



## Phytochemical Screening and In-vitro Antioxidant property of Ethanol Extract of *Stereospermum kunthianum* Root Bark

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### ABSTRACT

In this work, the phytochemical and antioxidant property of ethanol extract of *Stereospermum kunthianum* root bark have been investigated. Free radical scavenging assay DPPH(1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) were employed and the results revealed that the root bark of *Stereospermum kunthianum* has antioxidant property in agreement with its phytochemical constituents suggesting its traditional medicinal usage.

**Keywords:** Antioxidants, Phytochemicals, *Stereospermum kunthianum*

### INTRODUCTION

Medicinal plants are continuously used in the treatment of various illnesses such as asthma, hypertension, typhoid fever, and pile (FAO, 1996). One of such plants is *Stereospermum kunthianum* which belongs to the family *Bignoniaceae*. It is a deciduous tree found in Asia and Sudano-Guinea savanna regions of Africa. The tree grows up to 12 – 15 m high but generally 5-6 m high in Sahel. It is almost leafless in dry season with attractive pink or purplish flowers. The stem bark is grey-black with smooth or flaking patches. The trunk is rarely straight and often has forks and twisted branches (Ken-Fern, 2014).

*Stereospermum kunthianum* is known as pink Jacarnda in English, Sansami among the Hausas and Umanatumba by the Tiv tribe of north central Nigeria (Agishi, 2010). Among the different traditional medicinal uses of the plant is that the decoction of its stem bark is used for bronchitis, pneumonia, cough, rheumatic arthritis and dysentery. More so, the root, leaves and the pods chewed with salt are used to treat cough, ulcer, leprosy, skin eruption and venereal diseases (Gill, 1992).

Scientific evidence have shown that *S. kunthianum* leaves, stem bark as well as root bark have antibacterial, analgesic, antioxidant, anticonvulsant, antiarrhoea and antiplasmodial activities respectively (Ching *et al.*, 2013; Comparoe *et al.*, 2011; Aliyu *et al.*, 2009; Onegi *et al.*, 2002; Ching *et al.*, 2009;). Oloche *et al.*, 2016 reported the presence of coumarin, fatty acid, sterols, flavonoids, tannins, polyphenols, glycosides terpenoids, naphthaquinones and anthraquinones from the parts of *S. kunthianum*.

Generally, living cells generate free radicals and other reactive oxygen species (ROS)

as a results of physiological and biochemical changes. These could lead to many disease conditions like cancer, diabetes and aging or may exacerbate an already existing condition that could lead to death (Harman, 1998). Interestingly, however, it has been found that antioxidant compounds could stabilize or prevent deleterious effects of reactive oxygen species by scavenging and converting them to less harmful compounds. Although synthetic antioxidants could be efficacious in this regard, but there is a concern that they could also cause some undesirable effects. Hence the need to search for natural antioxidants form plants becomes the focus of many workers (Zheng and Wang, 2001; Cai *et al.*, 2003).

Several scientific studies have been reported on the medicinal uses of *S. kunthianum*. We noted that no work has been reported on the antioxidant property of its root bark. Thus, the present study attempted to investigate the phytochemical constituents and antioxidant property of the ethanol extract of *S. kunthianum* root bark.

### MATERIALS AND METHODS

#### General

Centrifuge (Denley B5400, England), Jenway 6310 UV-visible Spectrophotometer were used. Folin-Ciocalteu phenol reagent and Gallic acid were obtained from Fluka, UK. 1,1-diphenyl-2-Picrylhydrazyl radical (DPPH) and Trichloroacetic acid were obtained from Sigma-Aldrich. Anhydrous ferric chloride, potassium ferricyanide, anhydrous sodium carbonate and Ascorbic acid were obtained from BDH Chemical Laboratory, England, UK. All chemical used were of analytical grade.

### Plant Collection

The roots barks of *Stereospermum kunthianum* were collected from wild in Mbarumun-Nanev, Kwande Local Government Area of Benue State, Nigeria. The plant was authenticated by Joseph Waya, Botany Department, Benue State University Makurdi, Nigeria and the specimen's Voucher No: 231 were deposited at the Herbarium unit.

### Preparation and Extraction of Plant Extract

The root barks of *Stereospermum kunthianum* were chopped and air dried under shade for one month. The dry sample was crushed to powder using a mortar and pestle. The powdered sample (600g) was macerated in two liters (2L) of ethanol with occasional shaking for 3 days (72hrs). The mixture was decanted and filtered using Whatmann number one filter paper using vacuum filtration. The filtrate was concentrated to dry mass (for use) by exposure to the atmosphere.

### Phytochemical Analysis

Qualitative and quantitative phytochemical analysis of the root bark extract were done using standard procedures described by Amita and Shalini, (2014) and Chukwuma and Chigozie, (2016) respectively.

### Antioxidant study

Determination of free radical scavenging activity of the crude ethanol stem bark extract was carried out using the DPPH (1,1-diphenyl-2-picrylhydrazyl) quenching assay and ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) cation decolorization assay as described by Aliyu *et al.*, 2010 and Re *et al.*, 1999 respectively.

### DPPH Radical Scavenging Assay

The extract solutions (25 µg, 50 µg, 75 µg, 150 µg and 300 µg) in ethanol (1 ml) were transferred into 1ml of DPPH solution (0.2 mM in ethanol) and allowed to stand at room temperature for 30 min. The absorbance of the solution was measured at 517 nm. The ability of the extract to scavenge the DPPH radical was calculated using the equation 1.

$$\text{DPPH scavenging activity (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100 \quad (1)$$

Where  $A_0$  is the absorbance of the blank (control) and  $A_1$  is the absorbance of the sample. The blank contained ethanol (1 ml) and sample solution (2 ml). Ascorbic acid was used as standard.

### ABTS Radical Scavenging Assay

ABTS cation radical was produced by mixing 14mM ABTS solution (5 ml) and 4.9mM potassium persulphate,  $K_2S_2O_8$ (5 ml), stored in the dark at room temperature for 16 hrs. The plant extract at various concentrations (25µg, 50µg, 75µg, 150µg and 300µg) in 1ml of ABTS solution were homogenized and their absorbance recorded at 734nm. Ethanol blanks were run at each concentration and all measurements were recorded after 7min. Similarly, the reaction mixture of the standard solution was obtained by mixing ABTS solution (950 µl) and ascorbic acid (50 µl). The ABTS scavenging ability was expressed as IC50 µg/ml. The inhibition percentage of ABTS radical was calculated using equation 2.

$$\text{ABTS scavenging activity (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100 \quad (2)$$

Where  $A_0$  is the absorbance of the blank (control) and  $A_1$  is the absorbance of the sample

### Statistical Analysis

All tests were conducted in triplicates and the data were presented as an average ± SD. The results were statistically analyzed using one-way ANOVA. Average values were considered statistically significant when  $P < 0.05$ .

## RESULTS AND DISCUSSIONS

Preliminary phytochemical screening (Table 1) revealed the presence of tannins, flavonoids, steroids, saponins, amino acids and proteins, diterpenes, phenols, alkaloids and carbohydrate glycosides in the ethanol extract of *S. kunthianum* root bark. This result was in conformity with that reported by Oloche *et al.* (2016). The quantity of phenol, flavonoids, alkaloids, tannins and saponins in the sample were as presented in Table 2. The result showed the sample has higher saponin content  $40.00 \pm 6.92\%$  followed by flavonoids ( $27.47 \pm 0.46\%$ ) and the least was tannins ( $0.28 \pm 0.33\%$ ).

**Table 1: Qualitative Phytochemical Screening of Ethanol extract of *S. kunthianum* root bark**

Class of Phytochemicals	Remarks
Alkaloids	+
Carbohydrates glycosides	+
Saponins	+
Phytosterols	+
Phenols	+
Tannins	+
Flavonoids	+
Diterpenes	+
Steroids	+

+ = present

**Table 2: Phytochemical Content of Ethanol extract of *S. kunthianum* root bark**

Class of Phytochemical	Yield
Alkaloids,%	3.73 ± 0.46
Flavonoids, %	27.47 ± 0.46
Saponnins %	40.00 ± 6.92
Phenols, mg/g	5.15 ± 2.10
Tannins, mg/100g	0.28 ± 0.33

Values expressed as mean ± SD (n=3)

Antioxidant properties of the ethanol extract of *S. kunthianum* root bark were indicated in Tables 3. The result showed that the antioxidants activity (%) was concentration dependant, and increased proportionally with increase in concentration of the extract similar to the standard compound, vitamin C. However, the % inhibition of the extract was significantly lower ( $p < 0.05$ ) in both assays at every concentration compared to the standard. Similarly, in both scavenging assays, the IC<sub>50</sub> of the extract was higher than those of the standard compound (vitamin C); that is 83.23 µg/ml as against 32.83µg/ml and 86.33 µg/ml as

against 32.83 µg/ml respectively. This showed that *S. kunthianum* extract has poor scavenging ability for DPPH and ABTS radical compared to ascorbic acid. The antioxidants activity of the extract might be attributed to the alkaloids and high flavonoids and saponnins contents which have been suggested for the antioxidant property in previous studies (Farhet *et al.*, 2013; Zheng and Wang, 2001). Thus the presence of these free radical scavenging molecules from *S. kunthianum* suggested its use in the traditional treatments of various health conditions.

**Table 3: DPPH and ABTS Scavenging Assay of Ethanol extract of *S. kunthianum* root bark**

Concentration µg/ml	DPPH %	Vitamin C %	ABTS %	Vitamin C %
25	2.08±0.01	29.29± 0.01	3.04±0.07	33.16 ± 0.02
50	5.62±0.01	62.43 ± 0.02	9.95±0.04	55.39 ± 0.14
75	8.36±0.00	80.27 ± 0.00	9.14±0.05	80.90 ± 0.0.05
150	13.01±0.01	86.66 ± 0.01	15.69±0.07	88.10 ± 0.05
300	32.15±0.00	92.08 ± 0.00	34.27±0.08	95.44 ± 0.00
	IC <sub>50</sub> =83.23µg/ml	IC <sub>50</sub> =32.83µg/ml	IC <sub>50</sub> =86.33µg/ml	IC <sub>50</sub> =32.83µg/ml

Values expressed as mean ± SD (n=3).

## CONCLUSION

Flavoniods, alkaloids, saponnins and phenols has been established to exhibit antioxidants property (Zheng and Wang, 2001). Consequently, their presence in the ethanol extract of *S. kunthianum* root bark may be responsible for its antioxidants property. Therefore, this indicated that the ethanol extract of *S. kunthianum* root bark could be a source of natural antioxidants.

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