

High Performance Liquid Chromatography (HPLC) Fingerprinting, Mineral Composition and *In Vitro* Antioxidant Activity of Methanol Leaf Extract of *Synsepalum dulcificum* (Sapotaceae)

Tajudeen Olabisi Obafemi^{1,2*}, Afolabi Clement Akinmoladun¹, Mary Tolulope Olaleye¹, Amos Onasanya², Kayode Charles Komolafe³, John Adeolu Falode³, Aline Augusti Boligon⁴, Margareth Linde Athayde⁴

¹Phytomedicine, Drug Metabolism and Toxicology Unit, Department of Biochemistry, The Federal University of Technology, Akure, Nigeria.

²Department of Chemical Sciences, Afe Babalola University, Ado – Ekiti, Nigeria.

³Department of Biochemistry, Federal University Oye-Ekiti, Nigeria.

⁴Phytochemical Research Laboratory, Department of Industrial Pharmacy, Federal University of Santa Maria, Santa Maria, Brazil.

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ABSTRACT

This study was carried out to identify the phytochemicals and *in vitro* antioxidant activity of methanol leaf extract of *Synsepalum dulcificum* (MSD). Standard protocols were used to evaluate the total phenols, total flavonoids and total antioxidants content of the extract. Nitric oxide (NO), hydroxyl radical (OH), ABTS⁺ and 2,2-diphenyl -1-picrylhydrazyl (DPPH) radicals scavenging activity, inhibition of lipid peroxidation and the ability of MSD to chelate ferrous ion as well its reductive potential were also evaluated. High Performance Liquid Chromatography (HPLC) was used to confirm the presence of polyphenols and carotenoids. Results showed the presence of flavonoids, saponins, terpenoids and cardiac glycosides in the extract. Among others, appreciable levels of potassium, calcium, sodium and magnesium were detected in the extract. The IC₅₀ of the extract for DPPH, NO, OH, and ABTS⁺ radicals scavenging assays were 139.45 µg/ml, 119.17 µg/ml, 147.65 µg/ml, and 135.83 µg/ml respectively. It could be inferred that MSD showed appreciable *in vitro* antioxidant activity and could be useful in preventing and ameliorating diseases in which free radicals are implicated.

INTRODUCTION

In recent times medicinal plants have occupied a unique position among the treatment modalities of a wide variety of diseases. This might not be unconnected with the presence of biologically active molecules in such plants especially molecules that have been proven to have therapeutic potentials in several

experimental disease models. Such molecules include flavonoids, tannins, steroids, terpenoids, saponins etc. These molecules have been shown to be responsible for the medicinal value of the plants in which they are found, including their antioxidant activity (Yildirim *et al.*, 2001).

The antioxidant activity of a compound has been attributed to various mechanisms viz: prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging ability (Saumya and Mahaboob, 2011).

* Corresponding Author

Obafemi T. Olabisi, Afe Babalola University PMB 5454, Ado – Ekiti, Nigeria . Phone: +234 8038059340; Email: oobafemi @ abuad.edu.ng

Moreover, The World Health Organization (WHO) stated that more than 80% of the population in developing countries use herbal and other traditional medicines to treat their common ailments (WHO, 1998). Free radicals are highly reactive chemical species regularly produced in the human system by normal biological reactions and also by various exogenous factors. Some of the radicals include superoxide O_2^- , nitric oxide (NO), peroxy (ROO), and hydroxyl (OH^\cdot) (Maritim *et al.*, 2003). These ROS play an important role in degenerative or pathological processes, such as aging, cancers, coronary heart diseases, Alzheimer's disease, neurodegenerative disorders, atherosclerosis, cataracts and inflammations (Huang *et al.*, 2005). Living organisms have endogenous antioxidant defense systems that protect against oxidative damage by removal or repair of damaged molecules (Sun *et al.*, 1998). The term 'antioxidant' refers to the activity of numerous vitamins, minerals and phytochemicals which provide protection against the damage caused by ROS (Khilfi *et al.*, 2006). Antioxidants interfere with the oxidative processes by scavenging free radicals, chelating free catalytic metals and by acting as electron donor (Gulcin *et al.*, 2005). More often than not, the endogenous antioxidant mechanisms may be overwhelmed in several diseased conditions thereby necessitating the supplementation of antioxidant levels through intake of dietary and plant derived antioxidants (Terao *et al.*, 1994). Plant-based antioxidants are now preferred to the synthetic ones because of safety concerns (Grice, 1986; Wichi, 1988). These factors have inspired the widespread screening of plants for possible medicinal and antioxidant properties, the isolation and characterization of diverse phytochemicals and the development and utilization of antioxidants of natural origin (Jayaprakasha *et al.*, 2001; Gulcin *et al.*, 2002). It is believed that the phytochemical composition and the *in-vitro* antioxidant activity of a plant might give a clue to its therapeutic potential.



Fig. 1: *Synsepalum dulcificum* leaves (George and Diejun, 2011).

The miracle fruit shrub, *Synsepalum dulcificum* Daniell (Sapotaceae) is indigenous to tropical West Africa. The fruit berries of the plant have unusual taste modifying properties of allowing sour substances to taste amazingly sweet after the inside

of the mouth has been thoroughly exposed to the fruit's mucilaginous pulp (Brouwer *et al.*, 1968). Miracle fruit has been studied to improve insulin resistance induced by fructose-rich chow in rats (Chen *et al.*, 2006). Researchers also reported that the skin contributed 43.96% of free phenolic compounds with 15.91% of freeze-dried solids due to its high phenolic content. They also observed that the free flavonoid content in the skin was tremendously higher than that in the seed and pulp and that the skin contributed about 52% of total flavonoid and 15.91% of dried solids (George and Diejun, 2011). However to the best of our knowledge the antioxidant activity of the methanol extract of *Synsepalum dulcificum* leaves has not been evaluated, hence this study.

MATERIALS AND METHODS

Chemicals

Acetonitrile, formic acid, gallic acid, chlorogenic acid, ellagic acid and caffeic acid were purchased from Merck (Darmstadt, Germany). Quercetin, quercitrin, isoquercitrin, rutin, catechin, epicatechin and kaempferol, DPPH (2,2-diphenyl-1-picrylhydrazyl) radical, gallic acid, ascorbic acid, quercetin and Folin-Ciocalteu reagent were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

Plant material and extraction

S. dulcificum leaves were collected from a farmland in Iseyin, Oyo-state in South-Western Nigeria. The leaves were identified and a voucher number- UIH-22457 was obtained for the leaf at Botany Department University of Ibadan, Nigeria. The leaves were air-dried for three weeks and pulverized. 700g of the pulverized sample was extracted in 80% methanol by maceration for 72 hours. The methanol extract was concentrated in a rotary evaporator and lyophilized. A total yield of the extract (98.7g, 14.1%) was obtained and preserved for further use.

Quantification of compounds by HPLC-DAD

Reverse phase chromatographic analyses were carried out under gradient conditions using C_{18} column (4.6 mm x 250 mm) packed with 5 μ m diameter particles. The mobile phase was water containing 1% formic acid (A) and acetonitrile (B), and the composition gradient was: 13% of B until 10 min and changed to obtain 20%, 30%, 50%, 60%, 70%, 20% and 10% B at 20, 30, 40, 50, 60, 70 and 80 min, respectively (Boligon *et al.*, 2012) with slight modifications. *Synsepalum dulcificum* (leaves) methanolic extract was analyzed dissolved in ethanol at a concentration of 20 mg/mL. The presence of eleven antioxidants compounds was investigated, namely, gallic acid, chlorogenic acid, caffeic acid, ellagic acid, catechin, epicatechin, quercetin, quercitrin, isoquercitrin, rutin and kaempferol. Identification of these compounds was performed by comparing their retention time and UV absorption spectrum with those of the commercial standards. The flow rate was 0.7 ml/min, injection volume 50 μ l and the

wavelength were 254 nm for gallic acid, 280 nm for catechin and epicatechin, 327 nm for caffeic, ellagic and chlorogenic acids, and 366 nm for quercetin, isoquercitrin, quercitrin, rutin and kaempferol. All the samples and mobile phase were filtered through 0.45 µm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use. Stock solutions of standards references were prepared in the HPLC mobile phase at a concentration range of 0.030 – 0.250 mg/ml for kaempferol, quercetin, isoquercitrin, quercitrin, catechin, epicatechin and rutin; and 0.030 – 0.250 mg/ml for gallic, caffeic, ellagic and chlorogenic acids. The chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200 to 400 nm). All chromatography operations were carried out at ambient temperature and in triplicate. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the responses and the slope using three independent analytical curves. LOD and LOQ were calculated as 3.3 and 10 σ/S, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve (Boligon *et al.*, 2013).

DPPH (1, 1, diphenyl 2-picryl hydrazyl) assay

The antioxidant activity by DPPH assay was assessed according to Molyneux method (Molyneux, 2004). To 1 ml of various concentration of the extract 1 ml of DPPH 0.1mM was added in a test tube. Tannic acid was used as the standard for comparison. After incubation for 30 mins in dark at room temperature, absorbance was recorded at 517 nm. The percent DPPH radical scavenging was calculated with the equation:

$$\% \text{ DPPH radical scavenging} = [(\text{Absorbance control} - \text{Absorbance sample}) / \text{Absorbance control}] \times 100.$$

ABTS radical scavenging activity

A TEAC assay was conducted based on the method of Ramos *et al.*, (1999). The ABTS aqueous solution (7 mM) was oxidized with potassium peroxodisulfate (2.45 mM) for 16 hours in the dark at room temperature. The ABTS^{•+} solution was diluted with 95% ethanol to an absorbance of 0.75 ± 0.05 at 734 nm. An aliquot (20 µL) of each sample (125 µg/mL) was mixed with 180 µL ABTS^{•+} solution and the absorbance was read at 734 nm after 1 min. Trolox was used as a reference standard. The percent ABTS^{•+} radical scavenging was calculated with the equation:

$$\% \text{ ABTS}^{\bullet+} \text{ radical scavenging} = [(\text{Absorbance control} - \text{Absorbance sample}) / \text{Absorbance control}] \times 100.$$

Nitric oxide scavenging activity

The nitric oxide scavenging activity was evaluated according to the modified method of Sreejayan and Rao (1997). Various concentrations of extract was prepared. Sodium nitroprusside (2.5 mL, 10 mM) in phosphate buffered saline (PBS) was added to 0.5 mL different concentrations of extracts. The reaction mixture was incubated at 25°C for 150 min. After incubation, 0.5 mL aliquot was removed and 0.5 mL of Griess reagent: (1% (w/v) sulfanilamide, 2% (v/v) H₃PO₄ and 0.1% (w/v)

naphthylethylene diamine hydrochloride) was added. The absorbance was measured at 546 nm. Ascorbic acid was used as reference standard and was treated the same way as that of fractions. Sodium nitroprusside in PBS (2 mL) was used as control. The nitric oxide radicals scavenging activity of the extracts and ascorbic acid was calculated according to the following equation:

$$\text{Percentage of inhibition} = [(A_0 - A_1) / A_0] \times 100, \text{ where } A_0 \text{ is the absorbance of sodium nitroprusside in PBS and } A_1 \text{ is the absorbance in the presence of the fractions and ascorbic acid.}$$

Iron chelating ability assay

The in vitro Fe²⁺ chelating ability of plant extract was assayed according to the method of Minnoti and Aust, 1987 with slight modification (Minnoti and Aust, 1987). Briefly, 900 µl of aqueous FeSO₄ (500 µM) and 150µl of extract were incubated for 5min at room temperature, 78 µl of 1,10- phenanthroline (0.25 % ,w/v , aqueous) was added. The absorbance of the orange colour solution was read at 510nm with a spectrophotometer. The principle of the assay is based on disruption of o-phenanthroline - Fe²⁺ complex in the presence of chelating agent. The in vitro of Fe²⁺ chelating ability of the sample was calculated by using the following formula:

$$\text{Chelating ability} (\%) = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100. \text{ Where;}$$

A_{control} = The absorbance of the control (reaction mixture in the absence of sample)(FeSO₄ alone).

A_{sample} = The absorbance of the reaction mixture (sample, FeSO₄ and 1,10-phenanthroline)

Reductive potential

Reducing power of the extracts were measured by the direct reduction of Fe³⁺(CN)⁻₆ to Fe²⁺(CN)⁻₆ and was determined by absorbance measurement of the formation of the Perl's Prussian Blue complex following the addition of excess Fe³⁺ (Oyaizu, 1986), as described previously (Gulcin 2006b). Different concentrations of extracts in 0.5mL of distilled water were mixed with 1.25 mL of 0.2 M, pH 6.6 sodium phosphate buffer and 1.25 mL of potassium ferricyanide [K₃Fe(CN)₆] (1%) the mixture was incubated at 50 °C for 20 min. After 20 min incubation, the reaction mixture was acidified with 1.25 mL of trichloroacetic acid (10%). Finally, 0.5 mL of FeCl₃ (0.1%) was added to this solution and the absorbance was measured at 700 nm in a spectrophotometer. Increased absorbance of the reaction mixture indicates greater reduction capability (Gulcin, 2006a).

Hydroxyl radical scavenging activity

The hydroxyl radical (OH) scavenging activity was measured by the method of Jin *et al.* (1996). The hydroxyl radical was generated in a mixture of 1.0 mL of 0.75 mM 1,10-phenanthroline, 2.0 mL of 0.2 M sodium phosphate buffer (pH 7.4), 1.0 mL of 0.75 mM FeSO₄ and 1.0 mL of H₂O₂ (0.01%, v/v). After addition of 1.0 mL sample solution, the mixture was incubated at 37°C for 30 min. Then, the absorbance of the mixture at 536 nm was measured. Deionized water and VC were used as

the blank and positive control respectively. The scavenging activity on $\cdot\text{OH}$ was calculated by the following equation:

$$\text{Scavenging activity (\%)} = \frac{(\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}})}{(\text{Abs}_0 - \text{Abs}_{\text{blank}})} * 100$$

where Abs_0 is the absorbance of the deionized water instead of H_2O_2 and sample in the assay system.

Inhibition of lipid peroxidation assay

In vitro inhibition of lipid peroxidation was estimated according to the method of Ruberto and Baratta (2000). In this assay, egg yolk homogenate served as lipid rich medium, and FeSO_4 acts as initiator of lipid peroxidation. Briefly, 50 μl of plant extract was mixed with 0.25 ml 10% egg yolk. This was followed by the addition of 10 μl FeSO_4 (0.07M, aqueous). The mixture was incubated at room temperature for 30 min. This was followed by the addition of 0.75 ml of glacial acetic acid (5%, v/v aqueous) and 0.75 ml of thiobarbituric acid 0.8% in Sodium dodecylsulphate (SDS) 1.1%. The mixture was incubated in a boiling water bath (90°C) for 60 min, cooled and centrifuged at 3000 rpm. One milliliter (1 ml) of the pink colour supernatant was read at 532 nm on a spectrophotometer.

In vitro inhibition of lipid peroxidation = $[(\text{A}_{\text{control}} - \text{A}_{\text{sample}}) / \text{A}_{\text{control}}] \times 100$

Determination of total phenolic content

The total phenolic content of the extract was determined according to the method described elsewhere (Hung *et al.*, 2001). In this assay, the phenolic group present in plant extract interacts with Folin – Ciocalteu in alkaline medium using Na_2CO_3 solution giving a blue colour, which has maximum absorption at 685 nm and correlates with total phenolic content. 0.1 ml of aqueous extract of the plant of different concentrations (2 – 10%) was rapidly mixed with 0.1 ml of Folin Ciocalteu reagent, followed by the addition of 0.3 ml sodium carbonate (15%, w/v) solution. The mixture was incubated in the dark for 30 mins. The absorbance of the blue colour was read at 760 nm after 30 mins on a spectrophotometer. The total phenolic content was extrapolated from a standard curve using tannic acid (graded doses, 50 – 250 $\mu\text{g}/\text{ml}$) as a standard phenol.

Determination of total flavonoid

Total flavonoid of the leaf extract was determined using the method described by Kumaran and Karunakaran, (2007) with slight modification. Briefly, 0.5 ml of extract solution (1 mg/ml) and standard (quercetin) at different concentrations were taken in test tubes. 3.0 ml of methanol followed by 0.1 ml of 10% aluminum chloride solution was added into the test tubes. Two hundred ml of 1M potassium acetate solution was added to the mixtures in the test tubes. Furthermore, each reaction test tube was then immediately diluted with 2.8 ml of distilled water and mixed to incubate for 30 min at room temperature to complete reaction. The absorbance of pink colored solution was noted at 415 nm using a spectrophotometer against blank methanol. TFC of the extract was expressed as quercetin equivalents (QE) after

calculation using the following equation: $C = (cV)/m$, where, C = total flavonoid contents, mg/g plant extract in QE, c = concentration of quercetin obtained from calibration curve (mg/ml), V = the volume of the sample solution (ml), m = weight of the sample (g). All tests were conducted in triplicate.

Determination of mineral composition

Copper, zinc, magnesium, lead, manganese, calcium and iron content of MSD were analyzed using atomic absorption spectrophotometer (Buck 210 VGD), while sodium and potassium content were analyzed using flame photometer (Gulfex medical and scientific, England).

Statistical analysis

All statistical analyses were performed using Microsoft excel package 2013 version. Results are expressed as mean \pm standard deviation. Independent sample t-test was used for data analysis. Linear regression analysis was used to calculate the IC_{50} values.

DISCUSSION

Before the advent of modern medicine herbs and herbal products were the mainstay of treatments around the world. The phytochemicals present in commonly consumed plant foods are normally non-toxic and have the potential for preventing chronic diseases. The plant extracts encompass high concentration of flavonoids and phenolic compounds. As potent antioxidants, flavonoids are especially important for protection against human diseases. The multiple properties of these phytochemicals have made them more attractive, as they can modulate various aspects of diseases like lipid peroxidation involved in atherogenesis, thrombosis, carcinogenesis, hepatotoxicity and a variety of disease conditions (Tiwari, 2001). Table 1 shows the phytochemical composition of the methanol extract of *S. dulcificum* leaves. Flavonoids, saponins, terpenoids and cardiac glycosides were present in the extract. Our result as presented in table 2 also showed that the total phenols and total flavonoids were estimated to be 58.67 mg/g TAE and 13.01 mg/g QE respectively.

Table 1: Phytochemical screening of methanol leaf extract of *Synsepalum dulcificum*.

Phytochemical	Result
Saponins	+ve
Tannins	-ve
Flavonoids	+ve
Alkaloids	-ve
Terpenoids	+ve
Anthraquinones	-ve
Cardiac glycosides	+ve
Steroids	-ve

Table 2: Total phenol and total flavonoids content of methanol leaf extract of *Synsepalum dulcificum*.

Total phenol	58.67 mg/g TAE
Total flavonoids	13.01 mg/g QE

The evaluation of the antioxidant activity of medicinal plants has become a key component of the processes employed in unraveling the therapeutic potentials of such plants. Researchers opined that no single assay accurately reflects the mechanism of action of all radical sources or all antioxidants in a complex system (Prior *et al.*, 2005) and that two or more methods should be employed in order to evaluate the total antioxidant activity (Ilhami *et al.*, 2005) due to various oxidative processes.

DPPH radical scavenging activity is usually used to estimate the *in-vitro* antioxidant activity of natural compounds or plant extracts (Philips *et al.*, 2010). As shown in figure 3 The DPPH scavenging activity of methanolic extract of *Synsepalum dulcificum* leaves compared favorably with tannic acid at all the concentrations tested. At the highest concentration- 400µg/ml the extract showed 91.84% inhibition while the standard antioxidant showed 93.53% inhibition of DPPH radical. The result also showed that the extract exhibited a dose-dependent inhibition of DPPH radical.

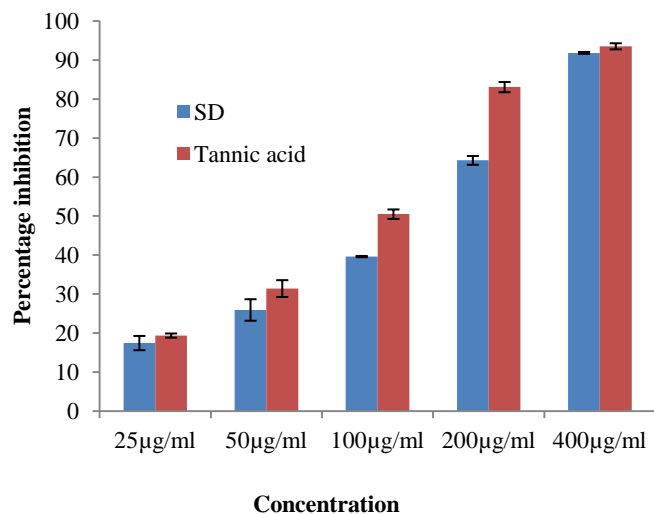


Fig. 3: Percentage DPPH radical inhibition of methanol leaf extract of SD.

ABTS⁺⁺ is a blue chromophore produced by the reaction between ABTS and potassium persulfate and in the presence of the plant extract or trolox. Preformed cation radical gets reduced and the remaining radical cation concentration after reaction with antioxidant compound was then quantified (Johnston, 2006). In the present study as presented in figure 4, methanol extract of SD leaves showed a remarkable ABTS⁺⁺ scavenging activity when compared with trolox. At 200 µg/ml and 400 µg/ml the extract showed 85.29% and 87.99% scavenging activity respectively against the ABTS radical while trolox showed 70.57% and 83.18% scavenging activity respectively at the same doses.

Nitric oxide is a free radical produced in mammalian cells, involved in the regulation of various physiological process including neurotransmission, vascular homeostasis, antimicrobial and antitumor activities. However, excess production of NO is associated with several diseases. It would be interesting to develop potent and selective inhibitors of NO for potential therapeutic use

(Hepsibha, 2010). Figure 5 shows that SD showed a dose-dependent increase in nitric oxide scavenging activity. At 400µg/ml which was the highest concentration tested the extract showed 69.42% scavenging activity while ascorbic acid which was used as standard showed 88.27% nitric oxide scavenging activity.

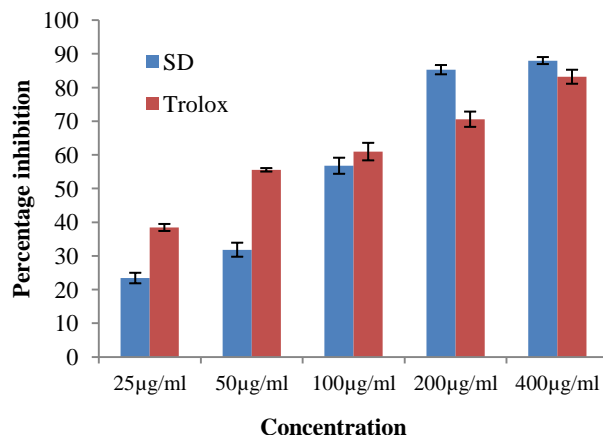


Fig. 4: Percentage ABTS radical inhibition of methanol leaf extract of SD.

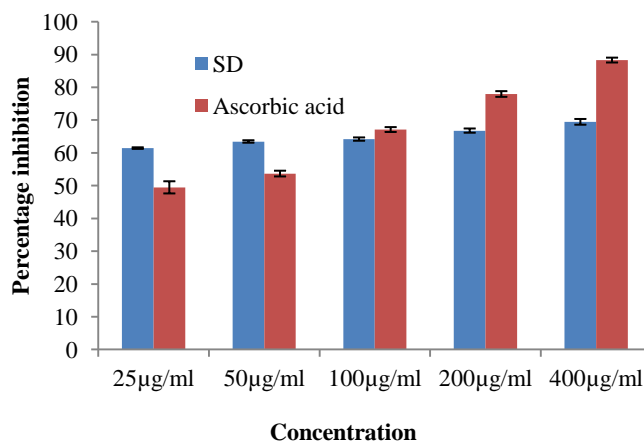


Fig. 5: Percentage nitric oxide scavenging activity of methanol leaf extract of SD.

Chelating agents form σ -bonds with a metals and are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion (Kumaran and Karunakaran, 2006). Figure 6 shows the percentage iron-chelating activity of our extract. The result shows that though the EDTA which was used as standard showed a better iron-chelating ability than the extract at all the doses tested, the extract still showed appreciable activity in this regard. At 400 µg/ml the extract and standard showed 48.86% and 60.52% iron-chelating activity respectively.

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Most antioxidants and pharmacologically therapeutic agents used in the treatment of oxidative stress related diseases have been shown to

have strong reducing power (Amin and Razieh, 2007). The result in figure 7 shows that the methanol extract of *Synsepalum dulcificum* leaves showed an appreciable reducing power when compared to quercetin which was used as the standard antioxidant. At 400 μ g/ml the extract and standard showed absorbance values of 1.21 and 1.80 respectively.

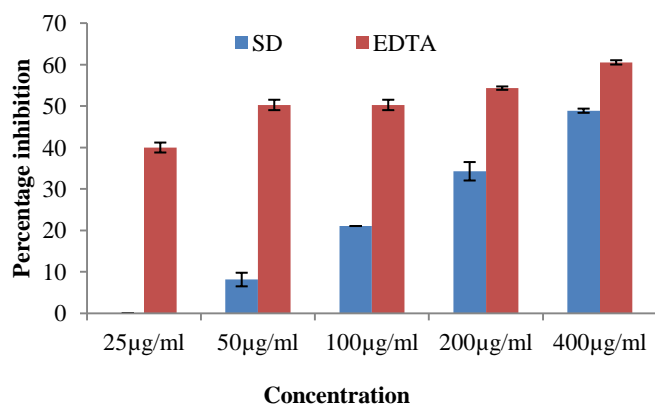


Fig. 6: Percentage iron chelating activity of methanol leaf extract of SD.

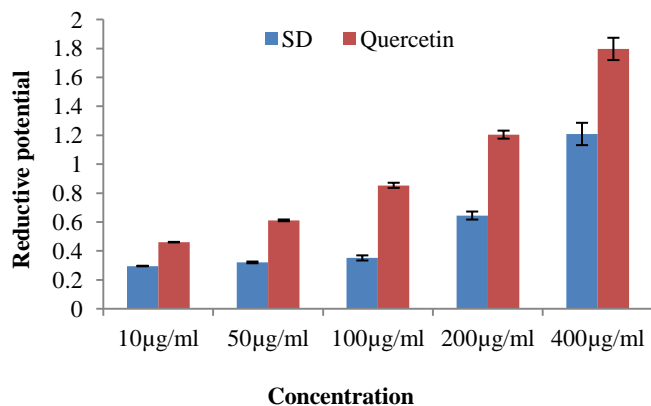


Fig. 7: Reductive potential of methanol leaf extract of SD.

This implies that the extract compared favourably with the standard in the reductive potential assay. The reducing capacity is generally associated with the presence of reductones and the antioxidant action of reductones is based on the breaking of the free radical chain by donating a hydrogen atom (Gordon and Hudson, 1990). The result indicated that the marked reducing power of the extract might be due to the presence of polyphenols which may act in a similar fashion as reductones.

The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule formed in living cells (Hochstein and Attalah, 1988). Hydroxyl radicals are also known to initiate peroxidation of lipid membranes (Halliwell, 1991). The radical has the capacity to form adducts with nucleotides in DNA and cause strand breakage which contributes to carcinogenesis, mutagenesis and cytotoxicity (Thirunavukkarasu *et al.*, 2011). Our result in figure8 shows that methanol extract of SD leaves has a high

hydroxyl radical scavenging activity which compared favourably with mannitol in a dose-dependent manner. At the highest concentration tested the extract and standard showed percentage hydroxyl radical scavenging activity of 79.86% and 80.56% respectively.

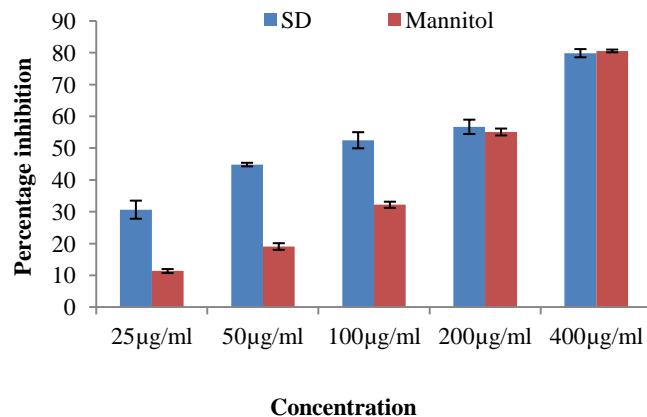


Fig. 8: Percentage hydroxyl radical scavenging activity of methanol leaf extract of SD.

Lipid peroxidation in biological systems has long been thought to be a toxicological phenomenon that can lead to various pathological consequences (Hochstein and Attalah, 1988). Lipid peroxidation is caused by the generation of free radicals from a variety of sources including organic hydroperoxides, redox cycling compounds and iron-containing compounds. The TBARS assay has been used to measure the degree of lipid peroxidation. TBA reacts specifically with malondialdehyde (MDA), a secondary product of lipid peroxidation to give a red chromogen, which may then be determined spectrophotometrically (Giri *et al.*, 2010). In this study, it was observed that methanol extract of SD leaves showed a moderate inhibition of lipid peroxidation. Our result as presented in figure9 shows that the extract has a dose dependent inhibition of lipid peroxidation with the highest concentration tested –400 μ g/ml showing a percentage inhibition of 48.79% while ascorbic acid had a percentage inhibition of 74.65% at the same concentration.

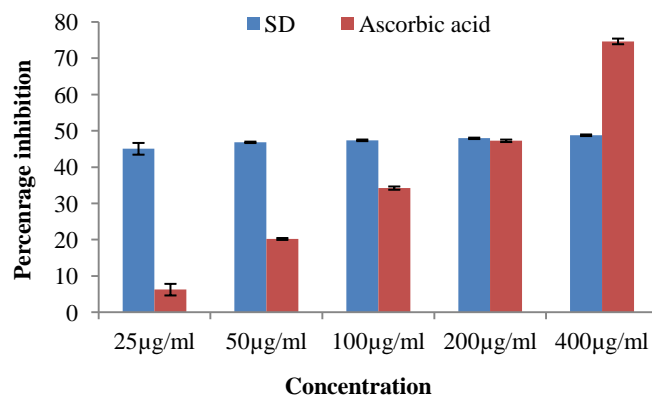


Fig. 9: Percentage inhibition of lipid peroxidation activity of methanol leaf extract of SD.

The IC₅₀ of the extract for DPPH, NO, OH⁻, and ABTS⁺ radicals scavenging assays were 139.45 µg/ml, 119.17 µg/ml, 147.65 µg/ml, and 135.83 µg/ml respectively, while its IC₅₀ for iron chelation and inhibition of lipid peroxidation were 345.03 µg/ml and 164.19 µg/ml respectively

HPLC/DAD fingerprint should be considered to evaluate the quality of herbal medicines all over the world considering the fact that multiple constituents are present in herbal medicines and its product (Coppen *et al.*, 1983). Our results as shown in tables 3 and 4 show that the extract has a high level of polyphenols in the order flavonoids (85.96 mg/g / 8.58%) > phenolic acids (63.5 mg/g / 6.33%) > tannins (23.87 mg/g / 2.38%).

Table 3: Composition of methanol leaf extract of *Synsepalum dulcificum*.

Compounds	<i>Synsepalum dulcificum</i>		LOD	LOQ
	mg/g	%	µg/mL	µg/mL
Gallic acid	6.18 ± 0.03 ^a	0.61	0.024	0.079
Catechin	5.95 ± 0.01 ^a	0.59	0.015	0.049
Chlorogenic acid	20.93 ± 0.01 ^b	2.09	0.007	0.023
Caffeic acid	14.82 ± 0.03 ^c	1.48	0.035	0.115
Ellagic acid	21.57 ± 0.01 ^b	2.15	0.028	0.091
Epicatechin	17.92 ± 0.01 ^d	1.79	0.021	0.070
Rutin	30.65 ± 0.03 ^e	3.06	0.042	0.138
Isoquercitrin	15.03 ± 0.02 ^f	1.50	0.010	0.032
Quercitrin	12.76 ± 0.01 ^f	1.27	0.019	0.063
Quercetin	21.90 ± 0.01 ^b	2.19	0.026	0.084
Kaempferol	5.62 ± 0.01 ^a	0.56	0.013	0.042

Results are expressed as mean ± standard deviations (SD) of three determinations. Averages followed by different letters differ by Tukey test at $p < 0.01$.

Table 4: Carotenoids compositions of methanol leaf extract *Synsepalum dulcificum*.

Carotenoids	<i>S. dulcificum</i> mg/g
Tocopherol	2.06 ± 0.01 ^a
β-Carotene	2.93 ± 0.04 ^a
Lycopene	0.57 ± 0.01 ^a

Results are expressed as mean ± standard deviations (SD) of three determinations. Averages followed by different letters differ by Tukey test at $p < 0.01$.

Among the flavonoids rutin was the most abundant followed by quercetin, isoquercitrin, quercitrin and kaempferol in that order. Ellagic acid is the most abundant among the phenolic acids present in the extract followed by chlorogenic acid, caffeic acid, and gallic acid in that order. For tannins our result also shows that the extract contains a higher level of epicatechin than catechin. The presence of carotenoids was also established in the extract. Figure 2 shows the chromatogram indicating the peaks of the polyphenols that were detected in the extract. HPLC is a sensitive, accurate and specific method for the separation and identification of phenolic acids, flavonoid glycosides and aglycones in plants. (Praven, 2012; Ana *et al.*, 2009). Moreover, many sources indicate good correlations between phenolics content and antioxidant capacity evaluated by different techniques, including HPLC (Benzie and Strain, 1999; Brand-Williams *et al.*, 1995), and Praven, (2012) asserted that plants exhibited medicinal properties due to the presence of flavonoids in it. We therefore opine that the presence of these plant polyphenols may contribute in part to the

significant antioxidant properties of MSD as observed in this study.

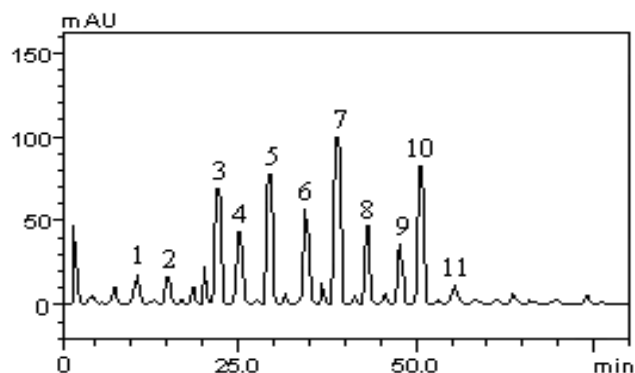


Fig. 2: HPLC chromatogram of methanol leaf extract of *Synsepalum dulcificum*. Representative high performance liquid chromatography profile of *Synsepalum dulcificum* at 327nm. Gallic acid (peak 1), catechin (peak 2), chlorogenic acid (peak 3), caffeic acid (peak 4), ellagic acid (peak 5), epicatechin (peak 6), rutin (peak 7), isoquercitrin (peak 8), quercitrin (peak 9), quercetin (peak 10) and kaempferol (peak 11).

The medicinal value of the plants lies in the chemical substances that produce a definite physiological action on human body (Hill, 1952). The distribution of minerals in common medicinal plants has important application for the health of people in addition to the basic need of developing countries.

Therefore there is need to evaluate the local herbs for mineral and nutrient composition, so as to determine their potential as indigenous source of medicine (Rahila *et al.*, 1994). Our result in table 5 shows that presence of some micro and macro elements. Potassium, calcium, magnesium and sodium are present in appreciable amounts while lead, a heavy metal is present in trace quantity.

Table 5: Mineral composition of methanol leaf extract of *Synsepalum dulcificum*.

Metals	mg/100g
Copper	1.10
Zinc	4.16
Magnesium	291.0
Lead	0.01
Manganese	8.70
Calcium	830.0
Iron	26.30
Sodium	102.0
Potassium	1850.0

CONCLUSION

From our study it could be safely concluded that the methanolic extract of *Synsepalum dulcificum* leaves has appreciable *in vitro* antioxidant activity and mineral content. This is a clear pointer to the possibility of the plant having potential health benefits especially in diseases in which oxidants have been established as the culprit. To confirm this possibility however, more research needs to be carried out using models of the diseases in question.

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