



# **Phytochemical Identification and Comparative *In-vitro* Anti-Oxidant Studies of Aqueous, Ethanol and Methanol Rootbark Extracts of *Simarouba glauca* DC (Paradise Tree)**

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## **Authors' contributions**

*This work was carried out in collaboration between both authors. Author SDEOE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SDEOE and MEO managed the analyses of the study. Author MEO managed the literature searches. Both authors read and approved the final manuscript.*

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## **ABSTRACT**

**Aims:** The study was conducted to determine the presence of selected phytochemicals and *in-vitro* antioxidant potency of aqueous, ethanol and methanol rootbark extracts of *Simarouba glauca*.

**Study Design:** True experimental study.

**Place and Duration of Study:** Department of Biochemistry, University of Benin, Benin City, Nigeria, between July and August 2017.

**Methodology:** Rootbark of the plant was harvested from a private farm at Ubiaja, Esan south-east part of Edo State, air dried, pulverized and extracted with water, ethanol or methanol solvents and freeze-dried to obtain respective fractions of extracts. An alkaloid, total phenol, tannins and Flavonoid content of rootbark extracts were evaluated by established standard experimental

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methods and principles. DPPH radical scavenging activity, reducing power activity, total antioxidant activity, hydroxyl free radical scavenging activity, ABTS<sup>+</sup> radical scavenging activity and nitric oxide radical scavenging activity were also evaluated based on established standard methods and principles.

**Results:** Alkaloids and flavonoids were observed in aqueous, ethanol and methanol rootbark extracts; flavonoid was, however, absent in the aqueous fraction. Total phenols and tannin were absent in all fraction. DPPH radical scavenging activity of aqueous, ethanol or methanol fraction extracts recorded no values at 50% inhibition concentration (IC<sub>50</sub>); although the extracts demonstrated the anti-DPPH radical effect at lower inhibitory concentrations, while BHT recorded an IC<sub>50</sub> of 18 µg. Reducing power activity of aqueous, ethanol or methanol fraction at IC<sub>50</sub> was 11 µg, 10 µg and 11 µg respectively while BHT was 11 µg. The total antioxidant activity of aqueous, ethanol or methanol rootbark extracts at IC<sub>50</sub> are 23, 21 and 58 µM FeSO<sub>4</sub> equivalent/µg of Fe<sup>2+</sup> liberated respectively relative to 388 µM FeSO<sub>4</sub> equivalent/µg of Fe<sup>2+</sup> liberated by BHT. Hydroxyl free radical activity of aqueous, ethanol and methanol fraction at IC<sub>50</sub> was 11 µg, 100 µg and 11 µg respectively while BHT was 11 µg. ABTS<sup>+</sup> radical scavenging activity of aqueous, ethanol or methanol fraction extracts at IC<sub>50</sub> was 29 µg, 25 µg and 34 µg respectively whereas BHT was 21 µg. Nitric oxide radical scavenging activity of aqueous, ethanol or methanol fraction extracts at IC<sub>50</sub> was 14 µg, 14 µg and 14 µg respectively whereas Quercetin was 16 µg. Butylated hydroxytoluene (BHT) and Quercetin was utilized as standard antioxidant.

**Conclusion:** The outcome of the research study revealed that the aqueous, methanol and ethanol rootbark extracts of *Simarouba glauca* possess significant phytochemicals and antioxidant potency, although, the methanol fraction appears to be more effective against investigated radicals.

**Keywords:** *Simarouba glauca*; rootbark; phytochemicals; oxidants; radical scavenging properties.

## 1. INTRODUCTION

Folk medicine has provided substantial information on the traditional health implications of a wide variety of plants. Plants provide naturally occurring phytochemicals as a rich source of antioxidants having free radical scavenging property [1]. Exploration of the plant kingdom for the presence of naturally occurring biologically active phytochemicals that can mitigate; in fact, cure and manage some diseases has led to the identification of several plants such as *Simarouba glauca*. *Simarouba* is belonging to the family Simaroubaceae; are widely documented in several pharmacopoeias for their medicinal value [2]. Among the thousands of naturally occurring constituents so far identified in plants and exhibiting a long history of safe use, there are none that pose – or reasonably might be expected to pose a significant risk in human health at low levels of intake when used as flavouring substances. Studies hitherto conducted on other vegetative parts of this plant has revealed the potent availability of phytochemicals and anti-oxidant properties [3], hence the need to explore the health benefits of the rootbark, as studies have shown that the root of plants can accommodate a significant amount of active principles of medicinal interest.

Antioxidants are reducing agents and limit oxidative damage to biological structures by passivating free radicals [4]. The antioxidant can be categorized into two main types called primary and secondary antioxidants where each type is responsible for different mechanisms [5]. Primary antioxidant acts to scavenge free radicals to inhibit chain initiation and to break chain propagation by donating hydrogen atoms or electrons that convert them into a more stable product. Secondary antioxidant functions by suppressing formation of radicals and protect against oxidative damage. Besides, a secondary antioxidant is also active in binding with metal ions and scavenging oxygen radicals. *Simarouba glauca* is a medium-sized evergreen tree with tap root system and cylindrical stem. The plants are polygamodioecious with about 5% of the population producing exclusively staminate (male) flowers and 40-50% producing mainly male flowers and a few bisexual (andromonoecious) while the remaining 40-50% produces only the pistillate (female) flowers. Common Names includes aceituno, paradise tree, negrito, Dysentery bark, palo blanco amongst others [6]. The study sought to identify selected phytochemicals and evaluate the comparative *in-vitro* antioxidant activities of the rootbark extracts of *Simarouba glauca*.

## 2. MATERIALS AND METHODS

### 2.1 Plant Materials

The rootbark of *Simarouba glauca* was harvested from a private farm located at Ubiaja, Esan - South East Local Government Area of Edo State, Identified at the Department of Plant Biology and Biotechnology and samples were transported to Department of Biochemistry, air dried for approximately 29 days and pulverised at the Department of Pharmacognosy Laboratory, at the University of Benin.

### 2.2 Preparation of Plant Extracts

A 400 g of the rootbark of *Simarouba glauca* was weighed and submerged in 5 litres of water, ethanol or methanol for 24 hours, after which the mixture was sieved off to obtain the filtrate. The filtrate was decanted into a clean, sterile container and the residue was subsequently re-submerged in a 3 L of water, ethanol or methanol of 99% purity for another 24 hours. The same procedure as earlier stated was carried out to obtain the filtrate. The filtrate was transported to the Department of Biochemistry, Adekunle Ajasin University, Akungba-Akoko, Ondo State; freeze-dried with the aid of a freeze dryer to obtain pure extracts of water, methanol and ethanol. These extracts were stored in sterile bottles and kept in the refrigerator at -4°C until required for analysis.

### 2.3 Identification of Alkaloids

Alkaloid of rootbark was determined by the principle described by Sani [7]. To 2 ml of the root extract of *Simarouba glauca*, 2 ml of 10% hydrochloric acid was added. In the acidic medium, 1 ml Hager's reagent (saturated picric acid solution) was added. Presence of Alkaloid is confirmed by formation of a yellow coloured precipitate.

### 2.4 Identification of Phenols

The qualitative determination of total phenol was conducted by the method described by Trease and Evans [8]. 2 ml of root extract of *Simarouba glauca* was mixed with few drops of 10% Ferric chloride solution. The formation of green-blue or violet or blue-black colouration indicates the presence of phenolic compounds.

### 2.5 Identification of Tannins

The identification of tannin was determined by the method described by Sofowora [9]. To 2 ml of

root extract of *Simarouba glauca*, five drops of 0.1% Ferric chloride was added. The formation of a brownish green or blue-black colouration indicates a positive presence of tannins.

### 2.6 Identification of Flavonoids

The total flavonoid in aqueous, ethanol and methanol rootbark extracts was determined by the methods described by Santhi and Sengottuvel [10].

#### 2.6.1 Lead acetate test

To 2 ml of root extract of *Simarouba glauca*, few drops of Lead acetate solution was added. Formation of yellow coloured precipitate indicates the presence of flavonoids.

#### 2.6.2 Sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) method test

To 2 ml of root extract of *Simarouba glauca*, few drops of the H<sub>2</sub>SO<sub>4</sub> solution was added. Formation of orange coloured precipitate indicates the presence of flavonoids.

### 2.7 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Assay

DPPH (2, 2-Diphenyl-1-picrylhydrazyl) is a stable free radical with red colour (absorbed at 517 nm). If free radicals have been scavenged, a yellow colour is generated by DPPH. This assay uses this character to show herbs free radical scavenging activity. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is widely used in plant biochemistry to evaluate the properties of plant constituents for scavenging free radicals. Butylated Hydroxyl Toluene (BHT) was used as the positive reference standard. It behaves as a synthetic analogue of vitamin E, primarily acting as a terminating agent that suppresses autoxidation [11]; BHT is a known synthetic antioxidant. The DPPH test showed the ability of the test compound to act as a free radical scavenger. This assay is based on the ability of DPPH to decolourize in the presence of an antioxidant. A serial concentration gradient of 0.01- 0.12 mg/ml which is equivalent to 10 µg/ml, 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, 100 µg/ml and 120 µg/ml was prepared from a stock of 1mg/ml. A solution of 0.1 mM DPPH in methanol was prepared. 1.0 ml of the solution was mixed with 3.0 ml of extracts in methanol of the concentration range of the extract. The mixture was vortexed thoroughly and kept in the dark at room temperature for 30 minutes. The

absorbance is read spectrophotometrically at 517 nm. The result of the radical scavenging activity of DPPH was expressed as percentage inhibition and inhibition concentration at 50% (percent) ( $IC_{50}$ ), i.e. the concentration of extract required to stop the radical chain reaction of DPPH by 50% ( $IC_{50}$ ) under the experimental conditions.

DPPH radical scavenging activity was calculated using the following equation:

$$\text{Percentage (\% inhibition)} = [(A_0 - A_1) / (A_0)] \times 100$$

## 2.8 Reducing Power Assay

The antioxidant can donate an electron to free radicals, which leads to the neutralization of the radical. Reducing power was measured by direct electron method, in the reduction of  $Fe^{3+}$  ( $CN^-$ )<sub>6</sub> to  $Fe^{2+}$  ( $CN^-$ )<sub>6</sub> [12]. A 1 ml of concentration gradient of extracts (0.02 - 0.12 mg/ml) in methanol is mixed with 2.5 ml of 0.2 M phosphate buffer pH 6.6, 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes. After that, 2.5 ml of 10% trichloroacetic acid was added to stop the reaction. 2.5 ml of distilled water and 0.1%  $FeCl_3$  were added and the absorbance was measured at 700 nm. Higher absorbance values indicated higher reducing power. Butylated hydroxyl toluene served as positive control.

$$\% \text{ Radical scavenging activity RSA} = [(A_0 - A_1) / (A_0)] \times 100$$

## 2.9 Total Antioxidant Assay (FRAP)

Total antioxidant activity is measured by ferric reducing antioxidant power (FRAP) assay described by Benzie and Strain [13]. It is based on the ability of the sample extracts to reduce Ferric tripyridyltriazine (Fe (III)-TPTZ) complex to Ferrous tripyridyltriazine (Fe (II)-TPTZ) at low pH. At low pH, reduction of ferric tripyridyl triazine (Fe III TPTZ) complex to ferrous form (which has an intense blue colour) can be monitored by measuring the change in absorption at 593 nm. The reaction is nonspecific, in that any half reaction that has lower redox potential, under reaction conditions, than that of ferric ferrous half reaction, will drive the ferrous (Fe III to Fe II) ion formation. The change in absorbance is, therefore, directly related to the combined or "total" reducing the power of the electron donating antioxidants present in the reaction mixture. The FRAP reagent was generated by mixing 25 ml of 300 mM acetate buffer pH 3.6,

2.5 ml of 10 mM 2,4,6- tripyridyl triazine (TPTZ) solution in 40 mM HCl and 2.5 ml of 20 mM Ferric chloride ( $FeCl_3 \cdot 6H_2O$ ) solution. Samples at different concentrations (10, 20, 40, 60, 80 and 100  $\mu\text{g/ml}$ ) were then added to 1.5 ml of freshly prepared FRAP solution and 7.5 ml of distilled water. The absorbance was read at 593 nm. Fresh solutions of  $FeSO_4$  were used as a standard.

$$\% \text{ Radical scavenging activity RSA} = [(A_0 - A_1) / (A_0)] \times 100$$

## 2.10 Hydroxyl Free Radical Scavenging Assay

The hydroxyl free radical scavenging activity was conducted according to the method described by Wenli and Yaping [14]. To 1 ml of the extracts with different concentrations (0.02-0.12 mg/ml) was added 1 ml of 0.75 mM 1,10-phenanthroline, 1.5 ml of 0.75 mM  $FeSO_4$ , 3.8 ml of 0.2 M phosphate buffer pH 7.4, 1 ml of 0.01%  $H_2O_2$  and 2.6 ml of distilled water. The absorbance was read at 536 nm.

$$\% \text{ Radical scavenging activity RSA} = [(A_0 - A_1) / (A_0)] \times 100$$

## 2.11 Abts<sup>+</sup> Radical Scavenging Activity

This assay was conducted with an improved 2,2-azino-bis-(3-ethylbenzothiazoline-6- sulfonic acid) diammonium salt (ABTS) based on the principle of decolourization described by Re and Pellegrini [15]. The ABTS<sup>+</sup> radical was generated by a reaction between ABTS (0.5 mM) and one mM potassium persulfate in 0.1 M phosphate buffer. To 0.5 ml of the extract with a concentration range of 0.04-0.14 mg/ml was added 3 ml of ABTS (2,2'- Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt). Absorbance was read at 734 nm.

$$\% \text{ Radical scavenging activity RSA} = [(A_0 - A_1) / (A_0)] \times 100.$$

## 2.12 Nitric Oxide Radical Scavenging Assay

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide ( $NO^{\cdot}$ ), which interacts with oxygen to produce nitrite ions. Scavengers of  $NO^{\cdot}$  compete with oxygen, leading to reduced production of  $NO^{\cdot}$  and a pink coloured chromophore is formed [16]. A 2 ml of 10 mM Sodium nitroprusside was

added to 0.5 ml of the extract with a concentration range of 0.02-0.10 mg/ml. 0.5 ml of phosphate buffer saline pH 7.4 was added to the reaction mixture. The mixture was incubated at 25°C for 150 minutes. After incubation, 0.5 ml is pipette from the tubes into a new set of tubes. To the new set of tubes, 1 ml of 0.33% Sulphanilic acid was added and allowed to stand for 5 minutes for complete diazotization. After that, 1ml of 0.1% Naphthylethylenediamine dihydrochloride was added and mixed thoroughly and allowed to stand for 30 minutes. Absorbance was read at 540 nm.

$$\% \text{ RSA} = \frac{1 - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

### 3. RESULTS AND DISCUSSION

#### 3.1 Alkaloids

The results of alkaloids detected in aqueous, ethanol and methanol rootbark extracts of *S. glauca* are shown in Table 1. The study showed that aqueous rootbark extract contained more alkaloid content compared to ethanol and methanol rootbark extract. Alkaloids, an amazing group of phytochemical, isolated from medicinal plants, have a varied spectrum of effects such as analgesics to addictive [17]. Alkaloids play a major role as anticancer agents by inhibiting the enzyme topoisomerase which is involved in DNA replication, inducing apoptosis and expression of p53 gene [17]. Alkaloids have potent anticancer activity against various cancers [17], and edible mushrooms have demonstrated anti-hypertensive potentials [18]. The strength of the yellow precipitate observed in the aqueous extracts revealed the strong presence of alkaloids.

#### 3.2 Flavonoids

The results of flavonoids detected in aqueous root extract, methanol and ethanol root extract of *S. glauca* are shown in Table 1. The study revealed that flavonoid was strongest in methanol rootbark extract compared with

aqueous or ethanol rootbark extract. Flavonoid promotes antioxidant activity that helps to maintain normal tissue growth.

#### 3.3 Phenol

Phenol compounds were not detected in the extracts of *S. glauca*.

#### 3.4 Tannin

The study showed that tannin was neither present in the aqueous, ethanol nor methanol rootbark extracts of *S. glauca*.

#### 3.5 2,2-Diphenyl-1-Picrylhydrazyl Scavenging Free Radical

In DPPH radical scavenging activity, the aqueous extract demonstrated maximum scavenging activity of 10.64% inhibition at 60 µg concentration of rootbark extract. The ethanol extract indicated maximum activity of 16.45% inhibition at 40 µg concentration of extract whereas BHT showed the maximum activity of 78.80% at 60 µg concentration. The methanol extract indicated maximum activity of 26.25 % at ten µg concentration of extract. The IC<sub>50</sub> indicates the concentration at which 50% of the test substance is significantly affected. Standard BHT was found to have an IC<sub>50</sub> value of 18 µg. The aqueous, methanol and ethanol extract indicated no IC<sub>50</sub> value. A higher IC<sub>50</sub> value indicates lesser free radical scavenging activity. Higher percentage (%) inhibition value indicates higher potent activity. From the result, it can be observed that aqueous, methanol or ethanol rootbark extracts were unable to scavenge free radicals at 50% inhibitory concentration unlike the results of the study reported by [1] where it was observed that IC<sub>50</sub> scavenging activity for aqueous, methanol and ethanol leaf extracts were 12.45, 9.38, 13.12 µg/ml respectively. However, the present study revealed that the activity of methanol rootbark extracts was significantly (P< 0.05) higher activity than aqueous and ethanol rootbark extracts

**Table 1. Phytochemicals constituents present in extracts of *Simarouba glauca***

Phytochemical	Aqueous extract	Ethanol extract	Methanol extract
Alkaloids	+++	+	++
Flavonoids	-	+	++
Phenols	-	-	-
Tannins	-	-	-

+++ = Strong ++ = Good + = Weak - = Absent

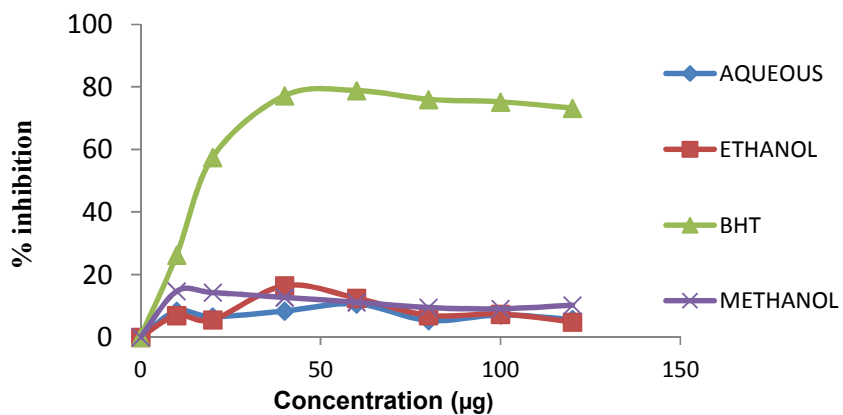


Fig. 1. DPPH radical scavenging activity of *S. glauca* Rootbark extracts and butylated hydroxytoluene (BHT)

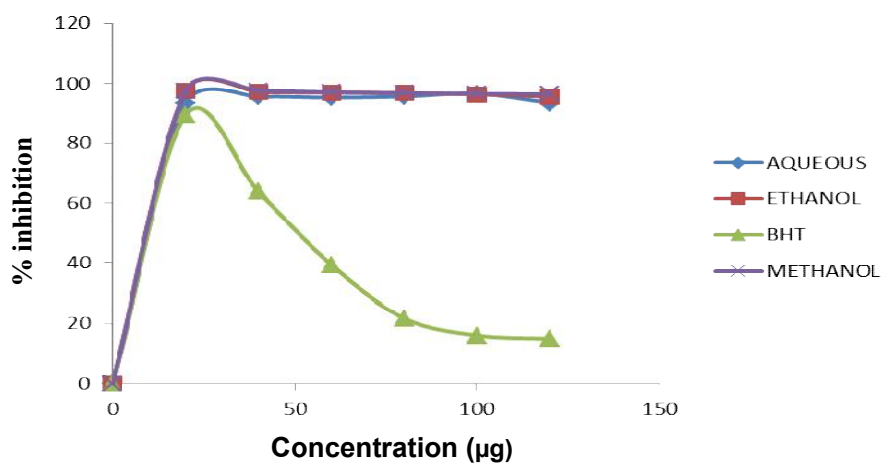


Fig. 2. Reducing power activity of *S. glauca* rootbark extracts and Butylated hydroxytoluene (BHT)

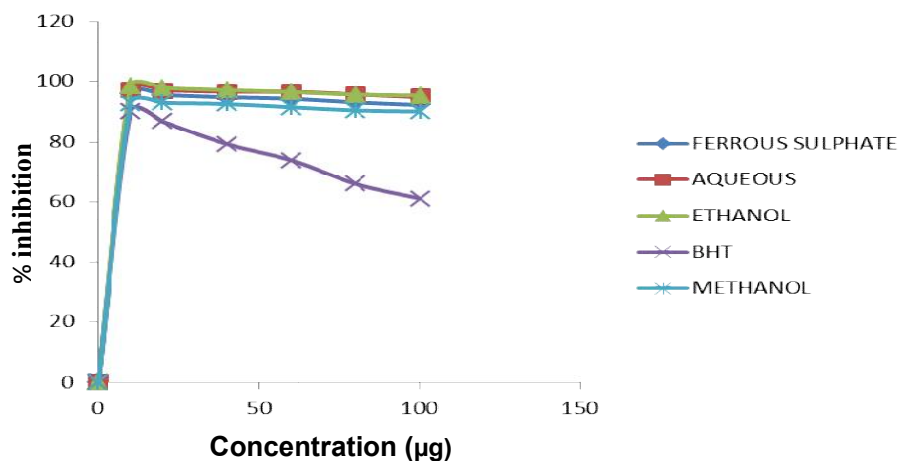


Fig. 3. FRAP of *S. glauca* rootbark extracts and Standard BHT vis-a-vis Fe (II) liberated

### 3.6 Reducing Power Activity

Reducing power activity of aqueous, ethanol or methanol root extracts of *Simarouba glauca* were compared to BHT. It was observed that control BHT had a concentration of 11.0  $\mu\text{g}$  at 50% inhibition. The  $\text{IC}_{50}$  values of aqueous, ethanol and methanol rootbark extracts were observed to be 11.0, 10.0 and 10.5  $\mu\text{g}$  respectively. The aqueous rootbark extract exhibited maximum reducing capacity of 96.58% inhibition at 100  $\mu\text{g}$ . Ethanol rootbark extract exhibited maximum reducing the capacity of 97.53% at 20  $\mu\text{g}$ , while the methanol rootbark extract exhibited maximum reducing the capacity of 97.45% at 40  $\mu\text{g}$ . Whereas, the activity of the ethanol or methanol extracts exhibited a significantly ( $P < 0.05$ ) better and higher reducing activity compared to the aqueous. However, the ethanol extracts demonstrated a significantly ( $P < 0.05$ ) better reducing power activity of 10.0  $\mu\text{g}$  at 50% inhibition concentration.

### 3.7 Total Antioxidant Activity (FRAP) Assay

*S. glauca* rootbark extracts reducing potential was estimated by its ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II) complex. Reducing activity increases as concentration increases. A standard curve of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  (10-100  $\mu\text{M}$ ) was plotted against concentration ( $\mu\text{g}$ ) wherein the amount of Fe (II) liberated by extract's reducing activities including BHT was extrapolated. At 50% inhibition concentration, aqueous rootbark extract liberated 22.84  $\mu\text{M}$   $\text{FeSO}_4$  equivalent/ $\mu\text{g}$  of  $\text{Fe}^{2+}$ , ethanol rootbark liberated 20.87  $\mu\text{M}$   $\text{FeSO}_4$  equivalent/ $\mu\text{g}$  of  $\text{Fe}^{2+}$  while methanol

rootbark extract liberated 58.03  $\mu\text{M}$   $\text{FeSO}_4$  equivalent/ $\mu\text{g}$  of  $\text{Fe}^{2+}$  vis-à-vis 388.23  $\mu\text{M}$   $\text{FeSO}_4$  equivalent/ $\mu\text{g}$  of  $\text{Fe}^{2+}$  liberated by BHT. This indicates that the methanol extract has a greater ability to reduce TPTZ- $\text{Fe}^{3+}$  to TPTZ- $\text{Fe}^{2+}$ . The findings in this report disagrees with the report of [3], who reported that the aqueous extract liberated the highest amount of Fe (II) (67.3101  $\mu\text{M/g}$ ) upon reduction of Fe (III) to Fe (II), followed by the ethanol extract (41.1320  $\mu\text{M}$ ) and lastly, BHT (39.0210  $\mu\text{M}$ ) at 50 percentage inhibition concentration ( $\text{IC}_{50}$ ). The results indicated that methanol extract significantly ( $P < 0.05$ ) reduced  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  when compared with the aqueous or ethanol rootbark extracts. Although, the synthetic antioxidant significantly maintained the lead in ferric reducing potential.

### 3.8 Hydroxyl Free Radical Scavenging Activity

At 20  $\mu\text{g}$ , it was observed that the aqueous extract, methanol extract and standard BHT had a remarkable scavenging activity of 93.83%, 91.63% and 93.49% respectively and are present in Fig. 4 and Table 2. The  $\text{IC}_{50}$  value of ethanol rootbark extract was observed to be 100  $\mu\text{g}$  compared to aqueous or methanol rootbark extracts with  $\text{IC}_{50}$  values of 11 and 11  $\mu\text{g}$  respectively. BHT also recorded 11  $\mu\text{g}$   $\text{IC}_{50}$ . The results indicate that aqueous or methanol rootbark extracts or BHT significantly ( $P < 0.05$ ) demonstrated similar anti-hydroxyl radical activities compared to the ethanol rootbark extract. The outcome of the study contradicts the findings reported by Nishaa et al. [16], although, the plant and plant part utilized in this study differs from that utilized in the study above.

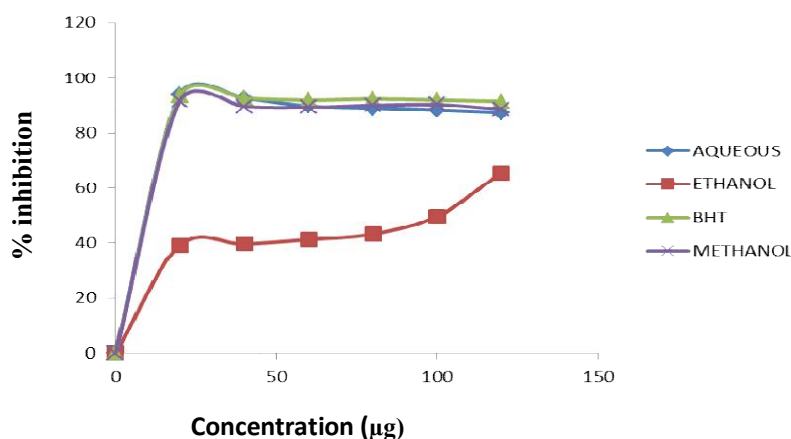


Fig. 4. Hydroxyl free radical scavenging activity of *S. glauca* rootbark extracts and Butylated hydroxytoluene (BHT)

### 3.9 Abts<sup>+</sup> Radical Scavenging Activity

The capacity of aqueous, ethanol or methanol rootbark extracts of *S. glauca* and standard BHT to reduce the ABTS<sup>+</sup> radical which is generated by a reaction between ABTS<sup>+</sup> and potassium persulphate in phosphate buffer are shown in Fig. 5 and Table 2 respectively. The scavenging capacity initially increases and then decreases with increase in concentration. The IC<sub>50</sub> values of aqueous, ethanol and methanol rootbark extract were observed to be 29.0, 25.0 and 34.0 µg respectively, whereas IC<sub>50</sub> value for BHT recorded 21 µg. Ethanol rootbark extract demonstrated a better inhibition effect of 25 µg at IC<sub>50</sub> compared to aqueous and methanol rootbark extracts with 29 and 34 µg at IC<sub>50</sub> respectively which is in line with the results of the study conducted by Osagie-Eweka et al. [3] in which the ethanol extract was observed to have the highest inhibition effect (IC<sub>50</sub> 45.2015 µg/ml) followed by the aqueous extract (52.0721 µg/ml) and standard Trolox being the least with 405.2314 µg/ml. It was observed in the present study that

ethanol rootbark extracts demonstrated a significantly ( $P < 0.05$ ) better ABTS<sup>+</sup> scavenging activity compared to aqueous or methanol. The synthetic standard anti-oxidant, however, demonstrated a far better activity compared to the three extracts understudied in this work

### 3.10 Nitric Oxide Radical Scavenging Activity

The aqueous, ethanol or methanol rootbark extracts and Quercetin being the standard antioxidant demonstrated significant nitric oxide Radical scavenging activity with IC<sub>50</sub> values of 14.0, 14.0, 14.0 and 16.0 µg respectively as shown in Fig. 6 and Table 2. All extracts remarkably displayed equipotent inhibition against nitric oxide radicals and in fact demonstrated a better anti-nitric oxide radical activity compared to Quercetin. The findings in this study are similar to the result of the study conducted by Osagie-Eweka et al. [3] where the IC<sub>50</sub> values determined for quercetin, aqueous extract, and ascorbate are 14.1201 µg/ml,

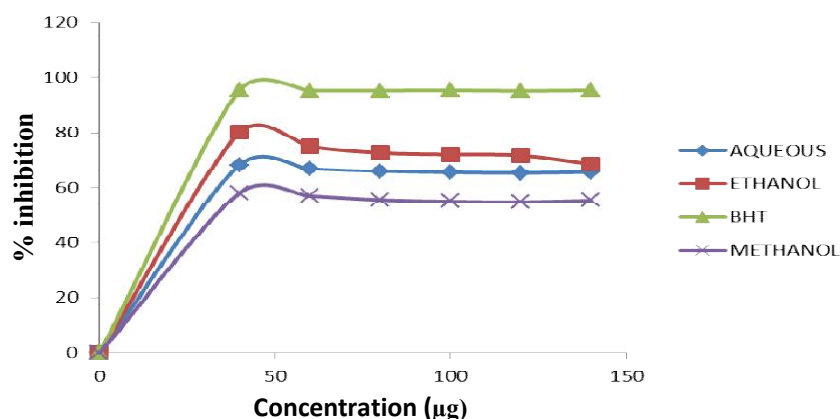


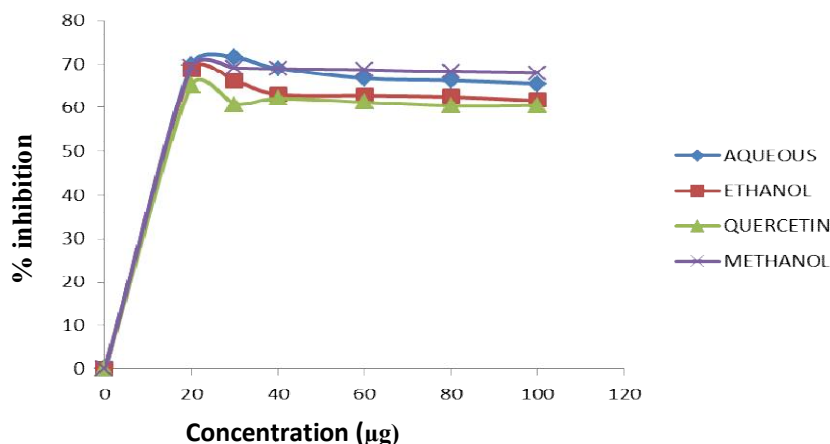
Fig. 5. ABTS<sup>+</sup> radical scavenging activity of *S. glauca* rootbark extracts and Butylated hydroxytoluene (BHT)

Table 2. Percentage (%) inhibition concentration of aqueous, ethanol and methanol rootbark extracts of *Simarouba glauca* against radicals and standard

Antioxidant/radical	Inhibition concentration (IC <sub>50</sub> )				
	Aqueous rootbark extract	Methanol rootbark extract	Ethanol rootbark extract	BHT	Quercetin
DPPH (µg)	NIL	NIL	NIL	17.90	-
Reducing power (µg)	11.00	10.50	10.00	11.00	-
FRAP(µg)	22.84	58.03	20.87	388.23	-
Hydroxyl radical (µg)	11.00	11.00	100.00	11.00	-
ABTS <sup>+</sup> radical (µg)	29.00	34.00	25.00	21.00	-
Nitric oxide (µg)	14.00	14.00	14.00	-	15.90

Results are Mean ± SD





**Fig. 6. Nitric oxide radical scavenging capability of *S. glauca* rootbark extracts and Quercetin**

14.2102 µg/ml and 16.0335 µg/ml respectively with no IC<sub>50</sub> value for ethanol. The Nitric Oxide radical scavenging activity of the aqueous, ethanol or methanol rootbark extracts were significantly not different ( $P > 0.05$ ). The extracts, however, demonstrated a significantly ( $P < 0.05$ ) better Nitric Oxide Scavenging potential compared to the standard (Quercetin).

#### 4. CONCLUSION

The results obtained from the study is evident that *S. glauca* possesses strong antioxidant potency and relevant phytochemicals capable of scavenging free radicals harmful to cells; also maintain the integrity of the cells. Such properties of plants have been implicated in the management and treatment of some diseases and conditions too numerous to mention. Therefore, the findings in this study serve a useful information on further studies of *Simarouba glauca* concerning pharmacological evaluation and potentials in prevention, management and treatment of related conditions and diseases.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

It is not applicable.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

#### REFERENCES

1. Umesh TG. *In-vitro* antioxidant potential, free radical scavenging and cytotoxic activity of *Simarouba glauca* leave. International Journal of Pharmacy and Pharmaceutical Sciences. 2015;7(2):411-416.
2. Moron J, Merrien MA, Polonsky J. Sur la biosynthesis des quassinoides de *Simarouba glauca* (Simarubaceae). Phytochem. 1971;71:10:585.
3. Osagie-Eweka SDE, Noghayin JO, Ekhaguosa DO. Comparative phytochemical analyses and *in-vitro* antioxidant activity of aqueous and ethanol extracts of *Simarouba glauca* (Paradise Tree). European Journal of Medicinal Plants. 2016;13(3):1-11.
4. Revathi D, Rajeswari M. *In-vitro* evaluation of nitric oxide scavenging activity of

- Guettarda speciosa* Linn. International Journal of Science and Research (IJSR). 2013;4:438.
5. Lim YY, Lim TT, Tee JJ. Antioxidant properties of several tropical fruits: A comparative study. Food Chemistry. 2007; 103:1003-1008.
  6. Technical data report for Simarouba (*Simarouba amara*) Sage Press, Inc. 2002;2.
  7. Sani I, Abdulhamid A, Bello F. *Eucalyptus camaldulensis*: Phytochemical composition of ethanolic and aqueous extracts of the leaves, stem-bark, root, fruits and seeds. Journal of Scientific and Innovative Research. 2014;3(5):523-526.
  8. Trease GE, Evans WC. Pharmacognosy. 15<sup>th</sup> Edition London, Saunders Publishers. 2002;42-44.
  9. Sofowora A. Medicinal plants and traditional medicine in Africa. 2<sup>nd</sup> Edition, Spectrum Books Ltd., Ibadan, Nigeria. 1993; 289. [ISBN-13:9782462195]
  10. Santhi K, Sengottuvel R. Qualitative and quantitative phytochemical analysis of *Moringa concanensis* nimmo. International Journal of Current Microbiology and Applied Sciences. 2016;5(1):633-640.
  11. Yen GC, Chen HY. Antioxidant activity of various tea extracts about their antimutagenicity. Journal of Agriculture and Food Chemistry. 1995;43:27-32.
  12. Nataraj L, Perumal S, Sellamuthu M. Antioxidant activity and free radical scavenging capacity of phenolic extracts from *Helicteres isora* L. and *Ceiba pentandra* L. Journal of Food Science and Technology. 2013;50(4):85-88.
  13. Benzie IFF, Strain JJ. The Ferric reducing ability of plasma (FRAP) as a Measure of antioxidant power: The FRAP assay. Analytical Biochemistry. 1996;239:70-76.
  14. Wenli Y, Yaping Z, Bo S. The radical scavenging activities of *Radix puerariae* isoflavonoids: A chemiluminescence study. Food Chemistry. 2004;86:525-529.
  15. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Evans C. Antioxidant activity applying an improved ABTS radical cation decolourization assay. Free Radical Biology and Medicine. 1999;26:1231-1237.
  16. Nishaa S, Vishnupriya M, Sasikumar JM, Hephizibah PC, Gopalakrishnan VK. Antioxidant activity of an ethanolic extract of *Maranta arundinacea* L. tuberous rhizomes. Asian Journal of Pharmaceutical and Clinical Research. 2012;5(4):85-88.
  17. Jeyachandran R, Karthik M. Deepa. Alkaloids as anticancer agents. Annals of Phytomedicine. 2012;1(1):46-53.
  18. Kotchen TA. Historical trends & milestones in hypertension research, a model of the process of translational research Hypertension. 2011;58(4):522-538.

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