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In vitro Antiplasmodial and Antioxidant Activities of *Entandrophragma cylindricum* (Meliaceae) Extracts

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Authors' contributions

This work was carried out in collaboration between all authors. Authors NACN, JWP, DS and MM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors NTA, DM and GM managed the analyses of the study. Author NACN managed the literature searches. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Malaria is still regarded as a major global infectious disease in the 21st Century with a high pediatric mortality toll in the developing world. In Africa, malaria is one of the diseases causing the most morbidity and mortality. These past 30 years, malaria parasites especially *P. falciparum* have rapidly developed resistance to commonly used antimalarial drugs. New, more effective and affordable anti-malarial drugs are needed. Medicinal plants play a key role in the control of malaria, especially where access to modern health services is limited. The aim of this study was to evaluate the antimalarial and antioxidant properties of *Entandrophragma cylindricum* stem bark extracts. Three types of extracts (methanolic, Ethyl Acetate and aqueous extracts) were prepared and tested on both Chloroquino-Sensitive 3D7 and Chloroquino-Resistant INDO strains of *Plasmodium falciparum*. These parasite strains were cultivated *in vitro* by the method of Trager and Jensen.

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Cultures were maintained in fresh O^+ human erythrocytes at 4% haematocrit in complete medium RPMI 1640 supplemented with 0.5% Albumax II at 37℃ under reduced O_2. The synchronized ring stage development of *P. falciparum Pf*3D7 and *Pf*INDO strains were incubated in a 96-well microplate for 48h with different concentrations (12.5, 25, 50 and 100 µg/ml) of plant extracts. Zero point four (0.4%) DMSO in RPMI was used as negative control, while Chloroquine (1 nM) was used as positive control and the results were obtained by the microtiter plate based SYBR Green I fluorescence assay. The antioxidant activity was determined by measuring ferric reducing-antioxidant power (FRAP), DPPH radical scavenging, nitric oxide (NO) radical scavenging and ferrous ion-chelating activities. Vitamin C was used as control. Of the extracts tested, the highest antiplasmodial activity was observed with Ethyl Acetate extract against the Chloroquinoresistant *Pf* INDO strain with IC_{50} of 16,05 ± 0,35 µg/ml then aqueous extract (16.85± 0,54 µg/ml) and methanol extract (18.93 ± 2.88 µg/ml). The same extract exhibited *in vitro* antioxidant property in FRAP, DPPH radical scavenging, NO radical scavenging and ferrous ion-chelating assays and can therefore prevent oxidative stress.

Keywords: Entandrophragma cylindricum; antiplasmodial activity; antioxidant; Plasmodium falciparum; Cameroon.

1. INTRODUCTION

Malaria is still the most important parasitic disease in the world [1]. It is caused by a protozoan parasite of the genus *Plasmodium*. Malaria is a major public health problem especially in tropical and sub-tropical regions. It is estimated that in 2006, 3.3 billion people were at risk of contracting malaria and that it causes nearly one million deaths each year; mostly of African children aged below 5 years who are susceptible to this disease [2]. In Sub-Saharan regions, 45 countries were endemic for malaria in 2008 [2]. The emergence and spread of resistance to chloroquine and sulfadoxine-pyrimethamine (SP) have led to recommend that they be replaced with artemisinin-based combination therapies (ACTs); which offer much improved efficacy. In Cameroon where drug resistance is widespread, malaria is the leading cause of morbidity and mortality [3]. Traditional treatments to cure malaria are under investigation by numerous teams. Significant successes were achieved with the new compounds extracted from plants like Qinghaosu (artemisinin) [4]. In Africa, indigenous plants play an important role in the treatment of a variety of diseases as shown by Phillipson [5]. Several authors have discussed the implications of free radicals through oxidative stress in the physiopathogenesis of malaria [6]. This involvement may be related to the pathogenic mechanisms triggered by the parasite [7], as well as free radical production and antioxidant defenses [8] in host cells to abate the infection. However, recent studies suggest that the generation of reactive oxygen and nitrogen species associated with oxidative stress plays a crucial role in the development of systemic complications caused by malaria [6]. Malaria infection induces the generation of hydroxyl radicals ($OH^•$) in the liver which most probably is the main reason for the induction of oxidative stress and apoptosis [9]. Additionally, Atamna et al. [10] observed that erythrocytes infected with *P. falciparum* produced $OH^•$ radicals about twice as much compared to normal erythrocytes. A potential source of free radical production in this disease is the host's hemoglobin molecule since the parasite uses this molecule as a source of amino acids for its own nutrition during the erythrocytic stage of the disease; resulting in the liberation of large amounts of circulating heme. By having Fe^{2+}-associated groups, these heme are able to induce intravascular oxidative stress; causing changes in erythrocytes and endothelial cells and facilitating the internalization of the parasite in tissues such as the liver and brain [11]. As a free radical species, which appears to be involved in this disease is nitric oxide (NO) [12], its role is still controversial. Some researchers claim that cerebral malaria is probably an unfortunate consequence of high amounts of NO production to promote the death of the parasites [13]. Therefore, the use of antioxidant supplements of synthetic or natural origin may constitute a far more effective adjuvant antimalarial strategy that causes less damage to the host. *Entandrophragma cylindricum* is a medicinal plant traditionally used in the Centre Region of Cameroon for the treatment of malaria, yellow fever, bacterial infections like typhoid fever, diarrhoea and symptoms like stomach-ache. Many medicinal plants have large amounts of antimalarial and antioxidants compounds

such as saponins, tannins and triterpenoids, polyphenols and others [14]. This work was therefore aimed at evaluating the antiplasmodial and antioxidant activities of the crude extracts of *E. cylindricum* in order to ascertain their potentials as an antiplasmodial drug.

2. MATERIALS AND METHODS

2.1 Plant Material

Stem barks, leaves, flour and fruit of plant species were harvested in Makenene, situated in Mbam Inoubou, Division, Centre Region of Cameroon, in March 2015. This plant was identified in Cameroon National Herbarium (Yaoundé) using a voucher specimen registered under the reference No 1716/SRFCam. After identification as *Entandrophragma cylindricum* (Sapeli), the stem barks of the plant were collected for the second time, air dried and reduced to powder before extractions were undertaken. Three types of extracts (methanolic, Ethyl Acetate and aqueous extracts) were prepared and tested on both Chloroquino-Sensitive 3D7 and Chloroquino-Resistant INDO strains of *Plasmodium falciparum*.

2.2 Preparation of Extract

The methanolic and Ethyl Acetate extracts were obtained using the procedure described by Wabo Poné et al*.* [15]. Briefly, 100 g of stored powder were macerated in 1.5 l of 95% methanol which removed the polar ingredient of the plants. The mixture was placed on a shaker. Seventy two (72) hours later, the suspension was sieved and filtered using filter paper of pore size 2.5 µm. The filtrate was aligoted in portion of 250 ml, introduced in a vial and concentrated for about 5 minutes using a rotavapor Buchi-R-210 model heated at 40℃. The concentration of all the filtrate took about 8 hrs. For Ethyl Acetate extract, the same quantity of the plant powder was macerated in 1.5 l of Ethyl Acetate for 72 hrs. After filtration, the same procedure was followed as for methanolic extract. For aqueous extract, a similar procedure was carried out, except for the fact that maceration in cold distilled water took 48 hrs. The filtrate was evaporated in a vacuum pump heated at 42℃ and the residues were lyophilized. The methanolic, Ethyl Acetate and aqueous extracts obtained were kept in a refrigerator at 4℃ for further usages.

2.3 Reference Drugs

The reference drugs, Chloroquine (CQ) and Artemisinin (ATR) were obtained from SIGMA and were used as positive controls for *Pf*3D7 and *Pf*INDO respectively. Zero point four (0.4%) DMSO used as diluent solvent and as negative control.

2.4 Dilution of Different Products

The dilution of tested products was done as described by Noumedem Anangmo et al. [3]. Briefly, 6.5 mg of plant extracts and ATR were dissolved in 250 µl of DMSO while the same quantity of CQ was diluted using water (Milli-Q grade) making a stock solutions of 25 mg/ml. Complete culture medium (RPMI 1640 with 0.2% Sodium Bicarbonate, 0.5% Albumax, 45 mg/l Hypoxanthine and 20 mg/l Gentamicin) was then added to solutions to prepare the desired concentrations ranging from 100 to 1.56 µg/ml with a reason of 2.

2.5 Maintenance of Culture

Plasmodium falciparum was cultivated *in vitro* by the method of Trager and Jensen [16] with minor modifications. Cultures were maintained in fresh O^+ human erythrocytes at 4% haematocrit in complete medium at 37°C under reduced O_2 (gas mixture 5% O_2, 5% CO_2 , and 90% N_2).

2.6 Synchronization of *Plasmodium falciparum* Parasite Development Using Sorbitol

The procedure used here is as per Noumedem Anangmo et al. [3]. The cell culture was centrifuged at 1800 rpm for 5 minutes. After centrifugation, the supernatant was discarded and the pellet retained. Then, 10 pellet volume of 5% sorbitol solution was added. This solution was mixed and kept at 37℃ for 7 minutes. The sorbitol solution containing the cells was taken out of the incubator and centrifuged at 1800 rpm for 5 minutes. The supernatant (sorbitol) was discarded and the synchronized culture was suspended in fresh cRPMI and transferred to Petri dishes. The parasite culture was incubated at normal culture conditions (37℃ under reduced O_2). After one hour, a thin blood smear was prepared and stained slides were examined under a microscope at 100 X magnification for the parasite stages identification and parasitemia.

2.7 Antiplasmodial Assay

For drug screening, SYBR green I-based fluorescence assay was set up as described previously [17]. The *in vitro* antiplasmodial activity of *E. cylindricum* extracts was determined by fluorescence against CQ sensitive *Pf*3D7 and resistant *Pf*NDO strains of *P. falciparum*. Synchronized culture at 2% hematocrit and 1% parasitemia was aliquoted with test drugs [plant extracts (100, 50, 25, 12.5, 6.25, 3.125 and 1.56 µg/ml), 1Nm of CQ and ART] to 96-well flat-bottom tissue culture to a final volume of 100 µl. After 48 h of incubation, tested wells were supplemented with equal volume of Lysis buffer (Tris-20 mM, EDTA-5 mM, Saponin- 0.008%, Triton-X 100 – 0.08%) containing 1X SYBR Green I dye. After addition of lysis buffer, the plates were incubated for another one hour. After this period, the plates were read using 96-well fluorescence plate reader (Victor, Perkin-Elmer) with excitation and emission wavelengths of 497 and 520 nm respectively. The fluorescence readings were plotted against drug concentration and 50 percent inhibition concentration (IC_{50}) values were determined. In order to validate the SYBR green data, thin blood smears of treated and untreated wells were prepared, stained and examined at 100 X magnification.

2.8 Antioxidant Assay

2.8.1 DPPH radical scavenging assay

The DPPH radical scavenging assay was used in order to evaluate the free radical scavenging activity of *E. cylindricum* Ethyl Acetate extract. DPPH was dissolved in methanol at a concentration of 100 µM. The DPPH solution (3 ml) was mixed with the same volume of various concentrations (12.5, 25, 50, 100 and 200 µg/ml) of Ethyl Acetate extract and incubated in a dark room at 27℃ for 30 min. After incubation, the absorbance of the samples was read at 517 nm using a spectrophotometer. Ascorbic acid (Vitamin C) at the concentration of 12.5, 25, 50, 100 and 200 µg/ml was used as positive control. Each assay was done in triplicate and the results, recorded as the mean ± standard deviation (SD) of the three findings, were presented in tabular form. The radical scavenging activity (RSA) was calculated as follows (1):

$$\text{RSA} = \frac{\text{Absorbance of DPPH} - \text{Absorbance of sample}}{\text{Absorbance of DPPH}} \text{x } 100 \quad (1)$$

The radical scavenging percentages were plotted against the logarithmic values of concentration of test samples and a linear regression curve was established in order to calculate the IC_{50} which is the concentration of sample necessary to decrease by 50% the total free DPPH radical [18].

2.8.2 Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power was determined by the Fe^{3+} - Fe^{2+} transformation in the presence of the extract. The Fe^{2+} was monitored by measuring the formation of Perl's Prussian blue at 700 nm. The method reported by Padmaja et al. [19] was used with slight modification. Briefly, 400, 200, 100, 50, 25 µl of solution of Ethyl Acetate extract (2090 µg/ml) were mixed with 500 µl of phosphate buffer solution (PBS) (pH 6.6) and 500 µl of 1% Potassium Ferricyanide and incubated at 50℃ for 20 min. After, 500 µl of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000 rpm for 10 min. The supernatant (500 µl) was diluted with the same volume of water and mixed with 100 µl of freshly prepared 0.1 % ferric chloride. The absorbance was read at 700 nm. All tests were performed in triplicate and the results were the average of three observations. Vitamin C (12.5 to 200 µg/ml) was used as a positive control.

2.8.3 Nitric oxide radical scavenging (NO) assay

Nitric oxide radical generated from sodium nitroprusside in aqueous solution interacts with oxygen to produce nitrite ions. The substitute generated is measured using the Griess reaction [20]. The method reported by Chanda and Dave [21] was used with slight modification. To 0.75 ml of 10 mM Sodium Nitroprusside dissolved in PBS was added to 0.5 ml of extract or reference compounds [(Vitamin C and Butylated hydroxytoluene (BHT)] in different concentrations ranging from 12.5 to 200 µg/ml. The resulting solutions were then incubated at 25℃ for 60 min. A similar procedure was repeated with methanol as blank which served as negative control. To 1.25 ml of the incubated sample, 1.25 ml of Griess reagent (1% Sulfanilamide in 5% Phosphoric Acid and 0.1% N-1-napthylethylenediamine dihydrochloride in water) was added. A final concentrations range of 12.5, 25, 50, 100 and 200 µg/ml were obtained. After 5 min of incubation in the dark at room temperature

(27℃), the absorbance of the formed chromophore was measured at 540 nm. The inhibition percentage of the nitrite oxide generated was measured by comparing the absorbance values of control and test samples. The inhibition percentage was calculated according to the following formula (2):

$$\text{Percentage of inhibition} = (1 - \frac{\text{absorbance of the extract or standard}}{\text{absorbance of the negative control}})\text{X}100 \quad (2)$$

2.8.4 Ferrous ion-chelating assay

The ferrous ion chelating (FIC) activity was used to assay the antioxidant activity of Ethyl Acetate extract. It was appreciated by the increase of absorbance at 562 nm of the iron (II) and ferrozine complex. The extract (1000 µg/ml) was twofold serially diluted with methanol. Two hundred (200) µl of the diluted extracts were mixed with 740 µl of methanol and 20 µL (2 mM) $FeCl_2$. The reaction was initiated by the addition of 40 µl (5 mM) ferrozine. A final extract concentrations of 12.5, 25, 50, 100 and 200 µg/ml was obtained. The mixture was incubated at room temperature (27℃) for 10 min and the absorbance read at 562 nm was recorded. Methanol without sample and without ferrozine were used as negative control and blank sample respectively. While Vitamin C and BHT at the concentration of 12.5, 25, 50, 100 and 200 µg/ml were used as standard for the assay.

2.9 Statistical Analysis

The data obtained were analysed using ANOVA one-way and presented as mean ± standard deviation (SD). The levels of significance considered at $P < 0.05$ were determined by Waller-Duncan test using the Statistical Package for the Social Sciences (SPSS) software version 22.0.

3. RESULTS

3.1 Antiplasmodial Activity

The result of antiplasmodial activity of aqueous, Methanol and Ethyl Acetate extracts of *E. cylindricum* was reported. The variation of the mean inhibition rate on the growth of *Pf*3D7 and *Pf* INDO strains of *P. falciparum* according to the different concentrations of extracts of *E. cylindricum* was shown in Table 1.

Table 1 shows that, 0.4% DMSO did not affect the development of 3D7 and INDO strains of *P. falciparum*. In general, the different extracts were more active on CQ resistant strains (INDO) of *P. falciparum* as compared to the CQ sensible (3D7) one. The higher inhibition rate (92. 8 ± 1,26%) was obtained with Ethyl Acetate extracts at 100 µg/ml concentration. On the 3D7 strain, a maximum inhibition rate of 87,4% was recorded with aqueous extract at the same concentration.

The IC_{50} obtained on 3D7 and INDO strains of *Plasmodium falciparum* are summarized in Table 2. According to Niharika Singh et al. [22] , plants are classified for their antiplasmodial potential as (a) highly active (IC50 ≤ 5 µg/ml, (b) Promisingly active (IC50: 5.1–10 µg/ml) (c) Good activity (IC_{50}: 10.1–20 µg/ml) (d) Moderate activity (IC50: 20.1–40 µg/ml), (e) Marginal potency

Table 1. Variation of the mean inhibition rate of the growth of *Plasmodium falciparum* 3d7 and INDO strains according to the different concentrations of *Entandrophragma cylindricum* extracts

Strains	Concentration (µg/ml)	*Entandrophragma cylindricum*		
		AE	ME	EAE
		Mean ± Standart deviation		
3D7	0.4 % DMSO	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}
	12.5	31.20 ± 6.41^{bA}	41.56 ± 2.39^{bB}	46.98 ± 1.91^{bC}
	25	45.00 ± 4.99^{cA}	60.72 ± 2.05^{cB}	56.45 ± 1.82^{cB}
	50	55.09 ± 2.61^{dA}	65.43 ± 1.89^{cB}	65.23 ± 3.02^{dB}
	100	87.44 ± 6.54^{eB}	70.01 ± 2.51^{dA}	84.86 ± 2.26^{eB}
INDO	0.4 % DMSO	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}
	12.5	49.8 ± 6.8^{bA}	45.1 ± 3.92^{bA}	51.9 ± 1.81^{bA}
	25	51.7 ± 12.4^{bA}	62.7 ± 11.60^{cA}	52.2 ± 3.46^{bA}
	50	58.3 ± 8.2^{bA}	66.3 ± 7.50^{cA}	60.3 ± 3.2^{cA}
	100	91.6 ± 6.2^{cB}	69.1 ± 5.82^{cA}	92.8 ± 1.26^{dB}

Legend: AE: Aqueous extract, ME: Methanolic extract, EAE: Ethyl Acetate extract

(IC_{50}: 40.1 –70 µg/ml. and (f) Poor or inactive (IC50 70.1 to 4100 µg/ml. The tested extracts studied presented good antiplasmodial activity *in vitro* against both 3D7 and INDO strains with an IC_{50} of 16,70 ± 0,48 and 16,05 ± 0,35 µg/ml respectively. It is interesting to note that the aqueous extracts, shows good activities.

3.2 Antioxidant Activity

3.2.1 DPPH radical scavenging activity

The model of scavenging the stable DPPH radical is a widely method used to evaluate the free radical scavenging ability of various samples [23]. DPPH is a stable nitrogen-centered free radical which the color changes from violet to yellow upon reduction by either the process of hydrogen- or electron- donation. It was found that the radical-scavenging activity of extracts increased with increasing concentrations. The mean IC_{50} for DPPH radical-scavenging activity was 14, 74 ± 0, 88 µg/ml. while this values was 10, 60 ± 0, 27 for ascorbic acid. In fact, according to Souri et al. [14], the antioxidant activities of plant extracts are significant when IC_{50} < 20 µg/ml, moderate when 20 µg/ml ≤ IC50 ≤75 µg/ml, and weak when IC_{50} > 75 µg/ml. There was no significant difference ($p > 0.05$) between IC50 values of the crude extract and ascorbic acid.

3.2.2 Ferric reducing power, nitric oxide scavenging and Ferrous ion-chelating activity

The presence of antioxidant in the samples result in the reduction of Fe^{3+} to Fe^{2+} by electron donation. The amount of Fe^{2+} complex can then be monitored by measuring the formation of Perl's Prussian blue at 700 nm scavengers. Increasing absorbance at 700 nm indicates an increase in reductive ability. The extract showed potent nitric oxide-scavenging activity between 12.5 and 200 µg/ ml. The inhibition percentage was increased with increasing concentrations of the extract (Table 3). The metal chelating ability of Ethyl Acetate extract was measured by the formation of ferrous ion ferrozine complex.

4. DISCUSSION

In the present study, All the tested extracts of *E cylindrixum* were more active against CQ resistant INDO strain than CQ sensitive 3D7 strain. These results are in contrast with those obtained by Noumedem Anangmo et al. [3] when tested the *B. pilosa* extracts. Nevertheless, Niharika Singh et al. [22] found the same results with *P. hysterophorus* extracts and fractions. The aqueous extracts of *E. cylindricum* shows good activities. Similar observations were reported by Douki et al. [24] with the aqueous extract of *Staudtia gabonensis*. This suggests that some active compounds were extracted with this solvent.

The high antioxidant activities of the Ethyl Acetate extract may be due to the hydroxyl group that could donate electron to the free radicals which showed high radical scavenging activities that could suppress the oxidative stress on the host. The hydroxyl group that could donate electron to the notorious free radical could be the reason why it possesses potent radical scavenging activities (DPPH) [25]. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers [26].

In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological conditions [27]. The plant products may have the property to counteract the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation in the human body. Further, the scavenging activity may also help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to human health.

Table 2. The IC_{50} obtained from the tests on the 3D7 and INDO strain of *Plasmodium falciparum*

Parasite	Strains	*Entandrophragma cylindricum*		
		AE	ME	EAE
		IC_{50} ± Standard deviation		
Plasmoduim falciparum	3D7	24.93 ± 4.53^{bB}	20.27 ± 0.48^{bB}	16.70 ± 0.49^{bA}
	INDO	16.85 ± 0.54^{aA}	18.93 ± 0.35^{bB}	16.05 ± 2.88^{bAB}

Legend AE : Aqueous Extract; EM : methanol Extract ; EAE : .Ethyl Acetate Extract;

Table 3. Ferric reducing power determination, nitric oxide scavenging capacity assay and ferrous ion-chelating assay

Concentrations (µg/ml)	EAE	Vitamin C	BHT
	Ferric reducing power activities		
12.5	0.050±0.011^{bc}	0.028±0.009^{ab}	
25	0.043±0.006^{ab}	0.044±0.0005^{ab}	
50	0.102±0.015^{bc}	0.056±0.021^{a}	NA
100	0.129±0.008^{a}	2.183±0.089^{d}	
200	0.271±0.041^{ab}	3.339±0.098^{e}	
	Nitric oxide (NO) radical scavenging		
12.5	77.044±1.168^{c}	92.427±3.627^{ef}	94.946±0.800^{f}
25	83.197±0.055^{c}	94.595±1.339^{e}	96.429±0.110^{e}
50	85.525±0.027^{c}	96.556±0.298^{ef}	97.274±0.526^{f}
100	87.804±0.095^{c}	96.556±0.895^{g}	97.624±0.027^{h}
200	89.701±0.138^{c}	94.595±2.032^{e}	99.410±0.055^{f}
	Ferrous ion-chelating		
12.5	0.128±0.08^{b}	0.507±0.06^{e}	0.366±0.06^{e}
25	0.159±0.01^{c}	0.771±0.15^{g}	0.539±0.05^{f}
50	0.206±0.01^{b}	1.039±0.06^{f}	0.740±0.01^{e}
100	0.273±0.02^{a}	1.223±0.02^{d}	0.960±0.04^{c}
200	0.512±0.03^{b}	1.298±0.02^{d}	1.358±0.03^{e}

Legend: EAE: .Ethyl Acetate extract, BHT: bultylhydroxytoluène, NA: Not applicable

It is worthy to note that *Plasmodium* parasites from malaria infection invade the host hemoglobin to make their own protein. Upon destruction, free heme (ferum atom) will be released and converted to hemozoin which is important for the survival of Plasmodium parasites. The killing of Plasmodium by binding to toxic free heme could prevent the formation of hemozoin [28]. The Ferrozine formed combines with ferrous ions forming a red colored complex which absorbs at 562 nm [29]. It was reported that the chelating agents which form σ bond with a metal are effective as secondary antioxidants because they reduce the redox potential thereby stabilising the oxidised form of the metal ion [30].

5. CONCLUSION

Malaria infection causes the formation of free radicals which subsequently lead to oxidative stress and apoptosis. The crude extract of *Entandrophragma cylindricum* exert substantial antiplasmodial and antioxidative effects through the DPPH free radical scavenging, FRAP, NO and metal chelating activities which could suppress oxidative stress that cause less damage to the host. Further investigation on the isolation and identifcation of antioxidant components in the plant may lead to chemical entities with the potential for clinical use. Therefore, the use of antioxidant supplements of synthetic or natural origin may constitute a far more effective adjuvant antimalarial strategy that causes less damage to the host.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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