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Phytochemicals, Antioxidant and Antimalarial Activity of the Leaves and Stem Bark Extracts of *Sterculia setigera* del. (Sterculiaceae)

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ABSTRACT

Medicinal plants are widely recognised for their therapeutic applications such as the treatment of malaria, diabetic, hypertension and bleeding etc. The leaves and stem bark fractions of *Sterculia setigera* were investigated for their metabolic profile (phytochemical screening), antioxidant activity (DPPH assay) and antimalarial activity against *Plasmodium falciparum*. Results of the antioxidant and antimalaria assays were compared with standards (i.e, ascorbic acid/gallic acid and artesunate respectively). The phytochemical analysis of the plant parts revealed the presence of vital antioxidant and antimalarial constituents that support the ethnomedicinal use of the plant. Both the leaves and stem barks displayed the presence of alkaloids, flavonoids, steroids and reducing sugar, while tannin was only observed in the leaves of the plant. The ethanol extract of the leaves showed the highest antioxidant activity (IC_{50}=13.78 µg/ml) compared to standards ascorbic acid (IC_{50}=11.28 µg/ml) and gallic acid IC_{50}=25.52 µg/ml). Additionally, the ethanol extract (LE1) and ethyl acetate extract (LE1-03) of the leaves of the plant displayed a highest antimalaria activity with a percentage elimination of the parasite of 80 % at 100 µg/ml and 88 % at 1000 µg/ml respectively compared to the control 94 % at 1000 µg/ml. These activities observed in the extract is correlated with the phytochemicals present in the extracts of the plant parts. The isolation and identification of the specific bioactive compounds that are responsible for the antioxidant and antimalaria activity is recommended.

Keywords: Antimalaria, Antioxidant, Phytochemical, Sterculiaceae, *Sterculia setigera*

INTRODUCTION

Malaria is a disease that is predominant in most underdeveloped and developing countries. It is caused by blood parasites; *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium vivax*. Recent survey shows most devastating and prevalent diseases of humans, infecting more than 247 million persons in 2021 in 84 malaria-endemic countries and 600,000 deaths each year with an estimated 95% of deaths in 2020 in African regions (WHO, 2022). The alarming increase in malaria drug resistance coupled with vector resistance has led to the failure of the prevention and eradication program put in place ((Balaska *et al.*, 2021). This resistance led to the promotion and use of medicinal plants to fight against circulating resistant strains, especially in sub-Saharan Africa (WHO, 2013).

Antioxidants have long been considered as compounds able to decrease oxidative stress. Dietary antioxidants exert a remarkably broad range of bioactivities, and many of these can be explained by the influence of antioxidants on the redox homeostasis. Such compounds help to modulate the levels of harmful reactive oxygen/nitrogen species, and therefore participate in the regulation of various redox signalling pathways (Hunyadi, 2019; Chaves *et al.*, 2021). In addition, antioxidants neutralize free radicals and stop the harm of reactive oxygen species (ROS) before they can damage the cells (Chaudhary *et al.*, 2023; Sidiki *et al.*, 2023). Therefore, it is important to discover an antimalarial agent that will equally possess antioxidant activity to prevent oxidative stress generated during malaria infection.

Sterculia setigera is a gummy deciduous tree with a spreading open crown leaf and large twisted branches. It is commonly found in tropical Africa and its natural distribution range stretches around West Africa (Ayodele *et al*., 2000; Zaruwa *et al.*, 2016). The roots were reported to be used as diuretic and were also analysed for their *in-vitro* trypanocidal activity against *T.b. brucei* and *T. congolense* at effective concentrations (Atawodi *et al*., 2003 and 2005). The stem bark also found to be useful in the treatment of snake bites, leprosy, syphilis, coughs, bronchitis, rickets, and diarrhoea (Sofowora, 1982). The gum is used as laxative, diuretic, antipyretic, and constipation. In addition, the leaves were traditionally found to be used as fodder for cattle and also in the treatment of trypanosomiasis and tuberculosis (Adelakun *et al.*, 2012). The antimicrobial activity of dried bark, dried fruits, and the root of *S. setigera* against

common microbes' species such as *Bacillus Subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Aspergillus niger* and *Candida albicans* have been reported (Hamidu 2012).

Despite the wide occurrence of the plant and its traditional importance, the antioxidant and antimalarial activity of the plant parts have not been investigated. Additionally, the resistive nature of malaria parasites and side effects of some drugs makes it necessary for continuous search for alternative bioactive constituents. Hence, this research is aimed to investigate the antioxidant and antimalarial activities of *S. setigera* leaves and stem bark extracts and to evaluate the phytochemicals present to confirm the bioactivity of the extract.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Fresh leaves and the stem bark of *S. setigera* were collected from the premises of Gwarmai, Bebeji local govt. of Kano State, Nigeria and were identified at the Department of Plant Science, Bayero University Kano, Nigeria. The plant materials were air dried in the laboratory at room temperature (26°C) for 2 weeks, after which they were pulverized into uniform fine powder. The powdered plant materials were weighed using an electric weighing machine and transferred into a conical flask for extraction.

Extraction of the Plant

Each of the powdered plant material (100 g) was percolated with 900 ml of distilled ethanol in a conical flask. It was allowed to stand for 2– weeks before it was filtered. The filtrate was concentrated using rotary evaporator to obtain the crude extract (ethanol fraction). The ethanol extract of each of the leaves and stem bark of the plant were labelled as LE1 and SB1 respectivelyand macerated using solvents of different polarities.

Fractionation of the Plant

Fractionation is a process of softening or separating the part of substance by steeping in a liquid with or without heat. The fractionation of the crude extracts obtained (ethanol fraction) was carried out according to protocols described by Semenescu and his co-workers (2023). The ethanol fraction (LE1) was macerated four times with 20 ml of each solvent; *n*-hexane, ethyl acetate, chloroform, and methanol and were labelled LE1-01–LE1-04 for the leaves and SB1-01–SB1-04 for the stem barks.

PHYTOCHEMICAL SCREENING

The extracts obtained from the plant materials were subjected to phytochemical screening to determine the presence of secondary metabolites in the plant material using standard proceduresto test the presence of alkaloids, flavonoids, tannins, saponins, steroids and reducing sugar (Fatope *et al*., 1993; Cruilci, 1994; Sofowora, 2013).

ANTI–PLASMODIUM ACTIVITY

Preparation of Solution

The extracts (20 mg) were dissolved in 2 ml Dimethylsulphoxide (DMSO) and transferred into a sterilized sample vial to prepare a stock solution of 10,000 ug/ml. Three different concentrations 250 ug/ml, 500 ug/ml and 1000 ug/ml were prepared from the stock solution.

Sourcing of Malaria Parasites for the Assay

Malaria parasites infected blood samples from Bayero University, Kano clinic laboratory provided clinical blood samples containing heavy parasitaemia of *Plasmodium falciparum.* The samples were immediately transferred into K_3–EDTA disposable plastic sample bottles with tightly fitted plastic corks and mixed thoroughly and then transported to the Microbiology laboratory at Bayero University in a thermos-flask containing water maintained at 4°C as demonstrated by Gruessner *et al*., (2021) and Mukhtar *et al.* (2006).

Determination of *Plasmodium falciparum* Positive Blood Sample Using Thin Smear Method

After thoroughly mixing the blood sample, a small drop of each sample was placed at the centre of a clean glass slide at least 2 mm from one end using a clean capillary tube. A clean cover slip was placed in front of each drop at an angle about 45°C and then drawn backward to be in contact with the blood. The drop was then allowed to run along the full length of the edge of the cover slip, with a fast and smooth movement, the cover slip was pushed forward to form a thin smear on each glass slide. The smear was air dried in a petridish containing absolute methanol for 15 minutes, and then covered with several drops Geimsa's stain for 10 minutes.

Then excess stain was washed with a clean tap water, and the smear were air dried on a rack with the glass slides kept upside down. The air-dried smears were observed under a microscope using a high-power objective (x 100) under an oil immersion. The smear was transverse thoroughly for *plasmodium falciparum* infected erythrocytes. An average parasitaemia was obtained from the reading of 3 microscopic fields. Blood samples with 5% parasitaemia were used for the research (Mukhtar *et al.*,2006 and Gruessner *et al*., 2021).

Preparation of the Erythrocytes (5% parasitaemia) from the serum of the Blood samples

A solution of 5% dextrose (0.05 ml) was added to each blood samples (5 ml) and defibrinated, and then centrifuged at 2500 rpm for

15 minutes in a centrifugation machine. The supernatant layers were discarded, and the sediment were diluted with normal saline (red blood cell diluting fluid) (Dikaso *et al*., 2006; Gruessner *et al*., 2021). Then centrifuged at 2500 rpm for 10 minutes. Similarly, the supernatant layer was discarded and samples with higher parasitaemia (above 5%) were diluted with fresh malaria negative erythrocytes (Gruessner *et al*., 2021).

Preparation of *Plasmodium falciparum* Culture Medium

Using a sterile 5ml disposable syringe, 2 ml of venous blood from the main vein of white rabbits' pinnae was withdrawn. This was defibrinated by allowing it to settle for at least one hour (Dacie, 1968; Gruessner *et al*., (2021). The defibrinated blood was centrifuged at 1500 rpm using spectre merlin centrifuge for 10 minutes and the supernatant layer was collected in a sterilized tube. The sediment was further centrifuged at 1500 rpm for five minutes, and the supernatant layer was added to the first test tube. The sediments were discarded, and the serum collected was supplemented with the salts of RPMI 1640 medium. The medium was sterilized by adding 40 ug/ml gentamicin sulphate.

***In–vitro* Assay of the Activity of the Extracts on *Plasmodium falciparum* Culture**

The tested solution (0.1 ml) and 0.2 ml of the culture medium were added into a vial containing 0.1 ml of 5% parasitaemia erythrocytes and mixed thoroughly. The sensitivity of the parasites to the tested fractions was determined microscopically after incubation for 24, 48 and 72 hours at 37°C. The incubation was undertaken in a glass bell jar containing a lighted candle to ensure the supply of required quantity of CO_2 (Gruessner *et al*., 2021).

Determination of Activity

At the end of incubation periods 24, 48 and 72 hours, a drop of a thoroughly mixed aliquot of the culture medium was smeared on microscopic slides and stained by Geimsa's staining techniques. The mean number of erythrocytes appearing as blue discoid cells containing life rings of the parasite (that appeared red pink) was estimated and the average percentage elimination by the samples was determined. The activity of the tested samples was calculated as the percentage elimination of the parasites at the end of incubation periods of 24, 48 and 72 hours using the formula below;

$$\%\ Elimination = \frac{N}{Nx} * 100$$

where N = Total number of cleared red blood cells, N_x = Total number of parasitized red blood cells

ANTIOXIDANT ACTIVITY

DPPH Radical Scavenging Assay

The free radical scavenging activity of the plant extracts against 2,2-diphe-nyl-1-picrylhydrazyl (DPPH) radical was determined according to the standard method with slight modification (Chaves *et al.,* 2021; Ibrahim *et al.,* 2023). The stock solutions of the extracts and standards (ascorbic acid (AA) and butylated hydroxytoluene (BHT)) were prepared (2.0 mg/mL) and diluted to final concentration of 1000, 500, 250, 125, 31.3, 15.63 and 7.82 µg/mL. 100 µM DPPH was also prepared by diluting 1 mg of the DPPH in 50 mL of methanol. 40 µL of the standards and samples were added to the microplate and their absorbance was measured at 517 nm. Then, a total of 160 µL of the DPPH was added to positive controls and sample solutions and allowed to react at room temperature for 30 min in dark. The absorbance of the mixtures and that of blank DPPH were measured at 517 nm. A sample is considered active if its reaction changes the colour of the DPPH from purple to yellow.

Lower absorbance of the reaction mixture indicates higher free radical scavenging activity, and vice versa. Inhibitions of DPPH radical in percent (I%) were calculated using the formula:

$$I\% = \frac{A_{blank} - A_{sample}}{A_{blank}}\ x\ 100$$

STATISTICAL ANALYSIS

All the methods were carried out in triplicate, and results were expressed as the mean of the values obtained for the replications. IC_{50} values and one-way analysis of variance (ANOVA) was performed using SPSS software. Statistical significance was established at $p < 0.05$.

RESULTS AND DISCUSSION

PHYSICAL PROPERTIES OF PLANT EXTRACTS

The colour, texture and weight of the leaves and stem bark extracts of *Sterculia setigera* as well as the secondary metabolites present were shown in Table 1 and 2 respectively. The phytochemical screening result showed the distribution of the presence of secondary metabolites in the extracts. From Table 2, the ethanol extracts of both the leaves (LE1) and stem bark (SB1) displayed the presence of the tested secondary metabolites and were reflected in the methanol fraction. The secondary metabolites were also reflected in the other fractions, i.e. flavonoids appeared in the fractions of the leaves, while steroids and reducing sugars were reflected in both the leaves and stem bark fractions as shown in Table 2.

Table 1: Physical Properties of the leaves and stem bark extracts/fraction of *S. setigera*

Fraction	Weight (mg)	Texture	Colour	Fraction	Weight (mg)	Texture	Colour
LE1	90.7	Oily	Dark green	SB1	92.0	Solid	Brownish
LE1-01	47.8	Oily	Dark green	SB1-01	52.0	Solid	Pale green
LE1-02	45.0	Solid	Dark green	SB1-02	57.9	S/solid	Brownish
LE1-03	52.6	Oily	Pale green	SB1-03	50.4	Oily	Pale green
LE1-04	96.8	V. liquid	Brownish	SB1-04	101.1	Solid	Brownish

Key: LE1 and SB1= crude ethanol extract, LE1-01 and SB1-01=n-Hexane fraction, LE1-02 and SB1-02= Ethylacetate fraction, LE1-03 and SB1-03 = Chloroform fraction, LE1-04 and SB1-04 = Methanol fraction. LE= Leaves and SB= Stem Bark.

Phytochemical Screening

Table 2: Phytochemicals of *Sterculia setigera* leaves extract and fractions

Phytochemical	LE1	LE2	LE3	LE4	LE5	SB1	SB2	SB3	SB4	SB5
Alkaloids	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	-	+	+	+	+	-	-	-	+
Tannins	+	-	+	-	-	-	-	-	-	-
Steroids	+	-	-	-	+	+	-	+	-	+
R. Sugar	+	-	+	-	+	+	-	+	-	+

Key: LE1=crude ethanol extract, LE1-01= n-Hexane fraction, LE1-02 = Ethylacetate fraction, LE1-03 = Chloroform fraction, LE1-04 = Methanol fraction. LE= Leaves; SB1=crude ethanol extract, SB1-01= n-Hexane fraction, SB1-02= Ethylacetate fraction, SB1-03= Chloroform fraction, SB1-04= Methanol fraction. SB = Stem Bark. + = Present, - = Absent

ANTIMALARIAL ACTIVITY

The leaves and stem bark extracts of *S. setigera* were investigated to discover new antimalarial agents against *P. falciparum*. In this study, the leaves of the plant extracts showed significant activity than the stem bark against the tested malaria parasite. The ethanol extract (LE1) and ethylacetate extract (LE1-03) of the leaves of the plant displayed a highest activity with a percentage elimination of the parasite of 80 % at 100 µg/ml and 88 % at 1000 µg/ml respectively compared to the control 94 % at 1000 µg/ml (Figure 2). The stem bark extract/fractions of the plant were found to be weakly active against the tested parasite compared to the standard control (Artesunate). The activity might be attributed to the presence of alkaloids or flavonoids which has been identified present in this work (Figure 2). The activity may also be linked to combined action of more than one metabolite (Gruessner *et al*., 2021). This study has also established the rationale for traditional use of this plant.

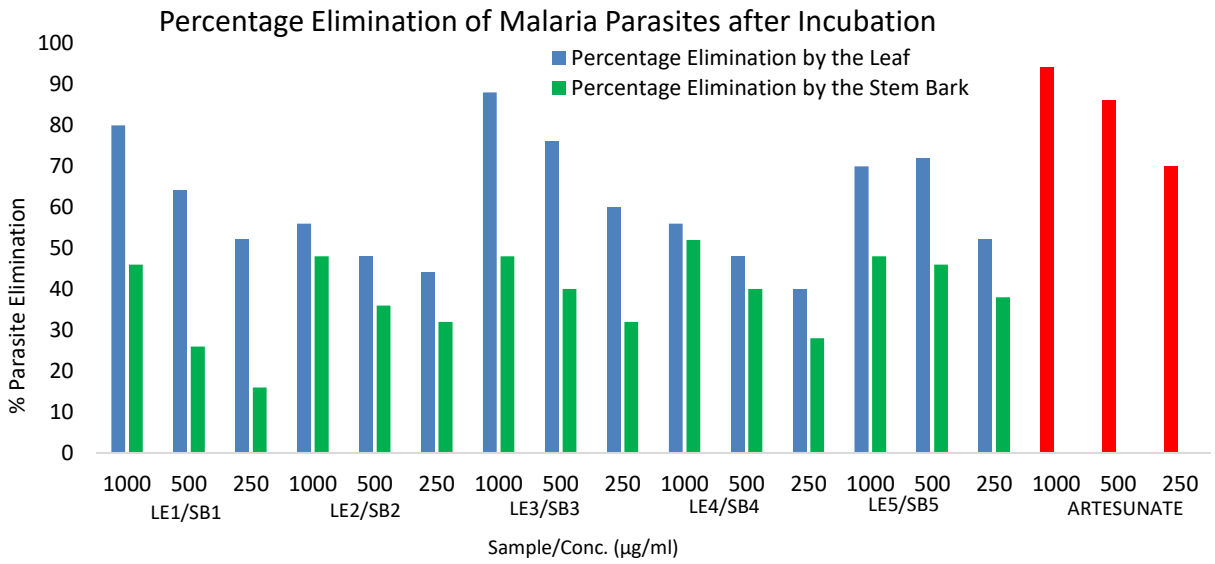

Figure 2: Antimalarial Activity of the Leaves and Stem Bark Extracts of *S. setigiera*

Key: LE1=crude ethanol extract, LE1-01= n-Hexane fraction, LE1-02 = Ethyl acetate fraction, LE1-03 = Chloroform fraction, LE1-04 = Methanol fraction. LE= Leaves; SB1=crude ethanol extract, SB1-01= n-Hexane fraction, SB1-02= Ethyl acetate fraction, SB1-03= Chloroform fraction, SB1-04= Methanol fraction. SB = Stem Bark. ART= Artesunate

ANTIOXIDANT ACTIVITY

DPPH Radical Scavenging Assay

Phytochemicals present in plants specifically phenols, can adjust the concentration of ROS, thus activating a network of biochemical events to increase tolerance and scavenge radical formations (Ibrahim *et al.*, 2023).

The antioxidant activity of the extracts of *S. setigera* showed the leaf extracts has the highest antioxidant capacity which is largely due to the presence of phenolic compounds. Figure 3 summarised the percentage inhibition of the plant extracts in comparison with positive standards ascorbic acid (IC_{50}=11.28 µg/ml) and gallic acid IC_{50}=25.52 µg/ml). From the leaf extracts, the ethanol extracts and ethyl acetate fraction displayed the strongest radical scavenging activity with higher percentage inhibition and lower IC_{50} values (LE1=13.78 µg/ml and LE1-02=60.79 µg/ml). From the stem bark extracts, the methanol fraction showed moderate activity (SB1-04=237.71 µg/ml). Extracts/fractions with IC_{50} values higher than 1000 µg/ml were regarded as not active (NA). The DPPH test relies on the elimination of DPPH, a stabilized free radical. Once, reduced and transformed into DPPH-H, the DPPH radical has a dark purple colour in solution, but when reduced as well as transformed into DPPH-H, it turns colourless or light yellow (Chaves *et al.*, 2021). The result of DPPH scavenging action of *S. setigera* could be related to the existence of flavonoids as well as other polyphenols throughout extraction, as shown in the current work.

There was a direct correlation between antimalarial activity and antioxidant activity, with the same order of activity of plant extracts observed in both assays.

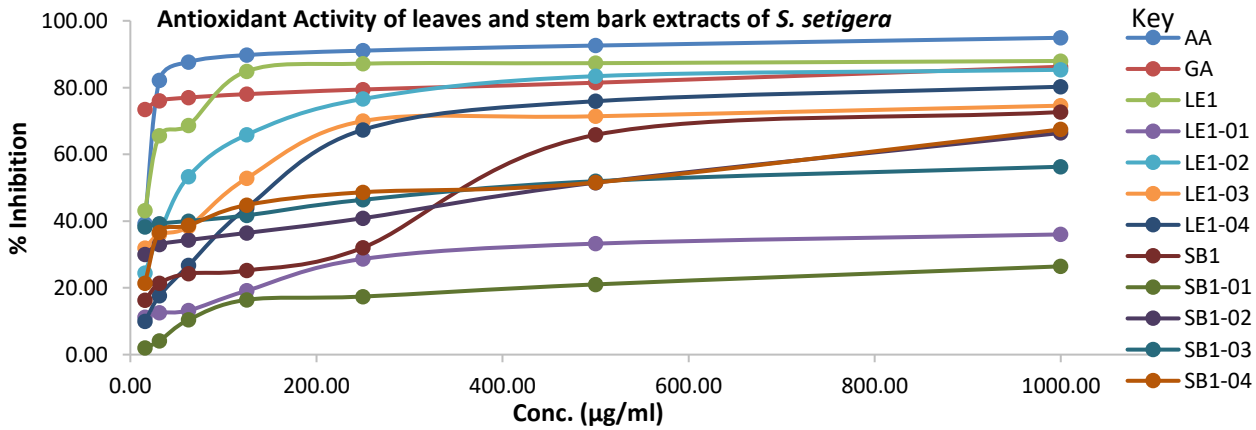

Figure 3: DPPH radical scavenging activity of the leaves and stem bark extracts of *S. setigera*

CONCLUSION

The present work has shown the efficacy of extracts of the leaves and stem bark of *Sterculia setigera* traditionally used to cure various infections in humans had shown significant antioxidant activity against DPPH assay and antimalarial activity against *Plasmodium falciparum*. The leaves extracts displayed a reasonable antioxidant and antimalaria activities compared to the standard control. A correlation between antioxidant and antimalaria activity was observed among the tested extracts/fractions. It would therefore be worthwhile to purify the active components by a bioassay-guided isolation. With the enriched fractions or the pure compounds, active components would be identified by spectroscopic techniques and their medicinal efficacy would be established.

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CONFLICT OF INTEREST

The Authors declare that there is no conflict of interest.

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