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In vitro Screening of Potential Antioxidative and Toxicity of the Stembark Extracts from *Sterculia setigera Del.*

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Abstract:
The current investigation deals with the *in vitro* antioxidative and toxicity effects of methanol and chloroform extracts from the stem bark of *Sterculia setigera*. The stem bark of the plant was extracted using organic solvents (methanol and chloroform) to afford methanol extract (ME) and chloroform extract (CE). The extracts were evaluated for antioxidative potentials using 2.2-diphenyl-1-picrylhydraxyl (DPPH) radical, also toxicity effect of the methanol extract was investigated against *Artemia salina larvae*. The antioxidative effects for the extracts uncovered promising activity on the DPPH with significant scavenging effects of 80.64% and 18.3% at 1000 and 7.813 µg/mL respectively. These values were found to be comparable with those of ascorbic acid (97.80 and 49.62%) and butylated hydroxytoluene (94.44 and 20.98%). The methanol extract was found to be as toxic as the positive control (potassium dichromate) with LC_{50} values of 1.64 µg/mL and 1.42 µg/mL, respectively. The antioxidative and cytotoxicity properties of methanol extract from *S. setigera* supported the ethno-medicinal claims on the plant as a curative agent of different diseases of clinical concern.

Keywords: *Sterculia setigera*; *Stembark extract;* Cytotoxicity; Antioxidant.

I. Introduction

Medicinal plants have been used in virtually all cultures as a source of medicine to treat health disorders and to prevent diseases including epidemics[1]. The active compounds produced during secondary metabolism are associated with the biological properties of plant species used for various purposes[2]. The safety, quality, and efficacy of medicinal plants and herbal products has now become a key issue in developed and developing nations[3].

Sterculia setigera Del. is a medicinal plant of sterculiaceae family. It is a deciduous savanna tree that grows up to 15 m high and characterized by pale purplish bark with thin scales which peel off to expose yellowish patches; slash crimson, exuding a gummy sap. The plant is known by different indigenous cultural communities in Nigeria: Hausa–"Kukuki"; Fulani–"bo"boli"; Yoruba–"Ose-awere"[4]. It is a savannah tree, widespread in savannah areas of tropical Africa. The seeds are with yellow aril and the tree is found in open savannah woodlands, often characterized by stony hills[4].

In ethnomedicinal practices in Sudan, the hot water of the dried stem bark extract is used for the treatment of jaundice, bilharzia and diarrhea, while the dried stem bark powder is used for treating wounds[5]. The Yoruba people of Nigeria use a black soap prepared from black powder obtained from burnt mixture of the fruits and seeds in the treatment of dermatosis[4].

Free radicals are atoms or group of atoms with unpaired electron. They are highly reactive due to their tendency to capture electrons from a stable molecule to reach their electrochemical stability, thereby causing a large number of diseases including cancer[6] Cardiovascular diseases[7] and neural disorders[8]. These number of diseases emanated when intracellular free radicals exceeds the antioxidant defense, leading to cell oxidative stress thereby causing damage to biomolecules such as lipids, proteins and nucleic acid, and protection against free radicals can be enhanced by ample intake of dietary antioxidants[9].

The current study was designed to evaluate the antioxidative and toxicity effects of chloroform and methanol extracts from stem bark of *Sterculia setigera Del* using DPPH scavenging and brine shrimp lethality techniques.

II. Materials and Methods:

II.1. Chemicals

All chemicals used in this work were purchased from Sigma Aldrich and used without further purification.

II.2. Sample Collection and Identification

The Fresh stem bark sample of *Sterculia setigera* was collected from Kankara Local Government Area of katsina State, Nigeria. The plant's leaves together with the stem bark were identified and authenticated at the Department of Plant Biology, Bayero University, Kano, Nigeria. The Voucher specimen of the plant was deposited at the herbarium unit of the University.

II.3. Sample Preparation

The stem bark sample of *Sterculia setigera* was washed with deionized water, diced into pieces, air dried under shade at ambient temperature for about two (2) weeks and then coarsely powdered. The powdered sample was stored in an air-tight container, cool and dry place away from any form of contaminant [10].

II.4. Extraction

The powdered sample (75 g) was percolated with chloroform (300 mL) in amber bottle with shaking at regular intervals for three (3) days. The filtrate was separated from the debris by filtration and then concentrated using a rotary evaporator (R110) at 40 ^{o}C to afford chloroform extract (CE) and kept away from any form of contaminant [11].

The debris was then percolated again with methanol (300 mL) and allowed to stand for 3 days with constant shaking and then filtered. The filtrate was also concentrated using same machine at same temperature to obtain methanol extract (ME).

The free radical scavenging activity of the two extracts (CE and ME) against 2.2-diphenyl-1-picrylhydrazyl (DPPH) was determined according to the method described by [12] with little modification. A methanol solution of 10 µg/mL DPPH was used for the assay. It is prepared by dissolving 1.0 mg of DPPH in 100 mL of methanol in amber bottle covered with aluminium foil paper and kept below 25 ^{o}C. The Stock solutions (1000 µg/mL) of the samples were prepared by dissolving 1.0 mg of each extract in methanol (1.0 mL). Solutions of the concentrations; 500, 250, 125, 62.5, 31.3, 15.63 and 7.81 µg/mL were prepared from the stock solution by serial dilution with methanol.

II.5. Antioxidative Effect Test

Test sample (100 µL) was transferred into a 96-well micro-plate using a sterile micropipette in triplicate according to concentration gradient. Their absorbance was determined at 517 nm using a microplate reader. Solution of DPPH (40 µL) was added to the test samples and allowed to react in darkness for 30 minutes at room temperature. The absorbance of the mixture (sample + DPPH) was determined at the wavelength of 517 nm. Percentage inhibition of the test samples were determined based on the equation 1;

$$\%\text{Inhibition} = 100 - \left(\frac{\text{ABS}_{\text{sample}} - \text{ABS}_{\text{blank}}}{\text{ABS}_{\text{Control}}}\right) \quad \text{x} \quad \frac{100}{1} \qquad (1)$$

Ascorbic acid and butylated hydroxytoluene were used as positive control. The concentrations of each sample that provides 50% Inhibition (IC_{50}) was determined using SPSS version 16 [12].

II.6. Hatching of *Artemia Salina* Eggs

Artemia Salina eggs (6.4 g) were hatched in sea water (100 mL). After 48 hours incubation at room temperature, the larvae was attracted to one side of the vessel with a light source and collected by pipette. Larvae were separated from eggs by aliquoting them three times in small beakers containing seawater[13] [14].

II.7. Preparation of Stock Solution and other working concentrations

About 1.0 mg each of the two extracts (CE and ME) was dissolved in methanol (1.0 mL) to give 1000 µg/mL stock solution. Solutions of concentrations 100 and 10 µg/mL were prepared by 10-fold dilution[15] [16].

II.8. Cytotoxicity Assay Procedure

Toxicity of the sample was monitored by the brine shrimp lethality test according to the method of [16] and [17] with slight modification. Each of the sample (1.0 mg) was dissolved in methanol (1.0 mL), from which 5 000, 500 and 50 µL of each solution was transferred into vials corresponding to 1.00, 0.10 and 0.01 mg/mL respectively. This was allowed to evaporate to dryness in about 24 h at room temperature. Each dosage was tested in triplicate (9 per test sample). Sea water (4 mL) and 10 larvae were introduced into each vial. The final volume of solution in each vial was adjusted to 5 mL with seawater immediately after adding the shrimps. A negative control was prepared as a drug-free and potassium dichromate was used as positive control. Survivours were counted after 24 h (equation 2), and LC_{50} was determined by probit analysis using SPSS version 16[18].

$$\%\text{Mortality} = \frac{\text{Total Number of nauplii} - \text{Number of nauplii survived}}{\text{Total Number of nauplii}} \times \frac{100}{1} \qquad (2)$$

III. Results and Discussion

III.1. Extraction

The weight, texture, colour and percentage yield of chloroform and methanol extracts of the stem bark of *Sterculia setigera* are shown in the Table 1. The percentage yield of the methanol extract appeared higher than that of the chloroform extract and both are brownish in colour of different texture. The solid nature of methanol extract entails that is more of polar compounds than the chloroform extract which is gummy in nature.

***Table 1**: Physical Properties of chloroform and methanol extracts*

Extract	Weight	%Yield	Colour	Texture
Chloroform	2.08	2.77	Brown	Gummy
Methanol	4.78	6.37	Brown	Solid

III.2. Antioxidative effect

The antioxidant effects of the chloroform extract (CE), methanol extract (ME), ascorbic acid (AA) and butylatedhydroxytoluene (BHT) were expressed as percent scavenging activity (Figure 1). The results uncovered promising antioxidative effects with percentage scavenging activities of 80.91 and 52.56% for chloroform extract (CE), 80.64 and 18.3% for methanol extract (ME) at concentrations of 1000 and 7.813 µg/mL, respectively. These values are comparable with those of ascorbic acid (97.8 and 49.62%) and butylatedhydroxytoluene (94.4 and 20.98%) used as positive controls at the same concentrations.

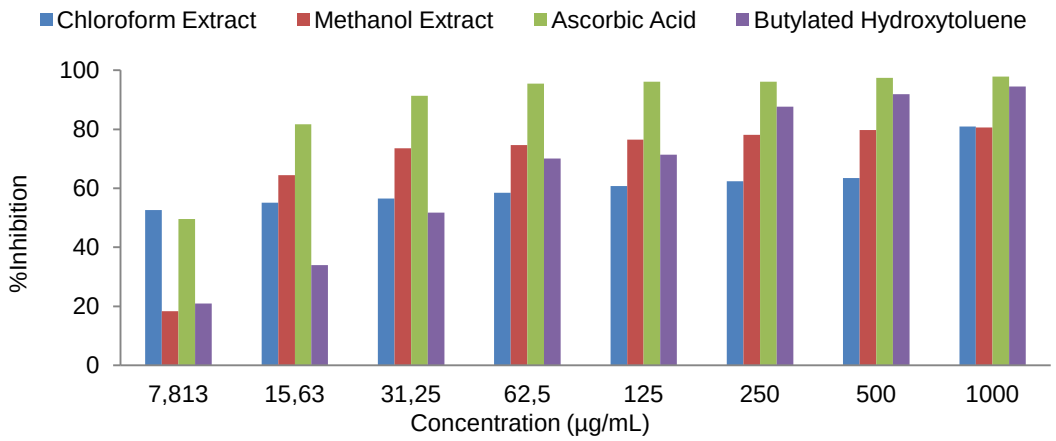

***Figure 1**: Percentage inhibition of S. setigera extracts and positive controls*

The free radical scavenging effects of the tested samples (Figure 1) were found to be concentration dependent, which is in agreement with the results obtained by [5]. They reported antioxidant activities of ethanol and petroleum ether extracts from the stem bark of *S. setigera* at 500 µg/mL with percent inhibitions of 86.00% and 37.48%, respectively.

Many synthetic antioxidative chemicals are toxic and their risk to health has increased the demand for natural antioxidant[19]. As seen from the results of this experiment, the stem bark of *Sterculia setigera* has potential antioxidative agents and if found non-toxic can be used as a natural source that can help to quench oxidative stress that occurs during physiological processes in a living system.

III.3. Cytotoxicity Assay against Brine Shrimp

The brine shrimp lethality test was carried out in order to determine the lethality of methanol extracts from *S. setigera* against the brine shrimp larvae. The percentage mortality at different concentrations and the IC_{50} values are shown in table 2. According to [20] [21] the brine shrimp lethality assay results are interpreted in accordance to the criterion by Meyer toxicity index that, LC_{50}

values>1000 µg/mL are considered nontoxic, LC_{50} values≥500≤1000 µg/mL are considered to have weak toxicity, while those having LC_{50} values <500 µg/mL are considered highly toxic.

***Table 2**: Brine shrimp lethality assay of methanol extract of S. Setigera*

		Methanol Extract			Potassium Dichromate			Sea water
Concentration (µg/mL)		1000	100	10	1000	100	10	
Shrimp Larvae used		10	10	10	10	10	10	10
Number of survived Shrimp Larvae	X_1	0	0	0	0	0	0	10
	X_2	0	1	2	0	0	1	10
	X_3	0	2	3	0	0	1	10
Total larvae survived		0	3	6	0	0	2	30
Number of dead larvae		30	27	24	30	30	28	0
Percentage mortality		100	90	80	100	100	93.33	0
LC_{50} (µg/mL)			**1.642**			**1.423**		

The LC_{50} values of methanol extract and potassium dichromate (positive control) were found to be 1.642 and 1.423 µg/mL respectively (Table 2). The toxicity value of the methanol extract (1.642 µg/mL) suggested that it is highly toxic. This indicates its potency as a source of antimicrobial and antitumor agents [22]. The toxicity results of methanol extract and potassium dichromate solution in comparable with that of negative control (seawater only) suggested that, the lethality of the shrimp larvae at the tested concentrations (1000, 100 and 10 µg/mL) was due to the activity of the extract.

IV. Conclusion

The findings of present study concluded that *Sterculia setigera* stem bark extracts possesses antioxidative and cytotoxic effects, suggesting the presence of potential bioactive chemical constituents. The cytotoxicity study uncovered that, methanol extract is highly toxic (LC_{50}=1.642 µg/mL), and could be employed as promising alternative in the treatment and management of tumors, since lethality bioassay against brine shrimp larvae is now serving as an indicator for the preliminary screening of bioactivity including for anticancer.

This toxicity results of methanol extract suggested that the extract contained highly active phytochemical ingredients which validates the primitive use of the plant's stem bark as a therapeutic agent for various infectious diseases. Therefore, proper conservation is needed for this medicinal plant (*Sterculia setigera Del.*) so as to adopt local usage and preparation modes of traditional remedies.

V. References

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