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Research Article

Antioxidant and Antiplasmodial Potentials of Methanol Bark Extract of *Entada africana* Via *in Vitro* Approaches

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Abstract

Plasmodium parasites, which cause malaria, continue to pose a serious threat to global health, necessitating the continuous search for novel antimalarial agents. Oxidative stress has also been linked to the pathophysiology of malaria. *Entada africana* is a plant known for its ethnomedicinal uses in treating various ailments associated with inflammation including malaria. This study aimed at evaluating the antiplasmodial and antioxidant potentials of methanol bark extract from *Entada africana* (MBEEA). *In vitro* approaches were adopted for the study. *Plasmodium falciparum*-infected erythrocyte samples were cultured in Roswell Park Memorial Institute (RPMI) 1640 media under anaerobic conditions for 72 hours. Eighteen test tubes were labeled and grouped into three replicates per group. Group I (untreated), Group II, and III were treated with chloroquine (CQ) and artemether (AR) at a concentration of 5 mg/dL. Group IV, V, and VI were treated with the extract at respective concentrations of 5 mg/dL, 10 mg/dL, and 20 mg/dL for 72 hours. The parasitemia count and the percentage parasitemia inhibition were determined by microscopic examination of Giemsa-stained smears. The antioxidant potential of the extract was assessed using *in vitro* assays, including superoxide radical scavenging activity (SRSA), hydroxyl radical scavenging activity (HRSA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and ferric reducing antioxidant power (FRAP). Microscopic examination of the treated samples revealed varying degrees of parasitemia inhibition. Group II and III treated with CQ and AR demonstrated a considerable reduction in parasitemia count with percentage inhibition of 100% and 83% respectively. The *E. africana* extract showed a concentration-dependent effect on parasitemia count. At 5 mg/dL, the extract exhibited 50% parasitemia inhibition, which increased to 100% at 10 mg/dL, and 20 mg/dL respectively. The MBEEA demonstrated significant *in vitro* antioxidant activities by scavenging DPPH, SRSA, and hydroxyl radical compared to the standard antioxidant (ascorbic acid). MBEEA thus exhibit potent antioxidant and antiplasmodial properties. This plant is therefore offers to be a promising medicinal plant in the treatment of malaria, hence it is recommended as potent antiplasmodial plant usable for treating malaria.

Keywords

Plasmodium falciparum-infected Erythrocytes, MBEEA, Antioxidant Potential, Parasitemia Counts, Chloroquine, Artemether

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1. Introduction

Public health concerns about malaria are widespread. In just 2012, there were an estimated 207 million cases and 627 000 deaths worldwide, according to the World Health Organization's (WHO) Malaria World Report [31]. 3.4 billion people worldwide are thought to be at risk of contracting malaria, with the disease being endemic in more than 70 countries. Malaria poses the greatest risk to people who live in Sub-Saharan Africa. Malaria primarily affects pregnant women and children under five years old, accounting for 90% of deaths and 80% of cases in the WHO African region. Female Anopheles mosquitoes are the vector by which humans contract the parasitic disease malaria. The four primary parasites that cause malaria are *Plasmodium vivax, Plasmodium ovale*, *Plasmodium malariae*, and *Plasmodium falciparum*. Furthermore, there have been reports of human infections with *P. knowlesi*, a species of *Plasmodium* that is endemic to simian populations in some regions of Southern Asia [17]. Malaria symptoms do not have a specific onset. Usually, they are characterised by a generalised weakness of the body, reocurring fever, nausea, vomiting, headaches, exhaustion, muscle aches, and abdominal pain.

However, failures in implementing the control interventions recommended by the World Health Organization (WHO) are leading to an increase in malaria incidences, mortality, and morbidity in some malaria endemic countries. Artemisinin based Combination Therapies (ACTs) are the first line antimalarial medication for both uncomplicated and severe malaria. However, there have been reports of treatment failures with ACTs for *falciparum* malaria, the most deadly and widespread form of malaria [4]. In addition to the inherent drawbacks of antimalarial medications, individuals who are most impacted by the disease may find it difficult to obtain and afford these medications, particularly in developing nations. Finding new antimalarials will require more in-depth investigation. According to Teixeira *et al.* [25], one technique involves generating novel compounds from guiding compounds that exhibit antiplasmodial activity. A drug may be selected to function as a guide compound based on how closely its chemical structure resembles that of other compounds with potent antiplasmodial effect.

Nevertheless, people in developing nations often resort to using natural resources like plants as remedies when they cannot afford modern allopathic medicines [6]. Since ancient times, people have been using plants for medical purposes, and this practice is still significant for primary healthcare today [3]. More than 80% of people in developing nations still use herbal remedies to treat common illnesses like malaria, according to the World Health Organisation [30]. Phytochemicals are created by plants during metabolism. It is known that many phytochemicals have pharmaceutical activity against a wide range of human diseases, even though their primary functions in plants are still unclear [22].

According to Bald é *et al.* [2], *Entada africana* may be the source of some of the compounds with potential for development as antiplasmodial substitutes. Malaria may induce oxidative stress in the body. Reactive oxygen species (ROS) production may be excessive due to the metabolic activities of the parasite and the host's immune response [9]. This oxidative stress may damage the host tissues and may also be a factor in the emergence of malaria complications like organ dysfunction and cerebral malaria [29]. Preventing heme from polymerizing is one of the antimalarial drug's actions. Heme becomes hemozoin through the process of polymerization. Chloroquine is one medication whose mode of action defies polymerization [23]. According to Openshaw *et al.* [16], heme complex formation and chloroquine may inhibit the production of hemozoin. The primary target of chloroquine is the heme. Cell membranes and *Plasmodium* proteolytic enzymes poison the free heme found in the parasite's digesting vacuoles [13]. The free heme is subsequently polymerized by the parasite into hemozoin, an innocuous material, to maintain *Plasmodium* survival [36]. Heme and hematin are related; however, hemozoin's structure is more akin to that of β-hematin. Thus, by employing the anti-heme polymerization mode of action, a molecule can be transformed into an antimalarial medication [20]. *In vitro* hematin has the ability to polymerize into β-hematin in acidic environments, possessing identical characteristics to the hemozoin found in *Plasmodium*.

Entada africana is used in African traditional medicine to treat liver-related disorders, inflammation, stomachaches, and malaria [35]. In contrast, its *in vitro* and computational antiplasmodial properties have gotten little or no attention. The antiplasmodial and antioxidant effects of the stem bark have primarily been studied using ethnomedical techniques.

This study thus combines both *in vitro* and *in silico* methods to examine *Entada africana's* potential for antiplasmodial and antioxidant activities.

2. Materials and Methods

2.1. Materials

2.1.1. Collection of Plant Materials and Authentication

Barks of *Entada africana* (Fabaceae) plant were collected from a local farm in Iwaro-Oka, Akoko South west local government area. Latitude 7.278PN and longitude 5.1167^{o}E, Ondo State, Nigeria. The plant bark materials were then identified by Dr. O. Obembe, and authenticated at Plant Science and Biotechnology Departmental Herbarium (PSBH), Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. Plant voucher specimen designated as PSB-479 for the *Entada africana*. Bark sample was later deposited at the Herbarium.

2.1.2. Chemicals

Reagents: Ascorbic acid, trichloroacetic acid (TCA), potassium ferricyanide, ferric chloride, pyrogallol, hydrogen peroxide, ethylene diamine tetra-acetic acid (EDTA), water, D-glucose, sodium bicarbonate, sodium chloride (NaCl), magnesium sulfate ($MgSO_4$), disodium hydrogen phosphate (Na_2HPO_4), and phenol red. All chemicals and reagents used were of analytical grade.

2.1.3. Equipment

Magnetic stirrer, UV-visible spectrophotometer (Spectrum lab uv/visible, Greece), spectramax Plus 385 micro plate spectrophotometer, freeze drier (Labfreeze Instrument Limited, China), water bath, and a bench centrifuge (Lw scientific centrifuge, America), Colorimeter (Jenway, UK), micropipettes (1000 µL and 500 µL), pH meter, analytical weighing balance (Mettler Toledo, Columbus), distiller (Bibby Scientific Limited, stone Stafford shire, England).

2.1.4. Parasitemia Counting

A compound microscope fitted with paired 10× oculars (eyepieces), 10×, 40× and 100× objectives and a mechanical stage (an objective marker and a 60× objective may also be fitted), a multiple tally counter or two-key tally counters, one to count malaria parasites and one to count white blood cells, Giemsa-stained blood slides to be examined, Immersion oil, type A, high quality, Lens paper, a pen and pencil and handheld calculator.

2.2. Methods

2.2.1. Preparation of Methanol Bark Extracts of *Entada africana*

Fresh bark of *Entada africana* were harvested, stored and washed properly to remove debris and dust particles. The bark of the plant were air dried at room temperature for 3 weeks and grinded to fine powder using electric blender. The ground particles were soaked in aqueous methanol (1:4 w/v) for 72 hours with intermittent stirring after which it was filtered. The crude extract is concentrated under reduced pressure using rotary evaporator to remove the methanol solvent. This results in a thick, concentrated crude extract.

2.2.2. Determination of DPPH Radical Scavenging Activity

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was measured by using the assay method described by Okawa *et al.* [15], with slight modifications. 1.0 ml of *E. africana* at different concentrations (0.5 - 2.5 mg/mL) was added to 1 mL 0.1 mM DPPH dissolved in 95% ethanol. The mixture was shaken vigorously and incubated in the dark and at room temperature for 30 minutes. The absorbance was read at 517 nm. Ethanol (95%) was used as blank. The control situation consisted of 0.1 mL of 95% ethanol and 2.9 mL of DPPH solution. Ascorbic acid was used as a standard antioxidant. Analyses were carried out in triplicates. Percentage inhibition of DPPH radical was calculated as follows:

$$\%\ \text{DPPH inhibition} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

2.2.3. Determination of Ferric Reducing Antioxidant Property (FRAP)

The reducing power of *E. africana* was measured according to the method reported by Yu *et al.* [34], with slight modification. An aliquot of 1 ml of different concentration (0.5 - 2.5 mg/mL) of *E. africana* (0.2 M PBS, pH 6.6) was mixed with 1 ml of 1% potassium ferric cyanide solution. The mixture was then incubated at 50°C for 30 minutes followed by the addition of 1 ml 10% (w/v) TCA. One (1.0) ml of the incubation mixture was added to 1 ml of distilled water and 0.2 ml of 0.1% (w/v) ferric chloride in test tubes. After a 10 minutes reaction time, the absorbance of resulting solution was read at 700 nm. Higher absorbance suggested stronger reducing power. Ascorbic acid was used as the reference antioxidant. An aqueous solution of known Fe (II) concentration ($FeSO_4$.7H2O; 2.0, 1.0, 0.5, 0.25, 0.125, 0.063 mM) were used for calibration. Results were expressed as mM Fe^{2+}/mg *E. africana*. All the tests were performed in triplicate.

2.2.4. Determination of Superoxide Radical Scavenging Activity (SRSA)

The method described by Mokrani and Madani [14], was used to determine SRSA. *E. africana* (0.5 - 2.5 mg/mL) were each dissolved in 50 nM Tris-HCl buffer. pH 8.3 containing 1 mM EDTA and 80 µL was transferred into a clear bottom microplate well; 80 µL of buffer was added to the blank well. This was followed by addition of 40 µL 1.5 mM pyrogallol (dissolved in 10 mM HCl) into each well in the dark and the change in the rate of reaction was measured immediately at room temperature over a period of 4 minutes using a spectrophotometer at a wavelength of 420 nm. Ascorbic acid was used as a standard antioxidant. The superoxide scavenging activity was calculated using the following equation:

$$\text{Superoxide scavenging activity}\ (\%) = \frac{(\Delta\text{Abs}/\text{min}_b - \Delta\text{Abs}/\text{min}_s)}{\Delta\text{Abs}/\text{min}_b} \times 100$$

Where b and s are blank and sample, respectively.

2.2.5. Determination of Hydrogen Peroxide Scavenging Activity (HPSA)

The ability of the extract to scavenge hydrogen peroxide was determined according to the method of Um *et al.* [28] with slight modifications. A solution of hydrogen peroxide (4 mM) was prepared in 0.2 M phosphate buffer (pH 7.4). Varying concentrations of the *E. africana* (0.5 - 2.5 mg/mL) in the

distilled water were added to 0.6 ml of 4 mM hydrogen peroxide solution. Absorbance value of test samples (As) were read at 230 nm after 10 minutes against a blank solution containing the phosphate buffer without hydrogen peroxide. Absorbance of hydrogen peroxide ~~(Ac)~~ was taken as the control. Ascorbic acid was used as a standard antioxidant. The percentage of scavenging effects was calculated by comparing the absorbance values of the control and test samples using:

$$\% \text{ Scavenging Capacity (\% Scavenged } [H_2O_2]) = [(Ac - As)/Ac] \times 100$$

2.2.6. Blood Samples Collection

Eighteen (18) *Plasmodium falciparum* erythrocyte samples were collected from the University Health Centre. into anticoagulant-containing tubes (EDTA) to prevent coagulation and maintain the viability of blood cells.

2.2.7. Incubation of Plasmodium Falciparum

The test tubes containing the *Plasmodium falciparum* infected blood sample was placed in a centrifuge to concentrate the infected red blood cells (RBCs), the RPMI media supplemented with essential nutrients, serum and human erythrocytes provides a supportive environment for parasite growth. The test tubes were placed in an incubator and the conditions were adjusted to mimic that of the human body, including temperature (37^{o}C) and a specific gas mixture (5% O_2, 5% CO_2, and 90% N_2) for 72 hours according to the method of Trager and Jensen [27].

2.2.8. Parasitemia Counts/Quantification

Thin and thick blood smear were prepared on clean glass slides, with the thick smear being denser for enhanced parasite detection. Blood smears were air-dried and fixed with methanol, preserving cellular morphology and preventing distortion during staining. The prepared blood smears were stained with Giemsa solution, following standard staining protocols, to enhance parasite visibility and differentiation from host cells. A high-quality compound light microscope with 100× oil immersion lens was utilized for microscopic examination, ensuring optimal resolution for parasite identification. Parasites were counted against a fixed number of 200 white blood cells (WBCs) to achieve statistically significant results and mitigate counting errors. The parasite density per microliter of blood was calculated by considering the average WBC count and converting the parasitemia percentage to parasites per microliter [19].

$$\text{Parasite / } \mu L = \frac{\text{Number of parasitized red cells} \times 5000000}{\text{Number of white cells counted}}$$

2.2.9. Statistical Analysis

The data was presented as mean ± SEM. A one-way analysis of variance (ANOVA) followed by Tukey's test were used to analyse the significant difference, where $p \leq 0.05$ was presented as statistical difference.

3. Results

3.1. Antioxidant Assays

3.1.1. Ferric Reducing Antioxidant Property

The ability of *E. africana* methanol bark extract (MBEEA) to reduce Fe (III) ions to Fe^{2+} in comparison to ascorbic acid, are shown in Figure 1. The result revealed that, the ferric reducing potential of the standard drug (ascorbic acid) was significantly ($p \leq 0.05$) higher when compared to MBEEA for all the concentrations assessed (0.5 mg/mL, 1.0 mg/mL, 1.5 mg/mL, 2.0 mg/mL, and 2.5 mg/mL) as shown in Figure 1. At a concentration of 0.5 mg/mL, the ascorbic acid showed 40% ferric reducing antioxidant capacity when compared to MBEEA, which showed 21% antioxidant capacity with significant difference ($p \leq 0.05$). At a concentration of 1.0 mg/mL, the ascorbic acid showed 50% ferric reducing antioxidant capacity when compared to MBEEA, which showed 22% antioxidant capacity. At a concentration of 1.5 mg/mL, MBEEA showed ferric reducing antioxidant capacity of 26% when compared to ascorbic acid, which showed an antioxidant capacity of 53% with a significant difference ($p \leq 0.05$). There was a remarkable difference in MBEEA ferric reducing potential at a concentration of 2.0 mg/mL, it showed an antioxidant capacity of 35% when compared to ascorbic acid with a antioxidant capacity of 54%. At a concentration of 2.5 mg/mL, ascorbic acid showed it highest antioxidant capacity of 73% when compared to MBEEA, which showed an antioxidant capacity of 49% with a significant difference ($p \leq 0.05$). MBEEA showed a moderate antioxidant activity in a concentration-dependent manner when compared to the control, ascorbic acid across all concentrations.

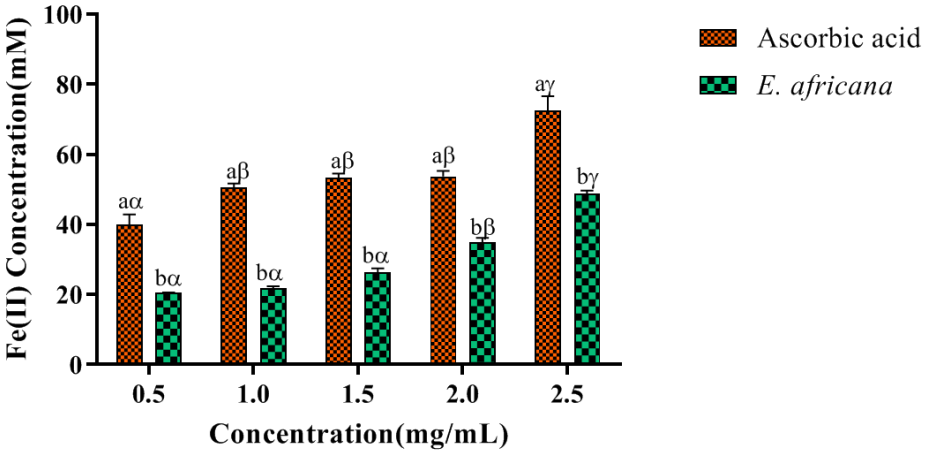


Figure 1. *Ferric Reducing Antioxidant Property (FRAP) of MBEEA and ascorbic acid.*

Results are expressed as mean ±standard deviation. Bars carrying the same Alpha-Greek superscripts are not significantly different at $p \leq 0.05$.

3.1.2. Hydrogen Peroxide Scavenging Activity

The relative ability of *E. africana* methanol bark extract (MBEEA) to scavenge the hydrogen peroxide radical in comparison to the control, ascorbic acid, are illustrated in Figure 2. For the various concentrations of MBEEA and ascorbic acid tested, MBEEA displayed lower ($p \leq 0.05$) hydrogen peroxide (H_2O_2) radical scavenging activities when compared to ascorbic acid for all concentration assessed (0.5 mg/mL, 1.0 mg/mL, 1.5 mg/mL, 2.0 mg/mL, and 2.5 mg/mL) as shown in Figure 2. There was a significant difference ($p \leq 0.05$) in the ability of MBEEA to scavenge H_2O_2 radical at a concentration of 0.5 mg/mL, as it showed 36% hydrogen peroxide scavenging ability when compared to ascorbic acid, with an antioxidant potential of 80%. At a concentration of 1.0 mg/mL, the ascorbic acid showed an antioxidant capacity of 84% while MBEEA showed a scavenging potential of 58%. At a concentration of 1.5 mg/mL, MBEEA and ascorbic acid showed an antioxidant capacity of 62% and 87% respectively with a significant difference ($p \leq 0.05$), while at a concentration of 2.0 mg/mL, ascorbic acid showed an antioxidant scavenging capacity of 89% when compared to 72% showed by MBEEA ($p \leq 0.05$). At a concentration of 2.5 mg/dL, ascorbic acid showed an antioxidant capacity of 91% when compared to MBEEA scavenging activity of 79%. There was an increase in the H_2O_2 radical scavenging activities of MBEEA in a concentration-dependent manner across all concentrations tested.

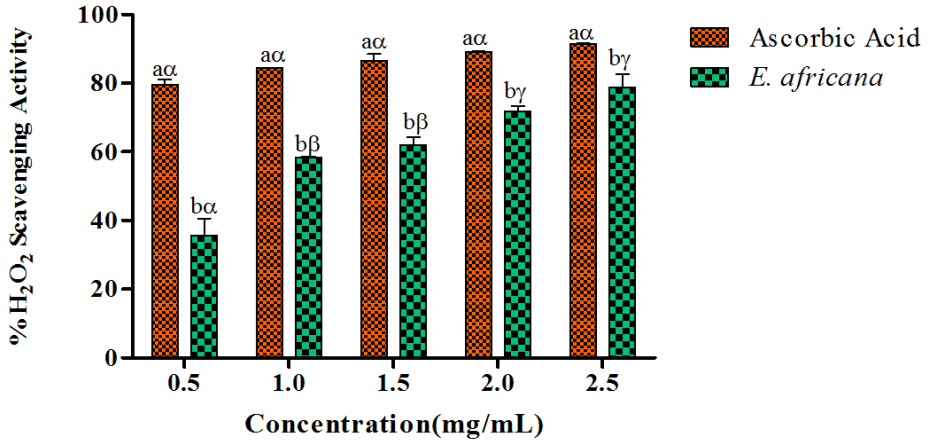


Figure 2. *Hydrogen Peroxide Scavenging Activity (HPSA) of MBEEA and ascorbic acid.*

Results are expressed as mean ±standard deviation. Bars carrying the same Alpha-Greek superscripts are not significantly different at $p \leq 0.05$.

3.1.3. Superoxide Radical Scavenging Activity

The abilities of MBEEA to scavenge the superoxide radical, relative to the control, ascorbic acid, are illustrated in Figure 3. The MBEEA demonstrated a significantly lower ($p \leq 0.05$) scavenging activities when compared to ascorbic acid (control)

for all the concentrations tested (0.5 mg/mL, 1.0 mg/mL, 1.5 mg/mL, 2.0 mg/mL, and 2.5 mg/mL).

At a concentration of 0.5 mg/mL, MBEEA showed superoxide radical scavenging activity of 22% while ascorbic acid, showed an antioxidant scavenging capacity of 68%. At a concentration of 1.0 mg/mL, ascorbic acid showed an antioxidant capacity of 76%, while MBEEA, showed an antioxidant capacity of 32% ($p \leq 0.05$). MBEEA showed an antioxidant capacity of 46% while ascorbic acid showed an antioxidant potential of 84% at a concentration of 1.5 mg/mL. Ascorbic acid showed an antioxidant scavenging activity of 91% when compared to MBEEA which showed an antioxidant capacity of 64% at a concentration of 2.0 mg/mL. At a concentration of 2.5 mg/mL, MBEEA showed its highest scavenging activity of 71%, while ascorbic acid, showed an antioxidant activity of 94%. The MBEEA showed an increase in its superoxide radical scavenging activity across all concentrations in a concentration-dependent manner.

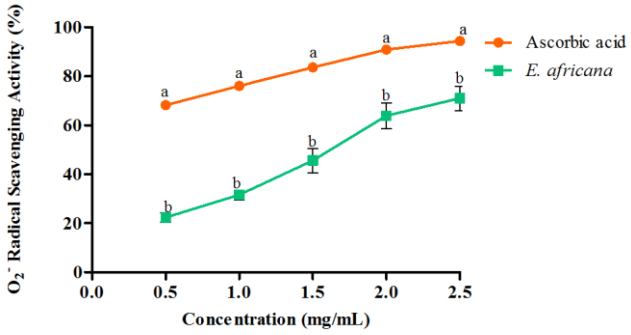
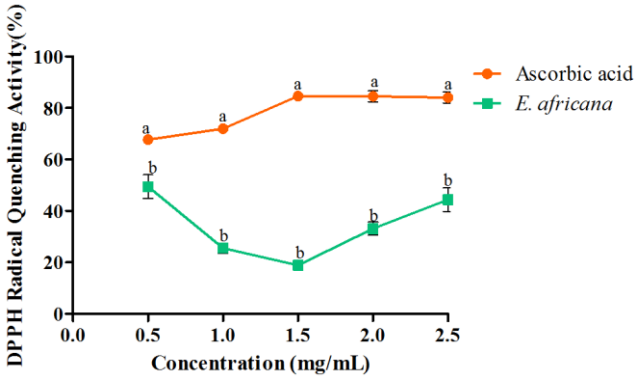


Figure 3. *Superoxide Radical Scavenging Activity (SRSA) of MBEEA and ascorbic acid.*

Dots on each line graph are expressed as mean ± standard deviation. Dots belonging to the fraction with same letters are not significantly different at $p \leq 0.05$.

3.1.4. DPPH Radical Scavenging Activity

The ability of MBEEA to scavenge the DPPH (1,1-dipheny-2-picryhydrazyl) radical in comparison to ascorbic acid are depicted in Figure 4. All MBEEA concentrations demonstrated significantly lower ($p \leq 0.05$) DPPH scavenging activities when compared to ascorbic acid at all concentrations (0.5 mg/mL, 1.0 mg/mL, 1.5 mg/mL, 2.0 mg/mL, and 2.5 mg/mL). The MBEEA showed its best percentage DPPH scavenging activity of 49% at its lowest concentration (0.5 mg/mL) when compared to ascorbic acid, 68%. At a concentration of 1.0 mg/mL, ascorbic acid showed a DPPH scavenging activity of 72% while MBEEA, showed a scavenging activity of 26%. MBEEA showed its lowest DPPH scavenging activity of 19% at a concentration of 1.5 mg/mL, when compared to 85% scavenging potential of ascorbic acid at the same concentration. At a concentration of 2.0 mg/mL, MBEEA displayed a scavenging activity of 33%, which is a slight increase from its antioxidant activity at a concentration 1.5 mg/mL when compared to ascorbic with an antioxidant activity of 85%. Ascorbic showed DPPH scavenging activity of 84%, while MBEEA, showed a scavenging activity of 44% at a concentration of 2.5 mg/mL with a significant difference ($p \leq 0.05$).



Results are expressed as mean ± standard deviation. $p \leq 0.05$

Figure 4. *DPPH scavenging activity of MBEEA and ascorbic acid.*

3.2. Parasitemia Counts

As shown in Figure 5, Group I, positive control (infected, untreated group), showed a high parasitemia level, the estimated number of *Plasmodium* parasites on the thick film of giemsa-stained microscopic slides as shown in Figure 7 before treatment in group II was 453 parasites/µL, showing high parasite density. After 72 hours of treatment with chloroquine (CQ) a standard drug, at a concentration of 5 mg/dL, there was a significant drop in parasitemia level from 453 parasites/µL to 0 parasites/µL at $P \leq 0.05$. In group III, treatment with artemether (AR) another standard drug, at a concentration of 5 mg/dL resulted in a decrease in parasitemia from 346 parasites/µL to 13 parasites/µL. While arthemeter was effective in reducing the parasite count, it was not as potent as chloroquine (CQ) in group II at the same concentration, as evidenced by the lower clearance rate. Group IV received treatment with 5 mg/dL of MBEEA and the parasitemia decreased from 560 parasites/µL to 40 parasites/µL. Group V received a higher concentration (10 mg/dL) of MBEEA, and it achieved complete parasite clearance from a parasite density of 586 parasites/µL before treatment to 0 parasites/µL after treatment, similar to the result in group II (the chloroquine (CQ) treated group). Group VI received the highest concentration (20 mg/dL) of MBEEA, and it also achieved complete parasite clearance, from 653 parasites/µL before treatment to 0 parasites/µL after treatment for 72 hours, similar to the result in group II and group V (chloroquine (CQ) treated group and the 10 mg/dL MBEEA treated group).

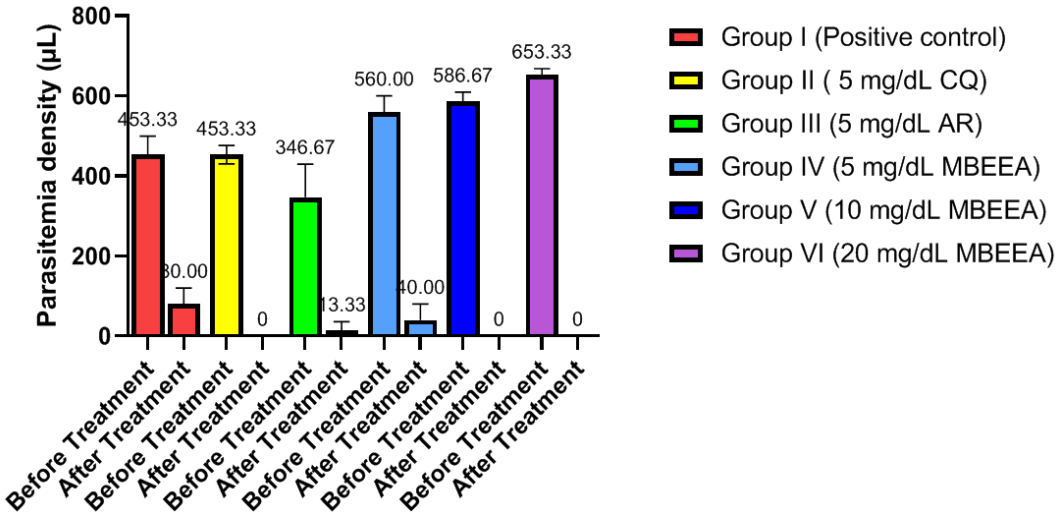


Figure 5. *The parasitemia density (µL) of the giemsa-stained microscopic slides.*

The data was presented as mean ±SEM. A one-way ANOVA followed by Tukey's test were used to analyse the significant difference between the groups, where $P \leq 0.05$ was presented as statistical difference. CQ (chloroquine), AR (arthemeter), MBEEA (methanol bark extract of *Entada africana*).

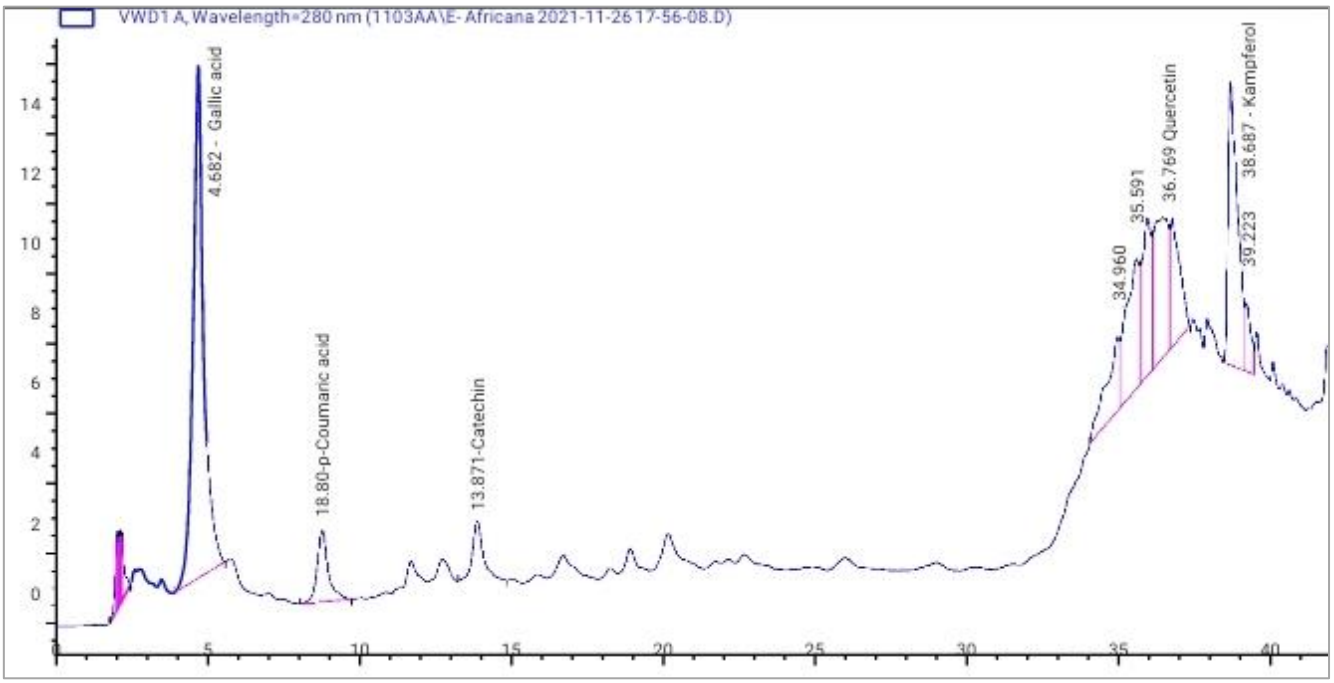


Figure 6. *HPLC chromatogram of Entada africana showing various peaks of flavonoid compounds at a wavelength of 280 nm.*

Percentage Parasitemia Inhibition

The percentage parasitemia inhibitory activity of two standard antimalarial drugs (chloroquine and artemether) at a concentration of 5 mg/dL, in comparison with different concentrations of MBEEA on parasite's activity was also evaluated as shown in Table 1. The positive control group represents the untreated samples, Chloroquine (CQ) treated group (Group II) achieved 100% parasitemia inhibition, while Artemether (AR) treated group (Group III) at a concentration of 5 mg/dL resulted in 83% parasitemia inhibition. Treatment with different concentrations of MBEEA showed varying parasitemia inhibitions, the treatment with 5 mg/dL MBEEA in group IV resulted in 50% parasitemia inhibition. Group V treated with a higher concentration (10 mg/dL) of MBEEA achieved a parasitemia inhibition of 100%, which is the same as chloroquine in group II. Similar to the 10 mg/dL concentration, treatment with MBEEA at 20 mg/dL resulted in 100% parasitemia inhibition, indicating that higher concentrations of the extract were equally effective in

inhibiting parasite growth. The results demonstrate the effectiveness of both chloroquine and MBEEA at higher concentrations (10 mg/dL and 20 mg/dL) in completely inhibiting parasite growth after 72 hours of incubation.

Table 1. *Percentage parasitemia inhibition.*

S/N	Grouping	%Parasitemia inhibition
1	Group I (Positive control)	____
2	Group II (5 mg/dL CQ)	100
3	Group III (5 mg/dL AR)	83
4	Group IV (5 mg/dL MBEEA)	50
5	Group V (10 mg/ dL MBEEA)	100
6	Group VI (20 mg/dL MBEEA)	100

The data was presented as mean ± SEM. A one-way ANOVA followed by Tukey's test were used to analyse the significant difference between the groups, where $P \leq 0.05$ was presented as statistical difference.

$$\text{Percentage parasitemia inhibition} = \frac{\text{Parasitemic control} - \text{Treatment}}{\text{Parasitemic control}} \times 100$$

Key;
CQ; (chloroquine treated group)
AR; (artemether treated group)
MBEEA; (methanol bark extract of *Entada africana* treated group)

Figure 7. *Giemsa-stained labeled microscopic slides.*

4. Discussion

Early and accurate parasitological diagnosis is the cornerstone of malaria treatment and control strategy. Light microscopy performed on peripheral blood smears remains the reference standard for malaria diagnosis [32]. Microscopic examination of the thick blood film remains the method of choice for examining blood for malaria parasites and estimating the parasite density in field studies [5]. The method of choice for parasite density estimation is dependent on the level of parasitemia. Parasite count is not only important for the assessment of the severity of malaria but also crucial for monitoring the therapeutic efficacy of drugs, emergence, and spread of drug resistance [7]. Efficacy outcomes depend on parasite clearance estimates and on the accurate determination of parasitemia status during follow-up. As shown in Figure 5, the estimated number of *Plasmodium* parasites on the thick film of giemsa-stained microscopic slides before treatment in group II showed high parasite density. After 72 hours of treatment with chloroquine (CQ) a standard drug, at a concentration of 5 mg/dL, there was a significant drop in parasitemia level ($P \leq 0.05$). This indicates the high efficacy of chloroquine as an anti-malarial agent. The complete clearance of parasites suggests that chloroquine effectively targeted and eliminated *Plasmodium* parasites by preventing the polymerization of heme into hemozoin in the *in vitro* culture. In group III, treatment with artemether (AR) another standard drug, at a concentration of 5 mg/dL resulted in a decrease in parasitemia. While arthemeter was effective in reducing the parasite count, it was not as potent as chloroquine (CQ) in group II at the same concentration, as evidenced by the lower clearance rate. Group IV received treatment with 5 mg/dL of MBEEA and there was a significant parasitemia reduction. The reduction in parasitemia suggests the effectiveness of MBEEA as an anti-malarial agent, although it was less effective than chloroquine (CQ) in group II and artemether (AR) in group III at the same concentration. Group V received a higher concentration (10 mg/dL) of MBEEA, and it achieved complete parasite clearance, similar to the result in group II (the chloroquine (CQ) treated group). This suggests that a higher concentration of 10 mg/dL, MBEEA was highly effective in eliminating *Plasmodium* parasites completely. Group VI received the highest concentration (20 mg/dL) of MBEEA, and it also achieved complete parasite clearance, after treatment for 72 hours, similar to the result in group II and group V (chloroquine (CQ) treated group and the 10 mg/dL MBEEA treated group) indicating the high potency of this concentration of the extract. The results indicate that chloroquine treated group (Group II) and high concentrations of MBEEA (group V and VI) were the most effective in reducing parasitemia level and achieving complete clearance. Artemether (group III) and lower concentrations of MBEEA (Group IV) were less effective but still showed significant anti-malarial activity. The antimalarial activity of the plant extracts is due to bioactive compounds, such as polyphenols, flavonoids, alkaloids, terpenoids, and saponins. Therefore, the antimalarial activity of the extract could have resulted from the single or combined action of the above compounds [21]. The possible actions of antimalarial activity might be through the antioxidant, immunomodulatory, intercalation in DNA,

blocking of protein synthesis, inhibition of erythrocyte invasion by parasites, disruption of hemozoin formation, or by other unknown mechanisms [24]. The results obtained from this study corroborate results from the previous studies by Ezenyi *et al.* [8] and Karou *et al.* [10].

The percentage parasitemia inhibitory activity of two standard antimalarial drugs (chloroquine and artemether) at a concentration of 5 mg/dL, in comparison with different concentrations of MBEEA on parasite's activity was also evaluated as shown in Table 1. The positive control group represents the untreated samples, serving as a baseline to compare the effectiveness of the treatments, but it would be expected to be close to zero because these samples did not receive any anti-malarial treatment. Chloroquine treatment in group II achieved complete parasitemia inhibition. This result suggests that the application of chloroquine at a concentration of 5 mg/dL completely prevented the growth or proliferation of *Plasmodium* parasites in the *in vitro* culture. Chloroquine is a well-established and highly effective anti-malarial drug [1]. Artemether treatment at a concentration of 5 mg/dL resulted in a significant parasitemia inhibition in group III. This demonstrates that artemether had a substantial inhibitory effect on the parasite's growth. Although not as effective as chloroquine in group II, this result still indicates artemether's anti-malarial activity. The treatment with 5 mg/dL MBEEA in group IV resulted in half parasitemia inhibition. This suggests that the extract has a moderate anti-malarial effect at this concentration, reducing the parasite count by half compared to the positive control. Treatment with a higher concentration (10 mg/dL) of MBEEA in group V achieved complete parasitemia inhibition, which is the same as chloroquine in group II. This indicates that at this concentration, the extract was as effective as chloroquine in inhibiting parasite growth. Similar to the 10 mg/dL concentration, treatment with MBEEA at 20 mg/dL also resulted in complete parasitemia inhibition, indicating that higher concentrations of the extract were equally effective in inhibiting parasite growth. The results demonstrate the effectiveness of both chloroquine and MBEEA at higher concentrations (10 mg/dL and 20 mg/dL) in completely inhibiting parasite growth. Artemether, though effective, was not as potent as chloroquine. MBEEA at 5 mg/dL showed moderate antiplasmodial activity. This indicates the potential of MBEEA as an anti-malarial agent, especially at higher concentrations. These findings are important for assessing the potential of the extract as an anti-malarial agent and for optimizing its concentration for maximal efficacy.

The presence of *Plasmodium* parasites in the bloodstream has been reported to trigger immune responses [18]. Immune cells, when activated, produce reactive oxygen species (ROS) as part of their defense mechanisms. The combination of ROS generated by both the parasites and the host immune system can lead to oxidative stress in the body [11]. Excessive ROS can damage cellular components, including lipids, proteins, and DNA [33]. In severe cases of malaria, this tissue damage can contribute to organ dysfunction and the development of complications. The intervention has now been to screen plant sources capable of preventing the cells from oxidation. Ferric reducing capability, hydrogen peroxide scavenging potency, superoxide scavenging potency, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging potency, and other antioxidant enzymes assays are few of the major ways to assess the antioxidant potentials of therapeutic plants. The result of the HPLC analysis of the methanol bark extract of *E. africana* (MBEEA) revealed flavonoid rich compounds such as gallic acid, ferullic acid, catechin, p-coumaric acid, rutin, apigenin, quercetin, and kaemferol, which were identified as the major compounds as shown in Figure 6. In this study, MBEEA was compared with ascorbic acid (Vitamin C) for its antioxidant property. Though, the plant extract was able to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). The result revealed that, the ferric reducing potential of the standard drug (Vitamin C) was significantly ($p \leq 0.05$) higher when compared to MBEEA for the various concentrations tested as shown in Figure 1. This implies that flavonoids present in the extract, such as quercetin and rutin, may function as reductones to adsorb ROS and end the free radical-mediated chain reactions. In similar manner, the extract was assessed for its capability to mop-up cellular levels of hydrogen peroxide and superoxide. For all the concentrations assessed, MBEEA showed a moderate antioxidant activity in a concentration-dependent manner with ascorbic acid outperforming the plant extract as shown in Figure 2 and Figure 3. MBEEA showed inhibitory activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) but significantly ($p \leq 0.05$) lower compared to the standard drug (ascorbic acid) as presented in Figure 4. The observation from the *in vitro* antioxidant assays showed the plant is capable of shielding cellular and tissue oxidation, but it is unable to reach the magnitude of effect seen with ascorbic acid. Overall, the plant showed good antioxidant potentials in a concentration-dependent manner when compared with known antioxidant (ascorbic acid). These results agree with results obtained from previous studies by Marquardt *et al.* [12] and Tibiri *et al.* [26].

5. Conclusion

This study has successfully established via *in vitro* experiments that *Entada africana* exhibits concentration-dependent antioxidant and antiplasmodial potentials. At higher concentrations, the extract demonstrated substantial parasite growth inhibition, ultimately reaching 100%. These findings suggest the plant's significant antiplasmodial potential, especially when used at optimal concentrations. Additionally, the extract's antioxidant properties indicate its ability to combat the oxidative stress associated with malaria, potentially contributing to a comprehensive therapeutic approach. Computational studies further illuminated the bioactive compounds within *Entada africana* by predicting their interactions with crucial *Plasmodium* proteins like heme polymerase, identified potential molecular mechanisms underlying the plant's antiplasmodial ef-

fects. This computational approach serves as a valuable tool for identifying and understanding the specific compounds responsible for *Entada africana's* antimalarial activity, offering a foundation for future drug development.

Abbreviations

MBEEA	Methanol Bark Extract from *Entada Africana*
RPMI	Roswell Park Memorial Insstitute
CQ	Chloroquine
AR	Artemether
SRSA	Superoxide Radical Scavenging Activity
HRSA	Hydroxyl Radical Scavenging Activity
DPPH	1,1-diphenyl-2-picrylhydrazyl
FRAP	Ferric Reducing Antioxidant Power
WHO	World Health Organization
ACTs	Artemisinin-Based Combination Therapies
ROS	Reactive Oxygen Species
TCA	Trichloroacetic Acid
EDTA	Ethylene Diamine Tetraacetic Acid
ANOVA	Analysis of Variance

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Conflicts of Interest

The Authors declare no conflict of interest.

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