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In vitro Antioxidant Activity of *Salacia lehmbachii* Ethanol Root Bark Extract

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Authors' contributions

This work was carried out in collaboration between all authors. Author FVU designed the study and supervised the work. Author GCA performed the experiments. Authors GAE and ADE wrote the first draft of the manuscript. Authors DEO, SKN and EMN did extensive literature review and performed the statistical analysis. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: This work was undertaken to investigate the *in vitro* antioxidant activity of *S. lehmbachii* ethanol root bark extract.
Place and Duration of Study: Department of Pharmacology, University of Calabar, Nigeria, between June, 2013 and October, 2015.
Methods: Antioxidant activity of the root extract was evaluated using DPPH (2, 2-diphenyl-1-picrylhdrayl) radical, Nitric oxide and hydrogen peroxide scavenging assays.
Results: A significant inhibition at different concentration of the extract was observed with DPPH radical, nitric oxides and hydrogen peroxide scavenging activities when compared with ascorbic acid.

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Conclusion: The results of the study show that the ethanol root bark extract of *S. lehmbachii* may serve as a good scavenger of free radicals, hence reduces the effects of oxidative stress in the body and could be explored as a therapeutic agent in free radical induced diseases.

Keywords: Free radicals; Salacia lehmbachii; DPPH; Nitric oxide; Hydrogen peroxide scavenging activity.

1. INTRODUCTION

Free radicals are believed to be involved in the cause of numerous disorders in human such as cancer, diabetes, atherosclerosis, ageing and other degenerative diseases [1]. To protect the adverse effects of free radicals, enzymes such as superoxide dismutase and catalase are generated by human cells or compounds like ascorbic acid and tocopherol [2]. Plants are endowed with free radical scavenging molecules including vitamins, flavoniods, tannins, terpenoid and alkaloids which are rich in antioxidant and protect the body from being damaged by free radicals. The antioxidants scavenge the free radicals which are generally present in biological systems [3]. The consumption of these natural antioxidants has been associated with reduced risks of chronic diseases [4]. There is so much interest towards the development of natural antioxidant which is found in plants as the phenols, flavonoids, alkaloids, tannins, vitamins, terpenoids and other secondary metabolites are essential in prevention of oxidative damage [5]. Recent finding has shown that consumption of natural antioxidants has been linked with reduced risk of many chronic diseases [6].

Salacia lehmbachii Loes (family celastraceae) is evergreen small tree of about three meters high and widely distributed in tropical area of East, West and Central Africa [7]. There are diverse therapeutic applications of *S. lehmbachi* which justify its folkloric background. The leaf extract of this plant has been evaluated for *in vivo* and *in vitro* antioxidant and antipyretic activities [8,9], while the root extract has demonstrated anticholinergic and anti-infertility activities [10,11].

The present study investigates the antioxidant potential of *S. lehmbachii* ethanol root bark extract in different assay methods.

2. MATERIALS AND METHODS

2. 1 Plant Material Collection

Salacia lehmbachii roots were collected from Ukanafu in Akwa Ibom State, Nigeria and were identified by a taxonomist in the department of Botany, University of Calabar, Calabar, Nigeria. A voucher specimen number 688 was deposited at the University herbarium for future reference.

2.2 Preparation of Extract

The roots were cleaned, cut into pieces and air-dried at room temperature, and ground to powder using mortar and pestle. The powdered root (350 g) was macerated in 1.5 L of 96% ethanol for 24 h and filtered. The filtrate was dried on a water bath at reduced temperature of 40℃ to recover the extract and the yield was calculated to be 9.8% w/w. The root bark extract was stored in refrigerator for subsequent use

2.3 Chemicals

The chemicals used for this study were of analytical grade. DPPH (2,2-diphenyl-1-picrylhdrayl radical, ascorbic acid, Sodium nitroprusside were obtained from Fluka chemicals (Germany). Sulphanilamide, O-phosphoric acid, napthylethylenediamine dihydrochloride, hydrogen peroxide (H_20_2), prepared phosphate buffer saline (PBS) and ethanol were sourced from Sigma Chemical Company Inc. (USA).

2.4 Antioxidant Activity Assays

2.4.1 DPPH radical scavenging activity method

The free radical scavenging activity of ethanol root bark of *S. lehmbachii* was analyzed by the DPPH Spectrophotometric method [12]. The ethanol solution of DPPH (200 µm) and the test extract (0.05 ml) were added at different concentrations (50, 100, 200, 300 and 400 µg/ml). Methanol was used as a control (negative) while Ascorbic acid was used as a reference standard. The absorbance was read after 30 minutes of incubation at 517 nm. The percentage inhibition was calculated by the formula:

$$\% \text{ inhibition} = \frac{\text{Abs control-Abs Test}}{\text{Abs Control}} \times 100$$

2.4.2 Nitric oxide scavenging radical assay

This was done by adopting the method described by [13] with slight modification. Nitric oxide was collected from Sodium nitroprusside and measured by Griess reaction. Sodium nitroprusside (5 mM) in standard phosphate buffer solution was incubated with different concentration (50-400 µg/ml) of ethanol extract dissolved in phosphate buffer (0.05 m, pH 7.4) and all tubes incubated at 25℃ for 5 hours. The buffer which was used as control was also incubated. Thereafter, 0.5 ml was removed and diluted with 0.5 ml of Griess reagent (1% sulphanilamide, 2% 0-phosphoric acid and 0.1% napthylethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with napthylethylene diamine was read at 546 nm. The experiment was repeated in triplicate.

2.4.3 Hydrogen peroxide scavenging assay

Hydrogen peroxide scavenging potential of the plant extract was determined using the method described by [14] with a slight modification to determine the hydrogen peroxide scavenging ability of the root bark extract. A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffer saline (PBS, pH 7.4). Different concentrations of the extract (50 to 400 µg/ml) in ethanol (1 ml) were added to 2 ml of hydrogen peroxide solution in PBS. After 10 min the absorbance was measured at 560 nm against a blank solution that contained hydrogen peroxide solution without the extract. The percentage of H_2O_2 scavenging of the plant extract was calculated as follows:

$$\% \text{ scavenged} = \frac{\text{Abs control-Abs Test}}{\text{Abs Control}} \times 100$$

2.5 Statistical Analysis

All experiments were performed in triplicate. The data were analyzed and expressed as mean ± standard deviation (n = 3). Statistical comparisons were made using the one-way analysis of variance (ANOVA) and the Tukey HSD tests. Differences were considered to be significant when the p-values were $p < 0.05$.

3. RESULTS

3.1 DPPH Scavenging Activity

The DPPH scavenging activities of the ethanol root bark extract of *S. lehmbachii* are presented in Table 1. At 50-400 µg/ml, the antioxidant activities of ethanol root bark extract and the standard ascorbic acid were 79.6 - 94.2% and 92.4 - 96.2%, respectively. The results clearly indicate the dose-dependent DPPH scavenging activity of *S. lehmbachii* extract with an IC_{50} value of 100.6 µg/ml, and ascorbic acid with an IC_{50} value of 48.9 µg/ml.

Table 1. DPPH radical effect of ethanol root bark extract of *S. lehmbachii* at different concentrations (µg/ml) and IC_{50} values

Conc. µg/ml	Ethanol root bark	Standard (Ascorbic acid)
50	79.6±1.4	92.4±2.2
100	90.3±2.4	94.3±1.5
200	91.2±3.2	95.5±1.3
300	91.4±1.4	96.3±0.7
400	94.2±1.5	96.2±0.9
IC_{50}	100 .6±1.8	48.9±0.9

Results are expressed as mean ± SEM, n= 3 replicate.

3.2 Nitric Oxide Scavenging Activity

The maximum *in vitro* nitric oxide scavenging activity of ethanol root bark extract of *S. lehmbachii* at 400 µg/ml concentrations was 91.9%, while the minimum activity at 50 µg/ml concentration was 72.44%. The root bark extract showed a dose dependent elevation in Nitric oxide scavenging activity (Table 2). However, ascorbic acid showed more activity than that of the root extract.

Table 2. Nitric oxide scavenging effect of ethanol root bark extract of *S. lehmbachii* at different concentrations (µg/ml) and IC_{50} values

Conc. µg/ml	Ethanol root bark	Standard (Ascorbic acid)
50	72.44±2.10	82.70±1.5
100	86.9±3.2	90.50±2.3
200	90.8±2.4	94.6±0.9
300	91.6±2.11	96.3±1.2
400	91.9±2.8	95.5±2.5
IC_{50}	96 .4±2.3	34.6±0.5

Results are expressed as mean ± SEM, n= 3 replicate.

3.3 Hydrogen Peroxide Scavenging Activity

As shown in Table 3, the root bark extract of *S lehmbachii* also demonstrated good hydrogen peroxide scavenging activity in a concentration dependent manner. At the higher concentration

(400 µg/ml) of extract and ascorbic acid, the H_2O_2 scavenging activity was found to be 94.2% and 96.9%, respectively. These results showed that ethanol root bark extract had effective H_2O_2 scavenging activity.

Table 3. Hydrogen peroxide scavenging effect of ethanol root bark extracts of *S. lehmbachii* at different concentrations (µg/ml) and IC_{50} values

Conc. µg/ml	Ethanol root bark	Standard (Ascorbic acid)
50	72.44±2.10	81.9±2.4
100	86.9±3.2	90.4±2.2
200	90.8±2.4	94.3±3.1
300	91.4±1.4	96.4±3.3
400	94.2±1.5	96.9±2.4
IC_{50}	88.4±2.6	34.9±0.5

Results are expressed as mean ± SEM, n= 3 replicate

4. DISCUSSION

Although, the presence of many antioxidant mechanisms in the body is to neutralize the reactive species when there are overproduction of them; sometimes these mechanisms are not enough to eliminate them from the body. This overproduction leads to an imbalance between the production and elimination of free radicals, which is called oxidative stress and can cause disturbs in many physiological processes [15-18]. Because of that, many studies have shown that consuming food rich in antioxidants is very important to reduce the damages caused by reactive species [19]. Numerous studies on natural products have shown various compounds with antioxidant activity [20]. Different diseases have been linked to free radicals [21]. Their use as supplements was shown to also minimize or reverse oxidative damage induced by disease and improve treatment outcomes [22,23]. Some plants extracts showed relative biological free radicals scavenging ability due to their flavonoids content [24,25]. The reducing properties of medicinal plants have been suggested to associate with the presence of reductones, which exerts antioxidant action by breaking the free radical chains through hydrogen atom donation [26-30].

Different concentrations of *S. lehmbachii* used in this study showed a dose dependent percentage of inhibition on the scavenging assays. Therefore, the ethanol extract showed significant antioxidant potential that may reveal its therapeutic potentials for several diseases.

5. CONCLUSION

The results obtained from this study strongly suggest that the ethanol root bark extract of *S. lehmbachii*, has significant antioxidant activity and can serve as natural rich antioxidant which may enhance the immune system against oxidative damage caused by reactive oxygen and nitrogen species.

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. Approval for this study was obtained from the appropriate ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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