ethanol was added to obtain 10 mL final stock containing 360 µmol/L (H). From the stock solution various dilutions were made with RPMI-1640 medium without sodium bicarbonate to give final well concentrations of chloroquine from 0.56 (Well, B) to 35.76 µmol/L (Well, H).

Quinine sulphate: 300 mg quinine sulphate tablet was dissolved in 100 mL distilled water to give 3 mg/mL or 3 g/L equivalent to 9,247.27 µmol/L. To 4.8 mL stock solution of Quinine, 5.2 mL distilled water was added to give a final stock solution containing 4,416 µmol/L (H). From this stock solution various dilutions were made with distilled water to get final well concentrations of 6.9 µmol/L (B) to 441.6 µmol/L (H).

In vitro antiplasmodial assay
The candle jar method as described by Moll *et al.*,[50] with slight modifications was used.

Dosing/Harvesting
For the negative control wells (A) for Chloroquine, 10 µL of each of the three strains of *Plasmodium falciparum* was pipetted into 3 separate wells and 90 µL of complete RPMI 1640 media with sodium bicarbonate (1 g/L) was added to each well. For the control wells (A) for Quinine, methanol stem extract of *Costus afer* and its residual aqueous fraction, 10 µL sterile distilled water, 80 µL complete RPMI media and 10 µL of each of the synchronized three *Plasmodium* strains were added to 3 separate wells.
For the dosing with the standard drugs, extracts and the parasites, pipetting started from the lowest dose (B) to the highest (H). For Chloroquine, 10 µL of chloroquine was added to each well, allowed to dry, then 90 µL of complete media was added to each well and finally 10 µL of synchronized *Plasmodium falciparum* was added to each well. For Quinine, methanol extract and residual aqueous fraction, 10 µL each of Quinine, methanol extract or fraction, 80 µL complete media and 10 µL synchronized *P. falciparum* were pipetted into each well starting from the lowest dose (B) to the highest dose (H). Parasitized cells were shaken gently before and during distribution. The microtiter plate was incubated in a lit and tightly closed candle jar dessicator to ensure the supply of required quantity of about 5% CO_2, 2% O_2 and 93% nitrogen, at 37°C for 30 h. After incubation, the microplate was taken out of the candle jar dessicator and the contents were harvested by removing the supernatant and the deposited red cells were used to make duplicate thick smears on clean microscope slides. The thick films were allowed to dry and carefully stained with 10% Giemsa solution diluted in phosphate buffer (pH 7.2) for 15 min before rinsing off with distilled water. The slides were allowed to dry and then examined microscopically to determine schizont growth in each well. Schizont growth in the test samples/standard drugs was compared with that of the negative control to calculate the percentage schizont inhibition as shown below;

$$\% \text{ schizont inhibition} = \text{Av. schizont count in negative control} - \text{Av. } \frac{\text{Schizont count in test sample/standard drug}}{\text{Av. Schizont in negative control}} \times 100$$

A graph of % schizont inhibition against log concentration was plotted to obtain the IC_{50} for the test samples and standard drugs against the three strains of *Plasmodium falciparum* used in this study.

Results and Discussion

Yield of methanol extract and fractions of Costus afer
The macerated 300 g of the powdered stem of *Costus afer* yielded 15.5 g crude methanol extract (5.2%). Fractionation of 10 g of the methanol extract with dichloromethane, ethyl acetate and n-butanol yielded 0.158 g, 0.127 g, 0.347 g of the various fractions respectively and 6.341g of residual aqueous fraction.

Effect of methanol stem extract of Costus afer and its residual aqueous fraction on chloroquine-sensitive Plasmodium falciparum strain
The methanol stem extract of *Costus afer* and its residual aqueous fraction as well as the standard drugs, chloroquine and quinine produced significant dose-dependent percentage inhibitions of schizont growth with the highest concentrations producing 79.9, 73.9, 77.9 and 80.7% schizont inhibition, respectively (Table1).
The concentrations of the extract, fraction and the standard drugs that inhibited 50% growth (IC_{50}) of the parasite were obtained from a plot of the percentage schizont inhibition against log concentrations.
The results of this study showed that the methanol stem extract of *Costus afer* and its residual aqueous fraction had significant *in vitro* antiplasmodial activity against chloroquine-sensitive *Plasmodium falciparum* strain with IC_{50} values of 8.86 µg/mL and 10.51 µg/mL, respectively. However, chloroquine phosphate with an IC_{50} value of 0.81 µg/mL showed the most potent antiplasmodial activity against this *plasmodium* strain. Quinine was also active against the chloroquine-sensitive *Plasmodium falciparum* strain and its IC_{50} value was 8.42 µg/mL. The order of antiplasmodial activity of the methanol stem extract of *Costus afer* and its residual aqueous fraction, chloroquine and quinine is as shown below;

Chloroquine > Quinine > Methanol stem extract > Residual aqueous fraction

Effect of methanol stem extract of Costus afer and its residual aqueous fraction on chloquine-resistant Plasmodium falciparum strain
The methanol stem extract of *Costus afer* and its residual aqueous fraction as well quinine produced significant dose-dependent percentage inhibitions of schizont growth with the highest concentrations producing 58.5, 75.6 and 87.2% schizont inhibition, respectively (Table 2).
The concentrations of the extract, fraction and quinine that inhibited 50% growth (IC_{50}) of the parasite were obtained from a plot of the percentage schizont inhibition against log concentrations.
The results of this study showed that the methanol stem extract of *Costus afer* and its residual aqueous fraction had significant *in vitro* antiplasmodial activity against chloroquine-resistant *Plasmodium falciparum* strain with IC_{50} values of 11.27 µg/mL and 15.05 µg/mL, respectively. Quinine had the lowest IC_{50} value of 8.80µg/mL indicating a higher antiplasmodial potency than the methanol extract and its residual aqueous fraction. The IC_{50} value obtained for chloroquine in this study was 3.70 µg/mL which was higher than its peak plasma concentration of 1.43 µg/mL, confirming that this *Plasmodium falciparum* strain was truly resistant to chloroquine.
The order of antiplasmodial potency of the methanol stem extract of *Costus afer* and its residual aqueous fraction and quinine on chloroquine-resistant *Plasmodium falciparum* strain is as shown below;
Quinine > methanol stem extract > residual aqueous fraction

Effect of methanol stem extract of Costus afer and its residual aqueous fraction on artemether-resistant P. falciparum strain
The methanol stem extract of *Costus afer* and its residual aqueous fraction and quinine showed significant dose-dependent schizont inhibition on artemether-resistant *Plasmodium falciparum* strain and they produced 76.8, 82.1% and 78.0% schizont inhibition, respectively at the highest concentrations tested (Table 3).
The concentrations of the extract, fraction and quinine that inhibited 50% growth (IC_{50}) of the parasite were obtained from a plot of the percentage schizont inhibition against log concentrations.
Quinine showed the most potent antiplasmodial activity (IC_{50} = 1.45 µg/mL) compared to the IC_{50} values of 10.30 and 11.23 µg/mL for the methanol stem extract of *Costus afer* and its residual aqueous fraction

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Table 1: Percentage schizont inhibition and IC_{50} values of methanol stem extract of *Costus afer* and its residual aqueous fraction on chlorquine-sensitive *Plasmodium falciparum* clinical isolate.

Sample	Concentration(µg/mL)	%Schizont inhibition	IC_{50} Value (µg/mL)
MSECA	1.56	20.48	8.86
	3.13	33.33	
	6.25	44.58	
	12.50	63.45	
	25.00	63.05	
	50.00	81.53	
	100.00	79.92	
RAFCA	1.56	30.18	10.51
	3.13	34.27	
	6.25	42.71	
	12.50	54.99	
	25.00	60.10	
	50.00	45.52	
	100.00	73.91	
Chloroquine	0.18	32.40	0.81
	0.36	46.11	
	0.71	46.73	
	1.43	53.89	
	2.86	11.21	
	5.72	77.65	
	11.44	77.88	
Quinine	2.21	27.04	8.42
	4.41	42.96	
	8.82	56.30	
	17.65	57.78	
	35.30	66.67	
	70.61	86.67	
	141.22	80.74	

MSECA = Methanol stem extract of *Costus afer,* RAFCA = Residual aqueous fraction of *Costus afer.*

respectively on artemether-resistant *Plasmodium falciparum* strain. The artemether-resistant *P. falciparum* strain used in this study was also resistant to chloroquine as the IC_{50} value of 5.52 µg/mL obtained was higher than the peak plasma concentration of chloroquine (1.43 µg/mL). The order of antiplasmodial potency of the methanol stem extract of *Costus afer* and its residual aqueous fraction and quinine on artemether-resistant *P. falciparum* strain is as shown below;

Quinine > methanol stem extract > residual aqueous fraction

Costus afer Ker Gawl is an indigenous West African medicinal plant of the family *costaceae* and its stem or leaf are often used as a medicinal herb in the treatment of various diseases including malaria, inflammation, rheumatoid arthritis, cough, epileptic attacks and haemorrhoids.[35,40,43] The result of the *in vitro* antiplasmodial activity assay showed that both the methanol stem extract of *Costus afer* and its residual aqueous fraction exhibited significant and dose-dependent antiplasmodial activity against chloroquine-sensitive, chloroquine-resistant and artemether-resistant strains of *Plasmodium falciparum*. Chloroquine phosphate demonstrated the highest potency (IC_{50} = 0.81 µg/mL) against the chloroquine-sensitive *Plasmodium falciparum* strain while quinine sulphate was the most potent against both the chloroquine-resistant *Plasmodium falciparum* and artemether-resistant *P.falciparum* strains with IC_{50} values of 8.86 and 1.45 µg/mL, respectively. The IC_{50} values for the methanol stem extract of *C. afer* and its residual aqueous fraction on the chloroquine-sensitive, chloroquine-resistant and artemether-resistant strains of *P. falciparum* used in this study ranged between 8.86 and 15.05 µg/mL (Table 4). According to the classification of *in vitro* antiplasmodial activity of plant extracts by Bickii *et al*.,[51] these plant extracts can be classified as being active in *in vitro* antiplasmodial activity. The presence of phytochemical constituents such as alkaloids, flavonoids, tannins, saponins and hydroxylanthraquinones in these extracts acting singly or synergistically may be responsible for the observed antiplasmodial activity. In a similar study of the *in vitro* antiplasmodial activity of ethanol extracts of four plants by Arnida *et al*.,[52] IC_{50} values of 2.86 ± 0.27, 9.38 ± 8.26, 25.48 ± 3.10 and > 250 µg/mL were reported for *Angiopteris evecta* tubers, *Ampelocissus rubiginosa* tubers, *Uraria crinite* roots and *Hydrolea spinosa* leaves, respectively against chloroquine-sensitive *P. falciparum* FCR3 strain. Zahari *et al*.,[53] reported potent *in vitro* antiplasmodial activity of two alkaloids out of the six isolated and charcaterized from from the bark of *Alseodaphne corneri* with IC_{50} values of 0.116 µM and 0.189 µM for

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Table 2: Percentage schizont inhibition and IC_{50} values of methanol stem extract of *Costus afer* and its residual aqueous fraction on chlorquine-resistant *Plasmodium falciparum* strain.

Sample	Concentration(µg/mL)	%Schizont inhibition	IC_{50} Value (µg/mL)
MSECA	1.56	33.66	11.27
	3.13	45.37	
	6.25	51.71	
	12.50	48.29	
	25.00	54.15	
	50.00	62.44	
	100.00	58.54	
RAFCA	1.56	26.70	15.05
	3.13	39.82	
	6.25	38.01	
	12.50	41.18	
	25.00	52.94	
	50.00	61.99	
	100.00	75.57	
Chloroquine	0.18	19.46	3.70
	0.36	23.98	
	0.71	28.51	
	1.43	31.67	
	2.86	45.70	
	5.72	58.82	
	11.44	64.25	
Quinine	2.21	21.71	8.60
	4.41	47.33	
	8.82	48.04	
	17.65	68.33	
	35.30	72.24	
	70.61	60.14	
	141.22	87.19	

MSECA = Methanol stem extract of *Costus afer,* RAFCA = Residual aqueous fraction of *Costus afer.*

(+)-norstephasubine and (+)-laurotetanine, respectively. These two potent compounds that revealed antiplasmodial activity also exhibited good antioxidant activities.[28]This synergism improves survival rates, reduces development of resistance and might decrease the transmission of drug resistant parasites.[53] Thus a single drug that possess both antiplasmodial and antioxidant activities might be a better choice compared to treatment with multiple drugs.[53]

The moderate *in vitro* antiplasmodial activity (IC_{50} = 10.30 -15.05 µg/mL) for the methanol stem extract of *Costus afer* and its residual aqueous fraction obtained in this research against the chloroquine-resistant and artemether-resistant *Plasmodium falciparum* strains is similar to that of do Nascimento *et al*.,[55] who reported an IC_{50} value of 23.68 ± 3.08 µg/mL, SI > 10.56, for the hydroethanolic extract *Aspidisperma excelsum* bark against chloroquine-resistant *P. falciparum* W2 strain. In the same study by do Nascimento *et al*.,[55] an alkaloid extract (Ae-Alk2) of the trunk bark of *Aspidosperma excelsum* showed a higher *in vitro* antiplasmodial activity with an IC_{50} of 8.75 ± 2.26 µg/mL and SI of 21.16 and the authors attributed the difference in the antiplasmodial activity of the hydroethanolic extract and that of the alkaloid extract to the lower content of alkaloids in the former. In an earlier work by Kassa *et al*.,[56] an IC_{50} value of 11.00 µg/mL was obtained for ethanol stem bark extract of *Bersema abyssinica* against *P. falciprum* tine-FAC-2/Ethiopia strain and chloroform extract of *Maesalanceolata* leaves had an IC_{50} value of 1.6 µg/mL against *P. falciparum* clinical isolates.[57]

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Table 3: Percentage schizont inhibition and IC_{50} values of methanol stem extract of *Costus afer* and its residual aqueous fraction on artemether-resistant *Plasmodium falciparum* strain.

Sample	Concentration(µg/mL)	%Schizont inhibition	IC_{50} Value (µg/mL)
MSECA	1.56	18.94	10.30
	3.13	34.88	
	6.25	47.51	
	12.50	57.81	
	25.00	60.80	
	50.00	71.76	
	100.00	76.41	
RAFCA	1.56	6.26	11.23
	3.13	43.84	
	6.25	48.16	
	12.50	59.40	
	25.00	56.16	
	50.00	64.79	
	100.00	82.07	
Chloroquine	0.18	15.70	5.52
	0.36	13.37	
	0.71	34.88	
	1.43	37.21	
	2.86	-13.95	
	5.72	52.91	
	11.44	53.49	
Quinine	2.21	51.15	1.45
	4.41	61.31	
	8.82	62.95	
	17.65	58.36	
	35.30	68.20	
	70.61	77.70	
	141.22	78.03	

MSECA = Methanol stem extract of *Costus afer,* RAFCA = Residual aqueous fraction of *Costus afer.*

Table 4: *In vitro* sensitivities (IC_{50} values) of three *Plasmodium falciparum* strains to methanol stem extract of *Costus afer* and its residual aqueous fraction, chloroquine and quinine.

	IC_{50} Values (µg/mL)		
Extract/Fraction/Standard drug	**CQ-sensitive *P. falciparum* Strain**	**CQ-resistant *P. falciparum* strain**	**Artemether-resistant *P. falciparum* strain**
MSECA	8.86	11.27	10.30
RAFCA	10.51	15.05	11.23
Chloroquine	0.81	**3.70^{R}**	**5.52^{R}**
Quinine	8.42	8.60	1.45

IC_{50} values with letter in red colour and R as superscript = Resistant (Inactive) , MSECA = Methanol stem extract of *Costus afer*, RAFCA = Residual aqueous fraction of *Costus afer*, CQ = Chloroquine.

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Conclusion

From the results of this study, it can be concluded that methanol stem extract of *Costus afer* and its residual aqueous fraction possess good *in vitro* antiplasmodial activity against the chloroquine-sensitive, chloroquine-resistant and artemether-resistant *Plasmodium falciparum* strains used in this research and this plant may be an effective remedy for chloroquine-sensitive, chloroquine-resistant and artemether-resistant *falciparum* malaria or it could be a source of lead compounds upon which to base organic synthesis for new antimalarial drugs. Further *in vitro* and *in vivo* screening supported by bioassay-guided isolation of bioactive molecules of this plant as well as cytotoxicity tests are suggested.

Conflict of Interest

The authors declare that there is no conflict of interest.

Authors' Declaration

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

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