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**Research Article**

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# A survey of the phytochemical and antioxidant potential of the fruit extracts of *Sarcocephalus latifolius* (Smith) bruce (Rubiaceae)

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**ABSTRACT**

*Antioxidants have the ability to protect organisms from damage caused by free radical-induced oxidative stress. A lot of research is being carried out worldwide directed toward finding natural antioxidants of plant origin. The antioxidant activity of the extract of S. latifolius fruit is being reported for the first time along with screening of its photochemical constituents. The antioxidant activity was tested spectrophotometrically, measuring the ability of the plant extract to scavenge a stable DPPH• free radical and reduce singlet oxygen in free hydroxyl radical. Preliminary studies showed the presence of carbohydrates, reducing sugars, flavonoids, steroids, saponins, terpenes and glycosides. The results of the antioxidant testing showed that the crude extracts demostrated potent scavenging ability on hydroxyl radicals generated by $H_2O_2$ and DPPH• free radicals, an indication that the presence of various phyto-compounds contribute to its anti-oxidative activity. Our findings further provide evidence that S. latifolius fruit is a potential source of natural antioxidants, and thus justifies its use in folkloric medicine.*

**Keywords:** *Sarcocephalus latifolius*, DPPH, $H_2O_2$ Scavenging, Phytochemicals.

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## INTRODUCTION

Both artificial and naturally occurring antioxidants have been reported to play major roles in protecting membranes and tissues from free radical and xenobiotic-induced oxidative damage (Burton, 1989; Carini *et al.*, 1990). Most living organisms harbour both enzymatic and non-enzymatic systems that protect them against excessive reactive oxygen species. However, various external factors (smoke, diet, alcohol and some drugs) and aging decrease the efficiency of these protective systems, thereby disrupting the redox equilibrium that is established under healthy conditions. Thus, antioxidants that scavenge reactive oxygen species may be of great value in preventing the onset and propagation of oxidative diseases (Willet, 1994). Recently, more attention has been paid to the role of natural antioxidants, mainly phenolic compounds, which may have higher antioxidant activity than do conventional antioxidants, such as vitamins C, E and $\beta$-carotene (Vinson *et al.*, 1995).

Antioxidant have the ability of protecting organisms from damage caused by free radical-induced oxidative stress [5]. Presently, the probable toxicity of synthetic antioxidants has been identified. It is strongly believed that regular consumption of plant-derived phytochemicals may drift the balance toward an adequate antioxidant status [6]. Thus, in recent years, interest on natural antioxidants, especially of plant origin, has increased manifolds. [7] Sarcocephalus latifolius variously known as "African peach" "Country fig" or "Strawberry tree," belongs to the sub-family cincoinideae of the Rubiaceae. it has an open canopy of flowers with small spherical head like cyme of small whitish flower. The fruit is a syncarp, the individual fruit fused together into a fleshy mass with characteristic pitted surface. The seeds are minute and are embedded in a pinkish flesh with strawberry scent [8]. The fruits are eaten by native of Kinshasha as a remedy for cough [9]. A review of the literature did not throw any light on the antioxidant study of the fruit. The aim of this study was to assess the *in vitro* antioxidant activity of the ethanol and aqueous

extract of *S .latifolius*. For this purpose, the DPPH free radical scavenging activity and hydroxyl inhibitory activity was evaluated. Attempts was made to quantitatively identify important phytochemicals and correlate their activity with the free radical scavenging reactions.

## EXPERIMENTAL SECTION

*Chemicals and Reagents*

2,2-diphenyl-1- picryhydrazyl (DPPH), ascorbic acid, ferric chloride ( $FeCl_3$ ), magnesium ribbon, acetic anhydride and ammonium hydroxide ($NH_4OH$), ethanol, chloroform, methanol, glacial acetic acid, benzene, hydrogen peroxide, potassium di-hydrogen phosphate ($KH_2PO_4$), potassium hydroxide (KOH), sodium hydroxide (NaOH), Fehling's solution, Mayer's reagent, sodium carbonate ($Na_2CO_3$), HCl and $H_2SO_4$ (Concn) were procured from Merck Sigma Aldrich. All chemicals and solvents were of analytical grade.

*Plant Sample and Collection*

*S. latifolius* fruit was collected from Gaya in Hong L.G.A of Adamawa State and identified by a Botanist at the Department of Biological Sciences, University of Maiduguri, Nigeria. A voucher specimen (CHM/LAB/SL544[a]) was kept in the research laboratory, Chemistry Department. Dried grounded fruits of *S .latifolius* (1.5 kg) was extracted in a sohxlet apparatus using 90% ethanol. The extraction was carried out at boiling temperature for six (6) hours. The extract obtained was evaporated under pressure at 50$^o$C to a constant weight. Also, distilled water was used to extract the fruit by refluxing and then filtered to obtain the extract concentrate.. The extracts were stored at 4$^o$C until required.

*Determination of Plant Extract Yield*

The yield of evaporated dried fruit extracts of *S .latifolius* based on dry weight was calculated from the following equation

Yield (g/100g of dry plant material) = (W1 × 100)/ W2

Where, W1 and W2 were the weight of the extract after the solvent evaporation and the weight of the dry plant material respectively.

*Preliminary Phytochemical Screening*

The presence or absence of the phytochemical constituents of the powdered plant material was analysed using the following standard methods

*Carbohydrates*: 200 mg of the powdered fruit sample was boiled in 30 ml DDW and filtered, 1 ml filtrate + 1 ml of Molisch's reagent + 1ml conc. $H_2SO_4$. The presence of carbohydrate is inferred by a reddish ring [10].

*Reducing sugars*: One ml of the above filtrate + 2ml of fehling's solution was boiled for 5mins. A brick red precipitate indicates the presence of reducing sugar [10].

*Tannins*: Two milliliters of the filtrate + 1ml $Fecl_3$. A blue-black or greenish-black precipitate confirms tannins [10].

*Saponins*: Frothing test: 0.5 ml filtrate + 5 ml DDW, shaken for 30 mins, persistent frothing indicated saponins [10].

*Flavonoids*: Shinoda's test; 200 mg plant material was extracted with 5 ml ethanol and filtered; 1 ml filtrate + magnesium + Conc. HCl was added to this. A pink or red color indicates the presence of flavonoids [11].

*Alkaloids*: Plant material (200 mg) was boiled in 20 ml of 1 % $H_2SO_4$ in 50% ethanol and filtered; filtrate + 5 drops conc. $NH_4OH$ + 20 ml chloroform were mixed and the two layers separated. The chloroform layer was extracted with 20 ml $H_2SO_4$. On addition of extract + 5 drops of Mayer's reagent, a creamy/ brownish-red/ orange-red precipitate indicates the presence of alkaloid [11].

*Steroids*: Liebermann-Burchard's; 200 mg of the plant material was extracted in 10ml chloroform and filtered. 2 ml filtrate + 2 ml acetic anhydride + 1 ml conc. $H_2SO_4$ were added to it. A blue-green precipitate showed the presence of steroids [11]

*Glycosides*: Keller-Killiani test; 2 ml filtrate + 1 ml glacial acetic acid + 1 ml $FeCl_3$ + 1 ml conc. $H_2SO_4$. A green-blue color indicates the presence of glycosides [11]

*Hydrogen Peroxide Scavenging*

This activity was determined according to a previously described method [12] with minor changes. An aliquot of $H_2O_2$ (2 mM) and various concentrations (100-1000 µg/ml) of samples were mixed (1:0.6 v/v) and incubated for 10 min at room temperature. After incubation, the absorbance of hydrogen peroxide at 230 nm was determined against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for background subtraction. The percentage scavenging activity of hydrogen peroxide by the *S .latifolius* extracts was calculated as follows: % scavenging activity [$H_2O_2$] = [Abs (control) - Abs (standard) / Abs (control)] X 100 where Abs (control): absorbance of $H_2O_2$ (2mM) as control; Abs (standard): absorbance of the extract/standard

*DPPH Radical Scavenging Activity*

The free radical scavenging capacity of the extracts was determined using DPPH [13]. The DPPH solution (0.3mM) was prepared in 95% methanol. The sample extracts of the *S. latifolius* was mixed with 95% methanol to prepare the stock solution (1 mg/ml). Freshly prepared DPPH solution was taken in test tubes and extracts were added followed by serial dilutions (100-1000 µg) to every test tube such that the final volume was 2 ml, and discoloration was measured at 517 nm after incubation for 30 min in the dark (SP001 Matemeter ,UV spectrophotometer, Thermo Electron Corporation, England, UK). Measurements were performed at least in triplicate. Ascorbic acid was used as a reference standard and dissolved in DDW to make the stock solution with the same concentration (1 mg/ml). The control sample which contained the same volume without any extract and 1ml of 95% methanol was used as the blank. Percent scavenging of the DPPH free radical was measured using the following equation.

DPPH Scavenging Effect (%) = $(Ao - A_1) / Ao \times 100$;

where, Ao was the absorbance in the presence of the crude samples (ethanol and aqueous fruit extract of *S .latifolius*).the actual decrease in absorption induced by the test compounds was compared with the positive controls.

*Statistical Analysis*

Results are expressed as mean ± S.E.M of triplets. The groups were compared by one way anova using Graph Pad Prism, Version 4.0 (Graph Pad, San Diego, CA, USA). P values < 0.05 was considered significant.

## RESULTS AND DISCUSSION

Yield: The extracts yield was 23.3% (ethanol) and 2.02% (aqueous). Preliminary phytochemical screening of the extracts revealed the presence of various bioactive components of which flavonoids, terpenoids and steroids prominent, the results of the phytochemical test are shown on Table 1.

**Table I. Phytochemical Screening of the fruit extracts (ethanol and aqueous) of *S.latifolius***

| S/No | Phytochemicals | Test | Result | |
|---|---|---|---|---|
| | | | Ethanol | Aqueous |
| 1 | Carbohydrate | Molisch's | + | + |
| | | Fehling's | + | + |
| 2 | Flavonoids | Shinoda's | + | - |
| | | Lead acetate | + | + |
| 3 | Steroidal nucleus | Salkowski | + | + |
| 4 | Terpenoid | L. Burchard | + | + |
| 5 | Saponins | Frothing | + | + |
| 6 | Tannins | Ferric chloride | - | - |

*Positive (+ve) = Present Negative (-ve) = Absent*

In this present study, the antioxidant activity of the ethanol and aqueous extracts of the *S. latifolius* fruit was investigated using the DPPH scavenging assay, which determines the reducing power of the extract compared with the reference standard antioxidant ascorbic acid. The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron that is responsible for the absorbance at 540 nm and also for the visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance [13]. Comparison of the antioxidant activity of the extracts and ascorbic acid is shown in [Figure 1]. The ethanol and aqueous extracts of *S. latifolius* exhibited a significant dose-dependent inhibition of DPPH activity. However, the aqueous extract seem to have shown better DPPH scavenging activity across the different concentrations except at the final dose where both extracts showed comparably significant inhibition when compared to the standard (ascorbic acid).

**Figure1.**

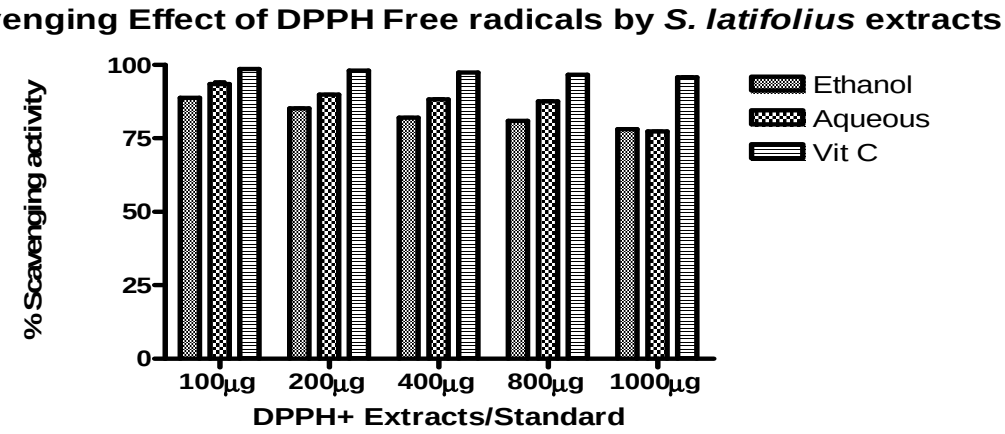
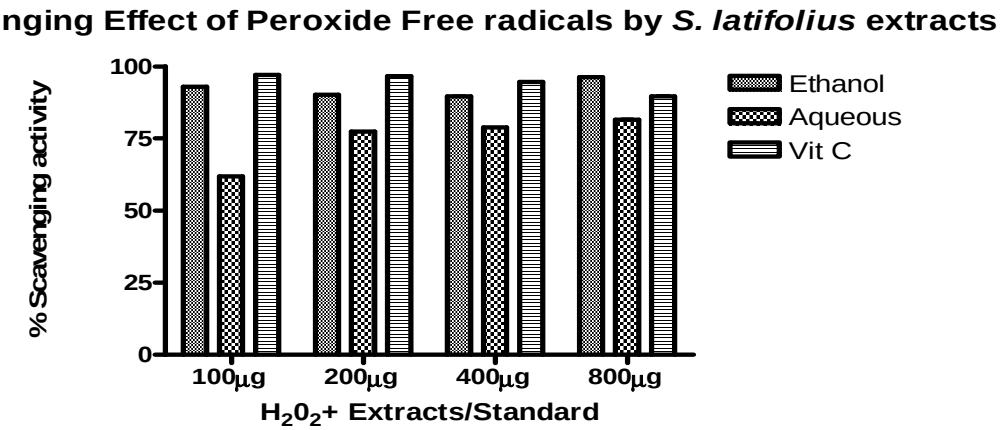



Figure 2 showed that the ethanol extract was more active than the aqueous extract in scavenging the peroxide ($H_2O_2$) radicals when compared with standard ascorbic acid. This was significant across the various concentrations.

**Figure 2.**




During oxidative stress and exposure to radiation, excessive free radicals are produced that are known to cause damage to biomolecules [6]. Although the specific roles of phytochemicals were not investigated in this study, it has been reported that most active ingredients in plants and vegetables are frequently saponins, tannins, alkaloids, flavonoids and phenols and these may be responsible for many of the pharmacological actions of such plants [14]. Specifically, Phenolic compounds have been reported to serve as antioxidants, and exhibit a wide range spectrum of medicinal properties such as anti-cancer, ant-iinflammatory and anti-diabetes [15]. In the dose-response experiment, it could be observed that total inhibition was not achieved. The maximum inhibition was in the range of 75-95% in the presence of the 100 mg/ml extract. With the addition of a larger amount of extract to the DPPH assay mixture, the degree of inhibition decreased, indicating a pro-oxidant effect. The explanation may be that better scavenging effect could be obtained at lower concentration of the extracts. The results showed that the ethanol extract had better scavenging effects on scavenging superoxide anions ($OH^-$), than the aqueous extract when compared to the standard antioxidant (ascorbic acid). The anti-oxidative effect could be ascribed to the phenolic components, such as phenolic acids, which are associated with most plant material [16]. The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging specie in free radical pathology, capable of damaging almost every molecule found in living cells [17]. This radical has the capacity to join nucleotides in DNA and cause strand breakage which contributes to carcinogenesis, mutagenesis and cytotoxicity. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides [18] In this respect, polyphenolic compounds like flavonoids and phenolic acids commonly found in plants have been reported to have multiple biological effects, including an antioxidant activity [19]. The presence of reductones is thought to be associated with the reducing properties, which in turn have been shown to exert an antioxidant action by donating

a hydrogen atom that breaks the free radical chain [20]. Reductones are also reported to react with certain precursors for peroxide thus preventing peroxide formation [21].

## CONCLUSION

The *S .latifolius* fruit extracts have shown a strong antioxidant activity by inhibiting DPPH and Hydrogen peroxide free radicals when compared with the standard L-ascorbic acid. In addition, it was found to contain noticeable amount of flavonoids, which plays a major role in controlling oxidation. The results of this study showed that *S .latifolius* could be used as an easily accessible source of natural antioxidant. However, the phyto-constituents responsible for the antioxidant activity of *S. latifolius* are not much clear. Therefore, a further study is needed to determine the mechanism behind the antioxidant study of the fruit of *Sarcocephalus latifolius.*

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