

In vitro* antioxidant activity of *Salacia lehmbachii

ethanol root bark extract

Abstract

Aim: This work was undertaken to investigate the *in vitro* antioxidant activity of *S. lehmbachii* ethanol root bark extract.

Methods: Antioxidant activity of the root extract was evaluated using DPPH (2, 2-diphenyl-1-picrylhydrazyl) 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.

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.

Key words: 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

22 diseases (Aiyegoro OA, Okoh). To protect the adverse effects of free radical,
23 enzymes such as superoxide dismutase and catalase are generated by human
24 cells or compounds like ascorbic acid and tocopherol [Niki et al., 1994]. Plants
25 are endowed with free radical scavenging molecules including vitamins,
26 flavonoids, tannins, terpenoid, alkaloids which are rich in antioxidant and
27 protect the body from being damaged by free radicals. The antioxidants
28 scavenge the free radicals which are generally present in biological systems
29 (Purbi et al., 2011). The consumption of these natural antioxidants has been
30 associated with reduced risks of chronic diseases (Gerber et al, 2002). There is
31 so much interest towards the development of natural antioxidant which is found
32 in plants as the phenols, flavonoids, alkaloids, tannins, vitamins, terpenoids and
33 other secondary metabolites are essential in prevention of oxidative damage
34 (Ames et al., 1995). Recent finding has shown that consumption of natural
35 antioxidants has been linked with reduced risk of many chronic diseases (Kris-
36 Etherton et al., 2002).

37 *Salacia lehmbachii* Loes (,family celastraceae) is evergreen small tree of about
38 three meters high widely distributed in tropical area of East, West and Central
39 Africa (Corstiaen and Sosef, 2007). There are diverse therapeutic applications
40 of *S. lehmbachii* which justify its folkloric background. The leaf extract of this
41 plant has been evaluated for *in vivo* and *in vitro* antioxidant and antipyretic
42 activities

(Okechi et al., 2015; Essien et al, 2015a), while the root extract has demonstrated anticholinergic and anti-infertility activities (Essien et al., 2015b; Essiet et al., 2016).

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 °C 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-picrylhydrazyl radical, Ascorbic acid, Sodium nitropruside were

64 obtained from Fluka chemicals, sulphanilamide, 0-phosphoric acid,
 65 naphthylethylenediamine dihydrochloride, hydrogen peroxide (H₂O₂), prepared in
 66 phosphate buffer saline (PBS), ethanol, were sourced from Sigma Chemical
 67 Company Inc., St. Louis, MO, USA.

68 **2.4 Antioxidant activity assays**

69 **2.4.1 DPPH radical scavenging activity method**

70 The free radical scavenging activity of ethanol root bark of *S. lehmbachii* was
 71 analyzed by the DPPH Spectrophotometric method (Onoja and Anaga, 2013).
 72 The ethanol solution of DPPH (200 µm) and the test extract (0.05 ml) were
 73 added at different concentrations (50, 100, 200, 300 and 400 µg/ml). Methanol
 74 was used as a control (negative) while Ascorbic acid was used as a reference
 75 standard. The absorbance was read after 30 min of incubation at 517 nm. The
 76 percentage inhibition was calculated by the formula: % inhibition = Control-
 77 Test X 100

78 Control

79 **2.4.2 Nitric oxide scavenging radical assay**

80 This was done by adopting the method described by Pacher et al., (2007) with
 81 slight modification. Nitric oxide was collected from sodium nitropruside and
 82 measured by GRiess reaction. Sodium nitropruside (5 mM) in standard
 83 phosphate buffer solution was incubated with different concentration (50-400
 84 µg/ml) of ethanol extract dissolved in phosphate buffer (0.05 m, PH 7.4) and all
 85 tubes incubated at 25 °C 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% naphthylethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with naphthylethylene 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 Jayaprakasha et al. (2004) 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 H₂O₂ scavenging of the plant extract was calculated as follows:

$$\% \text{ scavenged } [\text{H}_2\text{O}_2] = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100$$

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

107 made using the one-way analysis of variance (ANOVA) and the Tukey HSD
108 tests. Differences were considered to be significant when the p-values were $p <$
109 0.05.

110 **3. Results**

111 **3.1 DPPH scavenging activity**

112 The DPPH scavenging activities of the ethanol root bark extract of *S.*
113 *lehmbachii* are presented in Table 1. At 50-400 $\mu\text{g/ml}$, the antioxidant activities
114 of ethanol root bark extract and the standard ascorbic acid were 79.6 - 94.2 %
115 and 92.4 - 96.2 %, respectively. The results clearly indicate the dose-dependent
116 DPPH scavenging activity of *S. lehmbachii* extract with an IC_{50} value of 100.6
117 $\mu\text{g/ml}$, and ascorbic acid with an IC_{50} value of 48.9 $\mu\text{g/ml}$.

118 **3.2 Nitric oxide scavenging activity**

119 The maximum *in vitro* nitric oxide scavenging activity of ethanol root bark
120 extract of *S. lehmbachii* at 400 $\mu\text{g/ml}$ concentrations was 91.9 %, while the
121 minimum activity at 50 $\mu\text{g/ml}$ concentration was 72.44 %. The root bark extract
122 showed a dose dependent elevation in NO scavenging activity (Table 2).
123 However, ascorbic acid showed more activity than that of the root extract

124 **3.3**

125 **Hydrogen peroxide scavenging activity**

126 As shown in table 3, the root bark extract of *S lehmbachii* also demonstrated
127 good hydrogen peroxide scavenging activity in a concentration dependent

manner. At the higher concentration (400 µg/ml) of extract and ascorbic acid, the H₂O₂ scavenging activity was found to be 94.2 % and 96.9%, respectively. These results showed that ethanol root bark extract had effective H₂O₂ scavenging activity.

Table 1

DPPH radical effect of ethanol root bark extract of *S. lehmbachii* at different concentrations (µg/ml) and IC₅₀ 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	4.8±0.9

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

Table 2

Nitric oxide scavenging effect of ethanol root bark extract of *S. lehmbachii* at different concentrations (µg/ml) and IC₅₀ 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

152 IC_{50} 96.4±2.3 3.4±0.5

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

154 **Table 3**

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

157			
158	Conc. µg/ml	Ethanol root bark	Standard (Ascorbic acid)
159	50	72.44±2.10	81.9±2.4
160	100	86.9±3.2	90.4±2.2
161	200	90.8±2.4	94.3±3.1
162	300	91.4±1.4	96.4±3.3
163	400	94.2±1.5	96.9±2.4
164	IC_{50}	88.4±2.6	3.4±0.5

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

166

167 **4. Discussion**

168 Although, the presence of many antioxidant mechanisms in the body to
 169 neutralize the reactive species when there are overproduction of them;
 170 sometimes these mechanisms are not enough to eliminate them from the body.
 171 This overproduction lead to an imbalance between the production and
 172 elimination of free radicals, which is called oxidative stress and can cause
 173 disturbs in many physiological processes (Cervellati et al., 2014; Kumar, 2011;
 174 Lushchak, 2014; Sies, 2015). Because of that, many studies have shown that
 175 consuming food rich in antioxidants is very important to reduce the damages

caused by reactive species (Rezaire et al., 2014). Numerous studies on natural products have shown various compounds with antioxidant activity (Almeida et al., 2016). Different diseases have been linked to free radicals (Rafikali and Nair, 2001). Their use as supplements was shown to also minimize or reverse oxidative damage induced by disease and improve treatment outcomes (Ghashghaeinia *et al.*, 2010; Reis *et al.*, 2010). Some plants extracts showed relative biological free radicals scavenge ability due to their flavonoids content (Moure *et al.*, 2001; Jovanovic and simic, 2000). The reducing properties of medicinal plants have been suggested to associate with the presence of reductones, which exert antioxidant action by breaking the free radical chains through hydrogen atom donation (Gordon, 1990; Pin-Der-Duh, 1998; Stamler *et al.*, 1992; Galigniana *et al.*, 1999; Lee *et al.*, 2004).

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.

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

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