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Antiplasmodial and in vitro antioxidant potentials of crude aqueous and methanol extracts of *Chasmanthera dependens* (Hochst)

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Abstract

Background: Malaria is a major cause of death in tropical countries such as Nigeria. *Chasmanthera dependens* (Hochst) is a wild plant used by traditional healers for the treatment of malaria and other health challenges. This study evaluated the 'Antiplasmodial and in vitro antioxidant potentials of crude aqueous and methanol extracts of leaves, root and stem of *Chasmanthera dependens* (Hochst)'.

Results: Methanol extracts of leaves and roots of this plant exhibited higher antimalarial activities than other extracts. The level of parasitemia was significantly ($p < 0.05$) reduced in the treated animals when compared to untreated ones. Antiplasmodial activities of methanol extracts of roots and stem did not differ significantly ($p < 0.05$) from their aqueous extracts. An increase in body weight and higher survival rates were recorded in all the treated groups. Scavenging of DPPH, NO, H₂O₂ radicals and reducing power ability were concentration-dependent. Methanolic extracts of *C. dependens* root showed the best reducing activity. Phytochemical analyses revealed the presence of tannins, saponins, alkaloids, terpenoids, cardiac glycosides, flavonoids, phenols, phytate and oxalate.

Conclusion: Antimalarial and antioxidant properties of these extracts could be credited to the synergy between the phytoconstituents in this plant. Findings from this study justified the use of this plant in the treatment of malaria and other ailments.

Keywords: *Chasmanthera dependens*, Mice, *Plasmodium berghei*, In vivo study, Antimalaria

Background

Malaria has been implicated as a major reason for the sickness and death of about two million infants worldwide annually (Okafor and Oko-Ose 2012). Globally, this disease has a higher death rate than other infectious diseases (World Health Organisation 2015). It is common in the poor, less-developed countries of the world and

widely spread in tropical countries such as Nigeria (Okafor and Oko-Ose 2012).

About 92% of pregnant women and infants less than 5 years of age are very prone to malarial attacks due to their lowered immunity (Adebayo and Kareth 2010).

Treatment of this parasitic disease still relies on the administration of drugs whose efficacy is constantly frustrated by the continuous appearance of resistant strains of the parasite, coupled with the high cost of these orthodox drugs. Consequently, many caregivers, herbal homes and traditional medicine practitioners resort to the use of various traditional herbs and plants in the management of malaria. Herbal or traditional medicines are the first

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form of treatment given to many patients with suspected symptoms of malaria, especially in tropical countries, such as Nigeria, Ghana, Mali and Zambia (David et al. 2014). It is general knowledge that plants are the origin of some known orthodox antimalarial drugs such as quinine and artemisinin derivatives (Mustofa et al. 2007). It is therefore imperative to scientifically investigate the anti-plasmodial potentials of these traditional plants, which might become sources of new antimalarial drugs.

Antioxidants are substances that prevent cells from being damaged by the effects of Reactive Oxygen Species (ROS) known as 'free radicals.' They have sufficient electrons which they easily donate to compounds or molecules that are electron-deficient. These electron-deficient compounds have unpaired valence electrons (Aluko et al. 2013). In the bid to get more electrons, they may attack cells or biomolecules in the human body resulting in the generation of many diseases (Aluko et al. 2013). As they get sufficient electrons, their actions are stabilized. An imbalance between antioxidants and Reactive Oxygen Species could result in oxidative stress and cellular damage. According to studies by Uddin et al. (2018) and Jayasri et al. (2019), natural antioxidants serve the purpose of maintaining good health and well-being, as well as prevention of chronic and degenerative diseases, such as cardiac and cerebral ischemia, atherosclerosis, neurodegenerative disorders, carcinogenesis, rheumatic disorder, damage to DNA and aging.

Chasmanthera dependens (Hochst) is a flowering plant of the Menispermaceae, commonly called *Chasmanthera* (Fig. 1). It grows around forest margins, savannah or secondary forest. It thrives in moist and dense evergreen forest and grows up to 1500 m preferring well-drained soils. Usually, it is intentionally cultivated in homes or gardens as a medicinal plant. It is called 'aguru' in Igbo (South-East Nigeria), and 'atoo' in Yoruba (South West Nigeria).

It has been used in the treatment of health problems such as red-eye infections (Ogunlesi et al. 2008); venereal diseases, convulsions, snakebites, epilepsy, and in the management of fractures (Ogunlesi et al. 2008). It is also useful in controlling physical and nervous weaknesses (Iwu et al. 1999). According to Morebise et al. (2001), methanol extract of the dried leaves has anti-inflammatory and analgesic properties. Similarly, Adekunle and Okoli (2002) have reported antifungal effects of crude aqueous and ethanol extracts of the leaves. Antimicrobial activities of *C. dependens* stem on fungal yeast, Gram+ and Gram- bacteria were reported by Githinji et al. (2010). In vitro, antileishmanial and immunomodulatory activities of *C. dependens* stem and leaves were reported by Iwu et al. (1999). Aqueous extract of *Chasmanthera dependens* roots demonstrated pro-spermatogenic, fertility-enhancing, and androgenic activities in male rats



Fig. 1 *Chasmanthera dependens*

(Quadri and Yakubu 2017). However, the antimalarial and antioxidant activities of this plant are unavailable in the literature.

This study, therefore, evaluated the antimalarial and in vitro antioxidant potentials of aqueous and methanolic extracts of leaves, stem and roots of the plant.

Methods

Sample collection and identification

Fresh leaves, stems and roots of *Chasmanthera dependens* used in this study were collected from a fallow farmland at Ahiazu Mbaise L.G.A of Imo State, located between latitude 5°19' and 5°32'N and longitude 7°12' and 7°20'E (Ifeanyichukwu et al. 2016). They were transported to the laboratory where they were verified and authenticated by a taxonomist in the Department of Biological Sciences, Federal University of Technology, Owerri, Nigeria, with voucher number FUTOH- 006. The plant parts were sliced to reduce their size, air-dried at room temperature and pulverized using a laboratory mill. The coarse powder was then sieved, using a 2 mm mesh sieve, to obtain smooth samples.

Preparation of extracts

Using cold maceration, 200 g of each powdered plant material was separately soaked in 1000 ml of distilled water to get the aqueous extract. Samples were macerated for 48 h as described by Ene et al. (2013).

Methanol extraction was done according to Dejen et al. (2018). Two hundred (200 g) grams of the various plant materials were extracted with 1000 ml of 80%v/v methanol solvent using soxhlet extraction. After extraction, both aqueous and methanol extracts were filtered,

using a clean muslin cloth and Whatman number 1 filter paper to remove residues. Filtrates were concentrated and dried using a rotary evaporator (RE- 52A, Searchtech Instruments) set at a temperature of 50 °C to remove the solvent. Extracts were weighed and stored in well-labeled bijou bottles and kept at 4 °C in a refrigerator until needed.

Phytochemical screening

Crude aqueous and methanolic extracts of leaves, root and stem of *C. dependens* were screened for the presence of phytochemicals using standard methods (Trease and Evans 1989; Harborne 1973; Sofowora 1993).

Animal grouping

Fifty-four (54) male Swiss albino mice of 18–25 g weight were used for this study. They were obtained from the animal house of Veterinary Medicine, University of Nigeria, Nsukka. They were kept in animal cages in the laboratory environment for 14 days with feed (rat pellets) and water ad libitum and monitored under 12-h, light and dark cycles, in well-aerated cages. They were then separated into 9 groups of 6 mice each according to their body weights. On the basis of their position on the rack, cages were given numerical designation. For each treatment group, a cage was selected randomly from the pool of cages. The cages were then randomized within the treatment group. Animals in each group were assigned marks on different parts of their bodies using picric acid for easy identification. The cages were then randomized within the treatment group. Animals were grouped and treated as follows: Group 1- feed and water (normal control group), Group 2- artesunate (standard control group), Group 3- negative control, Group 4- methanolic extract of *Chasmanthera dependens* root, Group 5- methanolic extract of *Chasmanthera dependens* leaves, Group 6- methanolic extract of *Chasmanthera dependens* stem, Group 7- aqueous extract of *Chasmanthera dependens* root, Group 8- aqueous extract of *Chasmanthera dependens* leaves, Group 9- aqueous extract of *Chasmanthera dependens* stem. The study was carried out with ethical approval obtained from the Department of Biochemistry, Federal University of Technology, Owerri, with the reference number-FUTO/BCH/DEC/XXI/03/01.

In vivo culture of *P. berghei*

Before the experimental animals were inoculated with the infected blood, parasitized blood from a donor mouse was first diluted with normal saline as described by Kabiru et al. (2012). This inoculum was prepared such that each 0.2 ml contained approximately 1×10^7 parasitized erythrocytes. The animals were then injected with 0.2 ml of this suspension through the intraperitoneal

route. Animals in groups 2–9 were inoculated with chloroquine-resistant *Plasmodium berghei* (NK65) on day zero (D_0) through the intraperitoneal route.

Treatment of infected mice

A standard 4-day curative test of David et al. (2004) and Peter and Anatoli (1998) was used for the in vivo anti-malarial test. Only animals with percentage parasitemia of 60% or more were included in the treatment groups. Animals whose percentage parasitemia were below 60% were excluded in the treatment groups. Animals in group one were not infected with the parasites, while animals in groups two to nine were infected with *Plasmodium berghei*. Seventy-two hours (72 h) after infection, animals in the treatment groups (2–9) were orally given 200 mg/kg body weight of the standard antimalarial drug (artesunate) and 200 mg/kg body weight of the extracts once daily for 14 days. A dose of 200 mg/kg body weight was administered based on the result of the acute toxicity test which was earlier determined to be 5000 mg/kg body weight for the crude aqueous and methanol extracts of the leaves and roots and less than 3808 mg/kg body weight for the methanol and aqueous extracts of the stem of this plant. Treatment was administered based on the average body weight of the animals. The untreated control group received no treatment. The extracts were re-dissolved in distilled water to the required dose level. Parasitemia was accessed in all the groups on day 7 (4 days after treatment) and day 14 (7 days post-treatment) as described by Kabiru et al. (2012) and results were compared with the untreated group. Blood smears obtained from a cut on the tip of the tail of the mice were fixed for 15 min with methanol, Giemsa-stained for 25 min, washed off with phosphate buffer (pH 7.2), dried and viewed under the light microscope (magnification = $\times 100$) under a drop of immersion oil to determine parasitemia count. Percentage parasitemia in treated animals was determined as outlined by Iwalewa et al. (1997):

$$\% \text{ Parasitemia} = \frac{\text{No. of Parasitemia in treated}}{\text{No. of Parasitemia in control}} \times \frac{100}{1}$$

Euthanization of animals

At the termination of this experiment, animals used for the study were euthanized following the procedure described by Moody et al. (2014). They were kept in a transparent perspex chamber and euthanized with slow rising concentrations of carbon dioxide using a gradual fill (30% chamber volume per minute) technique. These animals were monitored closely till they lost consciousness.

In this experiment, three different investigators were involved as follows: the first investigator administered the

treatment based on the randomization table. This investigator was the only person aware of the treatment allocation. A second investigator unaware of the treatment was responsible for checking the toxicity and parasitemia level, while a third investigator was responsible for the euthanization procedure.

Determination of radical scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The method of Lim and Quah (2007) was used to determine the free radical scavenging activity of the plant extracts against 2,2-diphenyl-1-picrylhydrazyl radical. Various concentrations of the extracts (0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml) were mixed vigorously in 5 ml of DPPH solution (0.1 mM in methanol) in test tubes. These were allowed to stand for 30 min. in a dark chamber at room temperature. A standard solution of antioxidant (ascorbic acid) was treated in the same manner and incubated as well together with a reagent blank. At the end of the incubation process, the absorbance of the standard and extract was read with a spectrophotometer. Ability to decolourize DPPH was measured against blank at a wavelength of 517 nm. Percentage inhibition was then calculated thus:

$$\% \text{ Inhibition} = \frac{(\text{Abs Blank} - \text{Abs sample}) \times 100}{\text{Abs Blank}}$$

where Abs blank, absorbance of blank; Abs sample, absorbance of the sample.

Determination of nitric oxide scavenging activity

The methods of Panda et al. (2009) and Alisi and Onyeze (2008) were used to measure the nitric oxide scavenging activity of the plant extracts. 4 ml of the extract was added at different concentrations (0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml) to 1 ml of sodium nitroprusside (SNP) solution (5 mM) in test tubes, and the mixtures were incubated at 27 °C for 2 h. An aliquot (2 ml) of the incubated solution was taken and diluted with 1.2 ml of Griess reagent (1% sulfanilamide in 5% H₃PO₄ and 0.1% naphthyl ethylenediamine-dihydrochloride). The absorbance of the chromophore, which was formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with naphthyl ethylenediamine-dihydrochloride, was immediately read at 550 nm and compared with the absorbance of standard ascorbic acid.

$$\text{Nitric oxide scavenging activity (\%)} = \frac{\text{Abs(control)} - \text{Abs(sample)}}{\text{Abs(control)}} \times 100$$

where Abs (control), Absorbance of the control; Abs (sample), absorbance of the extract/standard.

Determination of hydrogen peroxide scavenging activity

This determination adopted the method of Ngonda (2013). A solution of hydrogen peroxide (2 mMol/l) was prepared in phosphate buffer (pH 7.4). Different concentrations of the extracts (0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml) were each added to hydrogen peroxide solution (0.6 ml) in test tubes. The absorbance of hydrogen peroxide at 230 nm was read after 10 min. against a blank solution (only phosphate buffer) and compared with the standard (ascorbic acid). The hydrogen peroxide activity of the extracts was then calculated using the equation:

$$\text{H}_2\text{O}_2 \text{ activity (\%)} = \frac{\text{Abs(control)} - \text{Abs(sample)}}{\text{Abs(control)}} \times 100$$

where Abs (control), absorbance of the control; Abs (sample), absorbance of the extracts/standard.

Determination of reducing power

The reducing power of the plant's extracts was determined using the ferric reducing antioxidant power (FRAP) assay as recorded by Sofidiya et al. (2006). Various concentrations (0.5, 1.0, 1.5, 2.0, and 2.5 mg/ml) of the extracts were each added to 1 ml of deionized water and were mixed with 2.5ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferrocyanide. The mixture was left to incubate at 50°C for 20 min. Then, 2.5 ml of 10% trichloroacetic acid was added and it was centrifuged at 3000 rpm for 10 min. Thereafter, 2.5 ml of the decant was mixed with 0.5 ml of 0.1% FeCl₃. Absorbance was measured at 700 nm. The reducing power of the extracts was determined from the graph of optical density against the concentration of the extracts. Reducing power (RP 0.5_{AU}) was determined as the concentration that gave a 0.5 absorbance reading.

Data analyses

Data obtained were expressed as mean ± standard error of the mean (SEM) and analyzed using analysis of Variance (ANOVA) procedure (statistical analysis system (SAS) package version 20 software). Mean differences were subjected to Duncan's multiple range test (DMRT) at a significant difference of *p* < 0.05. For in vitro antioxidant activities, the inhibition data (mean values

from triplicate determinations) were fitted into kinetic Eqs. (2–5) which include sigmoid ABC, logistic dose–response ABC, ABCD and Weibull cumulative ABCD models, respectively, using 2D Systat version 5.01, USA and Sigma plot 10. 0 Systat USA. Percentage inhibition was calculated relative to control using Eq. 1.

Equations

$$\% \text{ Inhibition} = \left[\frac{\text{Control}_{\text{ABS}} - \text{Test}_{\text{ABS}}}{\text{Control}_{\text{ABS}}} \right] \times 100 \quad (1)$$

Sigmoid a, b, c model

$$y = \frac{a}{1 + \exp\left(-\left(\frac{x-x_0}{b}\right)\right)} \quad (2)$$

$$y = \frac{a}{1 + \left(\frac{x}{b}\right)^c} \quad (3)$$

$$y = a + \frac{b}{1 + \left(\frac{x}{c}\right)^d} \quad (4)$$

$$y = a \left[1 - \exp \left[- \left[\frac{x + c(\ln 2)^{1/d} - b}{c} \right]^d \right] \right] \quad (5)$$

presence of tannins, saponins, alkaloids, cardiac glycosides, terpenoids, steroids, phenols, flavonoids, phytate and oxalate (Table 1). Alkaloids and phenols were not detected in the aqueous extract of the stem (Table 1).

In vivo antimalarial test

From the results obtained, administration of the extracts significantly reduced ($p < 0.05$) parasite count in all animals in the treated groups when compared to the negative control (group 2) whose percentage parasitemia significantly increased (Table 2). By the 14th day (7 days post-treatment), results obtained revealed that percentage parasitemia further reduced significantly ($p < 0.05$) in the treated groups but increased in the untreated control group. When compared to the standard antimalarial drug (artesunate), results obtained from the plant extracts were lower. Among the extracts, the methanol extract of the root of this plant showed the highest antimalarial activity. This extract reduced parasitemia from 75.2% on day 3 to 27.6% on day 14 (Table 2). In the negative control group, the percentage of parasitemia increased from 63.0% on day 3 to 79.2% on day 14. The bodyweight of animals in this group was also reduced (Table 3). Antimalarial activity observed in the methanol extract of the leaves of this plant differed significantly ($p < 0.05$) from that of the aqueous extract of the same plant part. Although methanol and aqueous extracts of the leaves, stem and roots of *C. dependens* significantly reduced the percentage of parasitemia in the treated animals, they did not completely clear the parasites in these treated groups as shown in Table 2.

Results

Phytochemical screening

Phytochemical analysis of methanol and aqueous extracts of leaves, roots and stem of *C. dependens* showed the

Table 1 Qualitative phytochemical analysis of crude aqueous and methanol extracts of the leaves, roots and stems of *Chasmanthera dependens*

Phytochemical	Specific test	CDLM	CDSM	CDRM	CDLA	CDSA	CDRA
Tannins	Ferric chloride test	+	+	+	+	+	+
Saponins		+	+	+	+	+	+
Alkaloids	Wagner’s test Dragendorff’s test	+	+	+	+	ND	+
Cardiac Glycosides	Keller-Killani test	+	+	+	+	+	+
Terpenoids	Salkowski test	+	+	+	+	+	+
Steroids	Liebermann-Burchard test	+	+	+	+	+	+
Phenols	Ferric chloride test	+	+	+	+	ND	+
Flavonoids	Sodium hydroxide test	+	+	+	+	+	+
Oxalate	Oxalate test	+	+	+	+	+	+
Phytate	Colourimetric test	+	+	+	+	+	+

+, present; ND, not detected; CDRM, *C. dependens* root methanol; CDLM, *C. dependens* leaves methanol; CDSM, *C. dependens* stem methanol; CDRA, *C. dependens* root aqueous; CDLA, *C. dependens* leaves aqueous; CDSA, *C. dependens* stem aqueous

Table 2 In vivo antimalarial activities of crude aqueous and methanolic extracts of the roots, leaves and stems of *Chasmanthera dependens* on the percentage parasitemia of *Plasmodium berghei* infected male Swiss albino mice

Groups/Extracts	Average %		Parasitemia	
	Before inoculation (Day 0)	Before treatment (Day 3)	After treatment (Day 7)	After treatment (Day 14)
1. NC	0.00	0.00	0.00	0.00
2. STD.C	0.00	69.0	4.6	0.00
3. NEG.C	0.00	63.0	72.4	79.2
4. CDRM	0.00	75.2	40.6	27.6
5. CDLM	0.00	68.2	40.2	30.2
6. CDSM	0.00	73.2	57.4	47.0
7. CDRA	0.00	67.0	42.8	31.2
8. CDLA	0.00	76.2	50.0	39.0
9. CDSA	00.00	76.4	61.4	49.4

NC, normal control; NEG.C, negative control; STD.C, standard control; CDRM, *C. dependens* root methanol; CDLM, *C. dependens* leaves methanol; CDSM, *C. dependens* stem methanol; CDRA, *C. dependens* root aqueous; CDLA, *C. dependens* leaves aqueous; CDSA, *C. dependens* stem aqueous

Table 3 Effects of crude aqueous and methanolic extracts of leaves, stem and roots of *Chasmanthera dependens* on percentage body weight and rate of survival of *Plasmodium berghei* infected male Swiss albino mice

Groups/Extracts	Number of animals	Initial body weight of animals (g)	Final body weight of animals (g)	% Body weight of animals	% Survival rate
1. NC	6	23.0	26.7	16.1	100
2. NEG.C	6	20.0	17.5	12.5	33.3
3. STD.C	6	18.0	20.8	15.6	100
4. CDRM	6	22.0	24.3	10.5	100
5. CDLM	6	22.0	24.1	9.6	100
6. CDSM	6	25.0	26.7	6.8	50
7. CDRA	6	24.0	26.4	10.0	100
8. CDLA	6	25.0	27.0	8.0	83.3
9. CDSA	6	24.0	25.4	5.8	50

NC, normal control; NEG.C, negative control; STD.C, standard control; CDRM, *C. dependens* root methanol; CDLM, *C. dependens* leaves methanol; CDSM, *C. dependens* stem methanol; CDRA, *C. dependens* root aqueous; CDLA, *C. dependens* leaves aqueous; CDSA, *C. dependens* stem aqueous

Radical scavenging activities of the extracts against 2,2-diphenyl-1-picrylhydrazyl (DPPH)

Activities of the extracts of *C. dependens* were dose-dependent in the scavenging of DPPH radicals. Aqueous extract of the root of this plant showed the highest activity, with an IC₅₀ value of 1.10 ± 0.12 mg/ml (Table 4). DPPH radical scavenging activities of the extracts were significantly (*p* < 0.05) lower than that of the standard antioxidant whose IC₅₀ value was 0.58 ± 0.05 mg/ml. Results obtained from this study show that the DPPH radical scavenging property of the aqueous and methanol extracts was also largely sigmoidal (Table 4). Figure 2 shows the effects of crude aqueous and methanol extracts of leaves, stem and roots of *C. dependens* and ascorbic acid (AA) on scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical.

Nitric oxide scavenging activities of the extracts

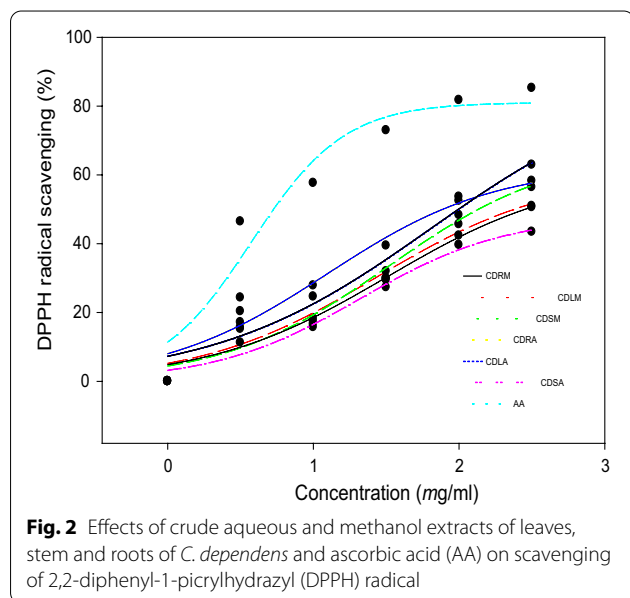
Nitric oxide radical scavenging activities of crude aqueous and methanol extracts of leaves, stem and roots of *C. dependens* and ascorbic acid (AA) standard are shown in Table 5. Results obtained show that methanol extracts scavenged more than the aqueous extracts as shown in their IC₅₀ values evaluated from the inhibition graph (Fig. 3). Activities of the extracts were however significantly (*p* < 0.05) lower than that of the standard. Results presented in Fig. 3 show the ability of extracts to scavenge nitric oxide, generated *in vitro* by sodium nitroprusside, followed by a sigmoid ABC model.

Table 4 Threshold Inhibitory concentration (IC₅₀) of crude aqueous and methanolic extracts of leaves, stem and roots of *C. dependens* and ascorbic acid standard on DPPH radicals

Extracts	Threshold inhibitory concentration (*IC ₅₀)	Mathematical model	Equation	R ²
CDRM	1.81 ± 0.32	Sigmoid (a, b, c)	$y = \frac{a}{1 + \exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.941
CDLM	1.44 ± 0.14	Sigmoid (a, b, c)	$y = \frac{a}{1 + \exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.956
CDSM	1.52 ± 0.10	Sigmoid (a, b, c)	$y = \frac{a}{1 + \exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.975
CDRA	1.10 ± 0.12	Sigmoid (a, b, c)	$y = \frac{a}{1 + \exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.939
CDLA	1.81 ± 0.32	Sigmoid (a, b, c)	$y = \frac{a}{1 + \exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.942
CDSA	1.51 ± 0.14	Sigmoid (a, b, c)	$y = \frac{a}{1 + \exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.966
AA	0.58 ± 0.05	Sigmoid (a, b, c)	$y = \frac{a}{1 + \exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.934

CDRM, *C. dependens* root methanol; CDLM, *C. dependens* leaves methanol; CDSM, *C. dependens* stem methanol; CDRA, *C. dependens* root aqueous; CDLA, *C. dependens* leaves aqueous; CDSA, *C. dependens* stem aqueous; AA, ascorbic acid

*IC₅₀ is the concentration of extract or standard that scavenged 50% of the generated free radicals



Effects of crude aqueous and methanol extracts of leaves, stem and roots of *C. dependens* and ascorbic acid on scavenging hydrogen peroxide (H₂O₂) radical

Table 6 shows the hydrogen peroxide radical scavenging activities of *C. dependens* and ascorbic acid (AA) standard. Result obtained also shows that scavenging activities of the extracts were largely sigmoidal, while the standard antioxidant followed a logistic dose-dependent pattern. Inhibitory effects of the extracts against hydrogen peroxide radicals were comparable to the standard. Hydrogen peroxide

radical scavenging activity of leaves, stem and roots of *C. dependens* was in the order AA > CDSA > CDRA > CDRM > CDLA > CDSM > CDLM. Figure 4 shows the hydrogen peroxide radical scavenging activities of crude aqueous and methanol extracts of leaves, stem and roots of *C. dependens* and Ascorbic acid.

Ferric reducing antioxidant power of crude aqueous and methanolic extracts of leaves, stem and roots of *Chasmanthera dependens*

Figure 5 shows the Ferric reducing antioxidant power of crude aqueous and methanolic extracts of *C. dependens* and ascorbic acid. The result obtained from the study shows that ascorbic acid was a more potent reducing agent with RP_{0.5} = 0.18 mg/ml. Results show that the root of *C. dependens* in methanolic solvent possesses reducing power activity (0.41 ± 0.05) comparable to the standard. Results obtained from extracts demonstrated a logistic dose-dependent response reducing effect on ferric chloride by AA, CDRM, and CDLA, CDLM followed a Weibull cumulative (a, b, c, d) model, while the reducing power of CDSA, CDSM and CDRA was sigmoidal (Sigmoid a, b, c) as shown in Table 7.

Discussion

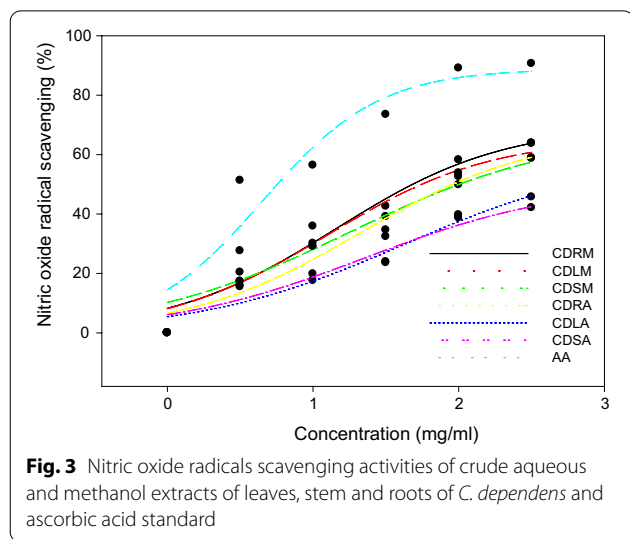
Extraction yield (% w/w) is a measure of the efficiency of a solvent to extract particular components from the original material. Aqueous (water) and methanol solvents were used for extraction in this study following a folkloric practice. Extract yield (% of dry wt.) from the studied

Table 5 Threshold inhibitory concentration (IC₅₀) of crude aqueous and methanolic extracts of leaves, stem and roots of *C. dependens* and ascorbic acid standard on nitric oxide radicals

Extracts	Threshold inhibitory concentration (*IC ₅₀)	Mathematical model	Equation	R ²
CDRM	1.16 ± 0.12	Sigmoid (a, b, c)	$y = \frac{a}{1 + \exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.95
CDLM	1.10 ± 0.11	Sigmoid (a, b, c)	$y = \frac{a}{1 + \exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.94
CDSM	1.25 ± 0.26	Sigmoid (a, b, c)	$y = \frac{a}{1 + \exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.89
CDRA	1.32 ± 0.14	Sigmoid (a, b, c)	$y = \frac{a}{1 + \exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.95
CDLA	1.62 ± 0.24	Sigmoid (a, b, c)	$y = \frac{a}{1 + \exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.94
CDSA	1.36 ± 0.22	Sigmoid (a, b, c)	$y = \frac{a}{1 + \exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.92
AA	0.65 ± 0.07	Sigmoid (a, b, c)	$y = \frac{a}{1 + \exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.91

CDRM, *C. dependens* root methanol; CDLM, *C. dependens* leaves methanol; CDSM, *C. dependens* stem methanol; CDRA, *C. dependens* root aqueous; CDLA, *C. dependens* leaves aqueous; CDSA, *C. dependens* stem aqueous; AA, ascorbic acid

*IC₅₀ is the concentration of extract or standard that scavenged 50% of the generated free radicals



plant was higher in the organic extracts (methanol) than in the aqueous extracts, which agrees with the findings of Pareck et al. (2015) that phytochemicals in plants are more soluble in organic compounds. The difference in yield may have been due to the polarity of the extracted compounds. The low polarity of methanol was probably accountable for the high yield of extracts because both polar and non-polar compounds were extracted. Results also suggest that crude powder extracts obtained from *C. dependens* could be affected by the extracting solvents.

Antimalarial activities were high in the roots and leaves and least in the stem of *C. dependens* (Tables 2, 3). Other

in vivo studies in mice revealed significant antimalarial activities in the roots of studied plants (Andrade-Neto et al. 2013; Murti and Mishra 2014). Leaves and roots of medicinal plants are extensively used in herbal remedies (Andrade-Neto et al. 2013). Methanol and aqueous extracts of the stem of *C. dependens* exhibited the least antimalarial activity on *Plasmodium berghei* infected mice. This could be attributed to the nature of the stem which is not woody, confirming an earlier report that plants with significant antimalarial activities in their stem extracts are trees with stem bark (Muthaura et al. 2017). Although antimalarial activities of methanol extracts of the leaves, roots and stem of *C. dependens* were more than that of the aqueous extracts, there was no significant difference except methanol extract of the leaf which significantly ($p < 0.05$) differed from the aqueous extract. This confirmed an earlier report that bioactive constituents are more soluble in methanol than in the aqueous medium (Pareck et al. 2015). Unlike the standard antimalarial drug (artesunate), extracts from this plant did not achieve total eradication of the parasites. Standard antimalarial drugs are more effective but they are not readily available and they are sold at exorbitant prices, justifying the use of these herbs and medicinal plants (Mgbemena et al. 2010).

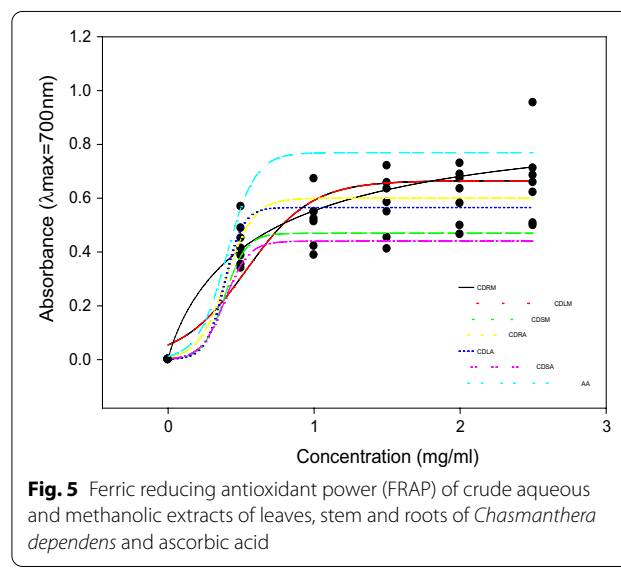
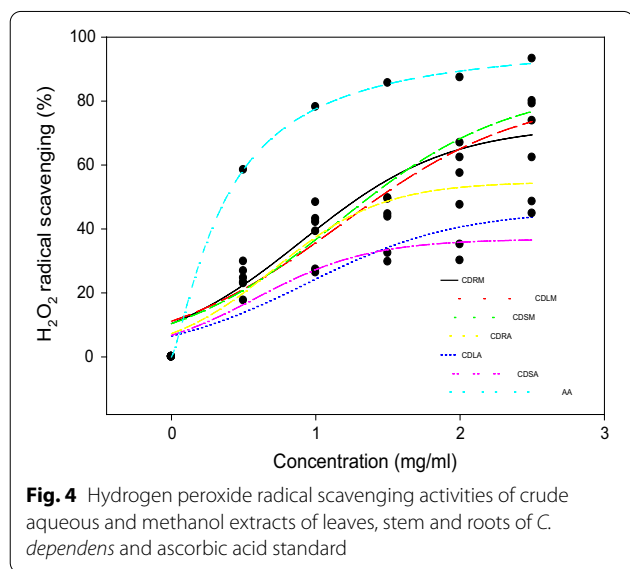
An increase in body weight was recorded in the infected and treated animals (Table 3), which could be attributed to the efficacy of the extracts on *Plasmodium berghei* infected mice. A decrease in percentage (%) body weight recorded in the untreated animals could be as a result of the parasites feeding on the blood cells of

Table 6 Threshold inhibitory concentration (IC₅₀) of crude aqueous and methanol extracts of *C. dependens* and ascorbic acid standard on hydrogen peroxide radicals

Extracts	Threshold inhibitory concentration (*IC ₅₀)	Mathematical model	Equation	R ²
CDRM	0.90 ± 0.09	Sigmoid (a, b, c)	$y = \frac{a}{1 + \exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.931
CDLM	1.17 ± 0.17	Sigmoid (a, b, c)	$y = \frac{a}{1 + \exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.920
CDSM	1.16 ± 0.15	Sigmoid (a, b, c)	$y = \frac{a}{1 + \exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.915
CDRA	0.71 ± 0.07	Sigmoid (a, b, c)	$y = \frac{a}{1 + \exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.915
CDLA	0.93 ± 0.11	Sigmoid (a, b, c)	$y = \frac{a}{1 + \exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.911
CDSA	0.59 ± 0.11	Sigmoid (a, b, c)	$y = \frac{a}{1 + \exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.810
AA	0.38 ± 0.01	Logistic dose response (a, b, c)	$y = \frac{a}{1 + \left(\frac{x}{b}\right)^c}$	0.999

CDRM, *C. dependens* root methanol; CDLM, *C. dependens* leaves methanol; CDSM, *C. dependens* stem methanol; CDRA, *C. dependens* root aqueous; CDLA, *C. dependens* leaves aqueous; CDSA, *C. dependens* stem aqueous; AA, ascorbic acid

*IC₅₀ is the concentration of extract or standard that scavenged 50% of the generated free radicals



the experimental animals bringing about a reduction in their body weight. This agrees with the reports of Ene et al. (2013). The survival rate of animals in the treatment groups indicated that extracts used in this study were not toxic to the experimental animals at the administered doses. Phytochemical constituents have been reported to have varying degrees of therapeutic activities (Gupta and Tandon 2014). The presence and therapeutic activities of phytochemicals in this plant could be accountable for the observed antimalarial activities of different parts of *C. dependens* as observed in the study.

The therapeutic properties of plants are credited to the antioxidant compounds and phytochemicals present in these medicinal plants (Padmanabhan and Jangle 2012). Antioxidants are known for their ability to scavenge free radicals, ameliorate free radical related diseases and conditions, such as oxidative stress. Extracts of the leaves, stem and roots of *C. dependens* showed appreciable antioxidant activities. Aqueous and methanolic extracts of the leaves, stem and roots of *C. dependens* showed DPPH scavenging activities in a concentration dependent-manner with DPPH scavenging activities increasing with an

Table 7 Ferric reducing antioxidant power (FRAP) of crude aqueous and methanolic extracts of leaves, stem and roots of *Chasmanthera dependens* and ascorbic acid

Extracts	Reducing power (RP _{0.5})	Mathematical model	Equation	R ²
CDRM	0.41 ± 0.05	Logistic dose response (a, b, c)	$y = \frac{a}{1+(\frac{x}{b})^c}$	0.992
CDLM	0.52 ± 0.07	WeibullCum (a, b, c, d)	$y = a \left[1 - \exp \left[- \left[\frac{x+c((ln2)^{1/d}-b)}{c} \right]^d \right] \right]$	0.998
CDSM	2.26 ± 0.11	Sigmoid (a, b, c)	$y = \frac{a}{1+\exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.973
CDRA	0.54 ± 0.05	Sigmoid (a, b, c)	$y = \frac{a}{1+\exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.965
CDLA	0.73 ± 0.04	Logistic dose response (a, b, c)	$y = \frac{a}{1+(\frac{x}{b})^c}$	0.997
CDSA	2.67 ± 0.23	Sigmoid (a, b, c)	$y = \frac{a}{1+\exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.954
AA	0.18 ± 0.00	Logistic dose response (a, b, c)	$y = \frac{a}{1+(\frac{x}{b})^c}$	0.953

CDRM, *C. dependens* root methanol; CDLM, *C. dependens* leaves methanol; CDSM, *C. dependens* stem methanol; CDRA, *C. dependens* root aqueous; CDLA, *C. dependens* leaves aqueous; CDSA, *C. dependens* stem aqueous; AA, ascorbic acid

increase in the concentration of extracts. This agrees with the report of Gyamfi et al. (1999). The high reduction in DPPH as recorded in this study is related to the high scavenging activity of a particular sample (Asadu et al. 2018).

Despite the beneficial effects of nitric oxide, it contributes to oxidative damage. This is because NO can react with superoxide ions to form a peroxy nitrite anion which is a strong oxidant that can decompose to produce OH and NO₂ (Alisi et al. 2018). The present study showed that aqueous and methanolic extracts of leaves, stem and roots of *C. dependens* have potent nitric oxide scavenging activities, efficiently converting nitric oxide to nitrites. Nitric oxide scavenging activities recorded in this study could be attributed to secondary metabolites, such as tannins, saponnins, flavonoids and carotenoids present in these plant extracts which agrees with Molyneux (2014).

This study also showed that aqueous and methanolic extracts of the leaves, stem and roots of *C. dependens* scavenged hydrogen peroxide (H₂O₂) effectively, compared to Ascorbic acid (standard antioxidant). Hydrogen peroxide (H₂O₂) easily decomposes into oxygen and water which may produce hydroxyl (OH) radicals that can initiate lipid peroxidation causing damage to DNA. Scavenging of H₂O₂ by extracts of *C. dependens* could be attributed to the presence of phenols which can donate electrons to H₂O₂ and neutralize it to water. Ferric reducing antioxidant power ability of extracts indicates the presence of reducing agents whose atoms can donate electrons or react with free radicals and convert them into stable metabolites, terminating radical chain reactions (Toykuni 2019). The plant extracts possession of ferric reducing antioxidant

power also indicated the ability of the bioactive components present in the plant extracts to donate hydrogen. This equally agrees with Shidduraju et al. (2012) that the ferric reducing power of most biologically active compounds is linked with antioxidant activity.

Conclusions

From this study, methanol and aqueous extracts of the leaves, roots and stem of *C. dependens* possess anti-malarial activities and antioxidant activities. Methanol extracts of leaves and roots were however more effective on *Plasmodium berghei* infected animals. Findings from this study also showed that both solvents used in the study are good for the extraction of antimalarial and antioxidant compounds from the plant parts. This study, therefore, supports the continued use of this plant in ethnomedicine for the management of malaria. This research equally revealed that these plants could be potential sources of natural antioxidants, preventing and/or slowing degenerative diseases. However, more work is required to identify and characterize antimalarial and antioxidant compounds from the leaves and roots of this plant.

Abbreviations

mg/kg: Milligram/kilogram; b.wt.: Body weight; H₂O₂: Hydrogen peroxide; NO: Nitric oxide; DPPH: 2,2 Diphenyl-1-picryl hydrazyl radical; AA: Ascorbic acid.

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Authors' contributions

CKE designed the study; JEE gathered the plant materials, conducted the experiment and prepared the manuscript; CEO, IAD and SOA supervised the research work; CON and MO carried out data analysis the data while OIO helped with the literature search. All authors read, corrected and approved the manuscript and gave consent to submit the manuscript for publication in *Bulletin of the Natural Research Centre*. All authors read and approved the final manuscript.

Availability of data and materials

Data sharing does not apply to this article as no datasets were generated or analyzed during the current study.

Declarations**Ethics approval and consent to participate**

The animals used for the experiment were handled following NIH guide for the care and use of laboratory animals Standard operating manual of NIPRD, approved by the Research Ethics Committee of Department of Biochemistry, Federal University of Technology, Owerri, Nigeria with the reference number- FUTO/BCH/DEC/XXI/03/01.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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