

Phytochemical and antioxidant properties of *Diodia sarmentosa* swartz leaves

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ABSTRACT

This research studied proximate, phytochemical and antioxidant properties of *Diodia sarmentosa* leaves. The ethanol and aqueous extracts of the leaves significantly inhibited 2,2-diphenyl-1-picrylhydrazyl (DPPH^o) radical formation with IC₅₀ values of 10.994 and 10.121 µg/mL respectively, compared to the ascorbic acid standard (IC₅₀ value = 17.916 µg/mL). The aqueous extract exhibited more inhibitory effect on thiobarbituric acid-reactive species (TBARS) with IC₅₀ values of 2.657 µg/mL while the ethanol extract had an IC₅₀ value of 8.53 µg/mL compared to butylated hydroxytoluene standard (IC₅₀ = 2.142 µg/mL). For the total antioxidant capacity assay, the aqueous extract had higher ascorbic acid equivalent values than the ethanol extract. However, the two solvent extracts showed antioxidant activity. *Diodia sarmentosa* leaves possess useful phytochemicals which are indicative of its antioxidant properties.

Keywords: Antioxidant; *Diodia sarmentosa*; phytochemical; proximate

INTRODUCTION

The use of plants for culinary and ethno medicinal purposes can be dated back to ancient history, even as far back as the “stone age” era. Plant leaves have been the most exploited for these purposes especially due to the proximate and phytochemical contents of these leaves. The proximate composition of these leaves reveal their nutritional status and make them suitable in food processing. The ethno medicinal uses of these leaves can be attributed to the phytochemical composition of the leaves which are also indicative of their antioxidant activities. Hence, a study on these vital components reveals the usefulness of a plant’s leaves. *Diodia sarmentosa* Sw commonly known as “Tropical buttonweed” [1], is a perennial herb without a true root system. It has a hairy leaf and stem which are about 7 cm and 4 m long respectively. It grows in bushy vegetation, riverine areas and on rocky grounds. It is majorly inhabited in tropical Africa, Asia, America and the Mascarene Islands. It is a dicot which belongs to the family Rubiaceae and the genus *Diodia*.

In some parts of Nigeria, especially the south western region, the leaves are crushed and used in preparing stew or soup and it is locally known as “Ewe Opaeyin” or “Ewe Ohaigbo”. *D. sarmentosa* leaves are used in treating injuries, oedema [2] and haemorrhoids (pile).

The whole plant is taken with pepper and salt for the treatment of dysentery in south western Nigeria [3, 4]. The antiulcer potential of *Diodia sarmentosa* (whole plant) [5] and its anti-inflammatory and analgesic activities [2] have been demonstrated. The anti-diabetic properties of *D. sarmentosa* has also been revealed [6]. This was determined using the n-hexane leaf extract of *D. sarmentosa*. Other species belonging to the genus *Diodia* has also gained wide applications. Studies on *Diodia scandens* revealed their use as poultry feed for ruminant animals [7]. There are also postulations that the plant has pharmacological properties, hence, could be used in the treatment of rheumatoid, oedema, inflammations [7] and brain thromboplastin [8]. *D. scandens* is also used traditionally in some parts of Eastern Nigeria to treat snake bites [8]. The plant also serves as a laxative and oxytocic agent in the treatment of uterine inertia and postpartum haemorrhage [9]. According to the same finding, the aqueous extracts are used to enhance micturition and sexual performance by rural inhabitants of Ikwere and Etche local government areas of Rivers State, Nigeria [9]. The anti-fungal properties of *D. scandens* have also been studied [10]. Presently, there is no data showing a comprehensive proximate composition, phytochemical and antioxidant properties of *D. sarmentosa* leaves.

EXPERIMENTAL

Plant collection and identification: Fresh samples of *D. sarmentosa* leaves were collected from farmlands and from natural vegetation within Federal University of Technology, Owerri (FUTO) premises. It was identified by Prof. Iloegbulam I.I. of the Department of Crop Science. A picture of a portion of *D. sarmentosa* plant is displayed in Figure 1.



Fig. 1. An aerial part of *D. sarmentosa* plant

Preparation of plant extract and extraction: Fresh leaves of *D. sarmentosa* were rinsed using distilled H₂O, dried at room temperature and then in a laboratory oven at 40 °C. The dried plant material was grinded into fine powder and 160 g was weighed, soaked in ethanol (800 mL) for 48 h and placed on an orbital shaker. Filtration was done using Whatman No.1 filter paper placed inside a funnel, then dried at 40 °C using a rotary evaporator.

Proximate analysis of sample: Determination of moisture content was carried out using a laboratory oven at 105 °C. Ash was determined using an electric furnace at 550 °C. Soxhlet apparatus was used in the determination of the crude fat. Crude protein was determined using Kjeldahl method. Percentage carbohydrate was done by difference. These were all done using AOAC method [11].

Qualitative phytochemical screening: The test sample (0.2 g) was placed in separate test tubes to assay for phytochemicals as follows:

Saponin: Frothing test was used in the detection of saponin, which was confirmed by constant foaming.

Flavonoid: Ammonium test method was used in the determination of flavonoid. A yellowish colouration confirmed the presence of flavonoid.

Carbohydrate: Molisch's method was used in the determination of carbohydrate. This was confirmed by the formation of a brown ring.

Phenol: Ferric chloride test was used in the determination of phenol. A greenish colouration was observed and this confirmed the presence of phenol.

Reducing Sugar: Reducing sugar was confirmed by a reddish colouration using Fehling's method.

Glycoside: Fehling's method was used in glycoside determination. Glycoside was confirmed by a reddish colouration.

Tannins: Ferric Chloride Method was used to determine the presence of tannins. A blue colouration was observed which confirmed the presence of tannins.

Alkaloid: Presence of alkaloid was determined using Wagner and Dragendorff's reagent. No precipitate was formed.

Steroids: To determine the presence of steroids, Lieberman-Buchard test was used. Steroids were detected by a greenish colouration.

Terpenoid: A violet colouration after dissolution in ethanol and treatment with acetic anhydride and conc. H₂SO₄ revealed the presence of terpenoids.

Quantitative phytochemical screening:

Determination of phenolics: Total phenolics was determined using Folin-Ciocalteu reagent method. Gallic acid was used as standard. Folin-Ciocalteu reagent was used in oxidizing *D. sarmentosa* leaves extract. This was then neutralized using Na₂CO₃. A portion (100 µL) of the different concentrations (15.63, 31.25, 62.5 µg/mL) of this extract was put into a mixture of 0.5 mL Folin-Ciocalteu reagent (0.1 dilution) and 1.5 mL sodium carbonate 2 % (w/v), then incubated for about 15 min at 25 °C. The absorbance of the blue coloured solution was read at 765 nm and results expressed in mg of gallic acid equivalent (GAE)/100 g of dry weight of plant powder.

Determination of tannins: The leaves extract (1 mL) of *D. sarmentosa* was put into a solution containing 0.5 mL Folin-Ciocalteu reagent, 1 mL sodium carbonate and 8 mL of distilled water. This was kept at 25 °C for about 30 min. It was then centrifuged to get a supernatant and absorbance read at 765 nm. The results were expressed as mg tannic acid/100 g of dry weight of plant powder.

Determination of flavonoid: Zhishen colorimetric method was used in the determination of flavonoid [12]. The diluted sample solution (0.5 mL) was put into a mixture containing 2 mL of distilled H₂O and 0.15 mL of sodium nitrate (5 %) solution. After a short while, 0.15 mL of 10 % AlCl₃ (aq) was put into the solution and kept for about 6 min. Diluted sodium hydroxide (4 %) solution (2 mL) was added to bring the total volume to 5 mL. The absorbance was taken after 15 min at 510 nm against water blank. The results were expressed as mg/100 g of dry weight of plant powder.

Determination of terpenoids: A portion of *D. sarmentosa* leaves (1 g) was extracted using ethanol (50 mL). The filtrate (2.5 mL) was mixed with 5% aqueous phosphomolybdic acid (2.5 mL) and 2.5 mL concentrated tetraoxosulphate (VI). After that, the volume of the solution was made up to 12.5 mL after 30 min using ethanol. The absorbance was read at 700 nm. The results were expressed as mg/100 g of dry weight of plant powder.

Determination of steroids: A portion of *D. sarmentosa* leaves (1 g) was extracted with 20 mL of ethanol. The filtrate (2 mL) was mixed with 2 mL of chromogen solution and kept at room temperature (25 °C) for about 30 min.

Then the absorbance was read at 550 nm. The results were expressed as mg/100 g of dry weight of plant powder.

Determination of saponin: Extraction of the sample (1 g) was done using 10 mL petroleum ether. After that, an additional 10 mL of petroleum ether was mixed with the solution and then subjected to dryness by evaporation. The residue obtained after dryness was dissolved in 6 mL of ethanol. Then, 2 mL of the solution was mixed with 2 mL of chromogen and left at room temperature for about 30 min. Absorbance of the solution was read at 550 nm. The results were expressed as mg/100 g of dry weight of plant powder.

Determination of glycosides: A sample of *D. sarmentosa* leaves (1 g) was extracted with 50 mL of distilled water. Alkaline picrate (4 mL) solution was mixed with 1 mL of the sample filtrate and heated for about 5 min. Absorbance was read at a wavelength of 490 nm. The results were expressed as mg/100 g of dry weight of plant powder.

Reducing sugar: Extraction of the sample (1 g) was carried out using 20 mL of distilled water. Alkaline copper reagent (1 mL) was mixed with the filtrate (1 mL) and heated for about 5 min. Then 1 mL of phosphomolybdic acid reagent and 2 mL of distilled water was mixed with the resultant solution and absorbance read at 420 nm. The results were expressed as mg/100 g of dry weight of plant powder.

Determination of soluble carbohydrates: Extraction of the sample (1 g) was done using 50 mL of distilled water. The filtrate (1 mL) was mixed with picric acid solution and the absorbance read at 580 nm. The results were expressed as mg/100 g of dry weight of plant powder.

In vitro screening for antioxidant activities:

Quantitative DPPH^o radical scavenging assay: Quantitative DPPH^o was determined according to a modified Gyamfi method [13]. The test was performed in triplicates. The sample extracts (1 mL each) were diluted 2-fold in 10 mL of water mixed with 0.5 mL of 0.076 mM DPPH^o. After that, it was mixed properly and kept away from sunlight at 25 °C for about 25 min. An aqueous solution (1 ml) of 0.076 mM DPPH^o was used as a negative control while the positive control was L-Ascorbic acid. Absorbance was read at 517 nm.

Thiobarbituric acid-reactive species (TBARS): Thiobarbituric acid-reactive species assay was carried out by a modified method of Banerjee [14]. This aimed at quantifying the amount of lipid peroxide formed. Egg yolk homogenate served as lipid-rich media [15]. The sample (100 µL) was mixed with egg homogenate (500 µL) in a test tube and distilled water was used to make up the volume to 1.0 mL. A mixture of 0.075 M FeSO₄ (50 µl) and 0.1 M L-Ascorbic acid (20 µL) were added to the solution, and kept at 37 °C for about an hour. Then 0.2 mL EDTA (0.1 M) and 1.5 mL of TBA reagent were put into each sample and heated for 15 min at 100 °C. After cooling, the samples were centrifuged for 10 min at 3000 rpm. The absorbance was read at 532 nm. Butylated hydroxytoluene (BHT) was used as the assay standard.

Total antioxidant capacity (TAC) assay: Phosphomolybdate method was used in assaying for total antioxidant capacity. A 0.1 mL aliquot of different concentrations (15.63, 31.25, 62.5, 125, 250, 500, 1000 µg/mL) of the extract and ascorbic acid (1000, 500, 250, 125, 62.5, 31.25, 15.63 µg/mL) was mixed with 1 mL of reagent solution (0.6 M H₂SO₄, 0.028 M Na₂HPO₄ and 0.004 M (NH₄)₂MoO₄). The test tubes were heated in a water bath at 95 °C for 90 min. After cooling, the absorbance was read at 765 nm. The reagent solution (1 mL) was used as blank and ascorbic acid was used as standard.

Statistical analysis: Pearson's correlation coefficient at 95% confidence interval was used in the determination of correlation coefficient between analytes. One way analysis of variance (ANOVA) was used in determining the standard deviation between means of values.

RESULTS AND DISCUSSION

Proximate analysis: The proximate analysis of *D. sarmentosa* leaves evaluated the moisture, ash, crude fat, crude fibre, crude protein and carbohydrate content. The percentage of carbohydrate was found to be (59.07 %); moisture content (10.66 %); crude protein (8.57 %); crude fibre (7.8 %); ash content (7.50 %) and crude fat (6.40 %).

Qualitative phytochemical analysis: *D. sarmentosa* leaves extract revealed the presence of saponin, flavonoid, and carbohydrate, phenol, reducing sugar, glycoside, tannin, steroid and terpenoid. However, alkaloid was absent (Table 1).

Table 1. Qualitative phytochemical analysis of *D. sarmentosa* leaves extract

Phytochemical	Observation	Bioavailability
Saponin	Persistent foaming	++
Flavonoid	A yellowish colouration	++
Carbohydrate	Brown ring formation at the interface	++
Phenol	A greenish colouration	+++
Reducing sugar	A brick red precipitate	+++
Glycoside	A brick red colouration	+++
Tannin	A blue black colouration	+++
Steroid	Colour change from violet to green	+++
Terpenoid	Colour change from pink to violet	+++
Alkaloid	No ppt formed	ND

Notice: + Slightly available; ++ Moderately available; +++ Very much available; ND Not detected

Quantitative phytochemical analysis: Quantitative phytochemical screening of the phytochemicals revealed a very high quantity of carbohydrate (3223 ± 3.95 mg/100 g) and reducing sugar (2410.87 ± 6.15 mg/100 g) in *D. sarmentosa* leaf extracts.

This suggest that the leaf extracts have high energy content and may be edible. High quantities of phenol (1121.02 ± 5.67 mg GAE/100 g), a potent anti-inflammatory phytochemical, was also observed. Relatively high quantities of flavonoid (320.15 ± 1.83 mg/100 g) and terpenoids (149.41 ± 3.64 mg/100 g), potent anti-inflammators were observed. The results of the quantitative phytochemical constituents of *D. sarmentosa* leaves extract are shown in Table 2.

Table 2. Quantitative phytochemical analysis of *D. sarmentosa* leaves

Phytochemical	Mean \pm std (mg/100 g)
Total phenolics	1121.02 ± 5.67
Total tannins	64.68 ± 1.08
Total flavonoid content	320.15 ± 1.83
Terpenoids	149.41 ± 3.64
Steroids	20.84 ± 0.13
Saponins	5.07 ± 0.86
Glycosides	50.38 ± 0.16
Reducing sugar	2410.87 ± 6.15
Soluble carbohydrates	3223 ± 3.95

In vitro antioxidant activities of leaves extracts of *D. sarmentosa*: **DPPH^o assay:** A graph of inhibition (%) was plotted against DPPH^o concentration ($\mu\text{g/mL}$) (Fig. 2). There was a significant ($r = -0.955$, $p < 0.05$) negative Pearson correlation between DPPH^o aqueous and concentration while a significant ($r = 0.704$, $p < 0.05$) positive correlation was observed between DPPH^o ethanol and concentration. DPPH^o aqueous was negatively ($r = -0.511$, $p < 0.05$) correlated with the standard (ascorbic acid) while DPPH^o ethanol was positively ($r = 0.257$, $p < 0.05$) correlated with the standard.

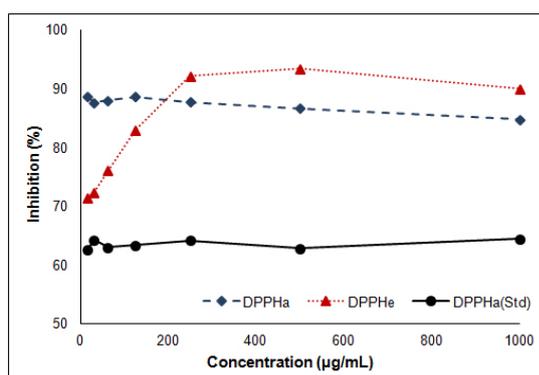


Fig. 2. Percentage DPPH^o inhibition of aqueous and ethanol extracts of *D. sarmentosa* leaves.

DPPHa - DPPH of aqueous extract; DPPHe - DPPH of ethanol extract; DPPH Std - DPPH of ascorbic acid standard

TBARS assay: A graph of inhibition (%) was plotted against concentration ($\mu\text{g/mL}$) (Fig. 3). There was significant ($r = 0.553$, $p < 0.05$) positive Pearson correlation between TBARS aqueous and concentration

and significant ($r = -0.834$, $p < 0.05$) negative Pearson correlation between TBARS ethanol and concentration. There was also significant ($r = 0.824$, $p < 0.05$) positive Pearson correlation between TBARS aqueous and BHT standard while there was significant ($r = -0.526$, $p < 0.05$) negative Pearson correlation between TBARS ethanol and BHT standard.

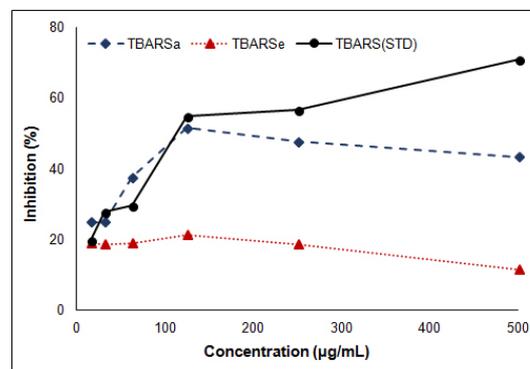


Fig. 3. Percentage TBARS inhibition of aqueous and ethanol extracts of *D. sarmentosa* leaves.

TBARSa - TBARS for aqueous extract; TBARSe - TBARS for ethanol extract; TBARS (Std) - TBARS for BHT standard

Total antioxidant capacity: A graph of ascorbic acid equivalent was plotted against concentration (Fig. 4). There was significant ($r = 0.987$, $p < 0.05$) positive Pearson correlation between total antioxidant capacity of aqueous extract and concentration. There was also significant ($r = 0.424$, $p < 0.05$) positive Pearson correlation between total antioxidant capacity of ethanol extract and concentration.

D. sarmentosa leaves contain protein (8.57 %), carbohydrate (59.07 %), fat (6.4 %), fibre (7.8 %), ash (7.50 %) and moisture (10.66 %).

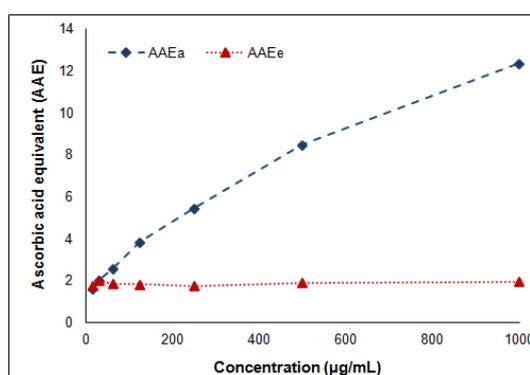


Fig. 4. Total antioxidant capacity of aqueous and ethanol extracts of *D. sarmentosa* leaves.

AAEa - Ascorbic acid equivalent of aqueous extract; AAEe - Ascorbic acid equivalent of ethanol extract.

This reveals the nutritional composition of the leaves and suggest that it could be used as part of a healthy meal.

Qualitative phytochemical screening of

D. sarmentosa leaves revealed the presence of saponin, flavonoid, terpenoid, phenol, reducing sugar, glycoside, tannin, steroid and carbohydrate except alkaloid which was absent (Table 1). Presence of saponin indicates that the leaves can be useful in treating yeast and fungal infections [16]. This agrees with the findings of Umoh [2] who reported the ability of *D. sarmentosa* leaves in preventing inflammation.

Flavonoids are natural antioxidants [17] which boost immune function, protect against microbial infections and prevent the formation of free radicals [18]. Phenols prevent inflammation and also boost the body immune system [19, 20]. This supports the findings of Umoh [2], who reported the anti-inflammatory properties of the leaves. Presence of glycosides suggest that the leaves extract have the ability to lower blood pressure [21]. Presence of tannins suggest that the leaves can be used in the treatment of wounds [22]. Results of this study validates that of Akah [5] regarding presence of tannins and of the antiulcer effect of *D. sarmentosa* leaves.

DPPH^o assay of the leaves extract of *D. sarmentosa* revealed significant antioxidant activity (Fig. 2). The IC₅₀ (inhibitory concentration at 50 %) value of aqueous and ethanol extracts of *D. sarmentosa* (10.121 and 10.994 µg/mL respectively) were significantly lower than the IC₅₀ value of ascorbic acid (17.916 µg/mL). Lower values reveal better antioxidant activity [23, 24]. In the Thiobarbituric acid assay (TBARS) (Fig. 3), the aqueous extract showed a significantly higher percentage inhibition compared to the ethanol extract. The IC₅₀ values for the aqueous and ethanol extracts were 2.657 and 8.53 µg/mL respectively compared to IC₅₀ value of BHT standard, which was 2.142 µg/mL. This suggest that water soluble phytochemicals possess a stronger potential to reduce malondialdehyde (MDA) formation. For the total antioxidant capacity assay (Fig. 4), the aqueous extract had higher ascorbic acid equivalent values compared to the ethanol extract. This is quite normal since the solubility of ascorbic acid increase with increasing polarity [25]. However, the two solvent extracts showed antioxidant activity.

CONCLUSIONS

The results of proximate assessment of *D. sarmentosa* leaves which revealed the edibility of the leaves, supports the use of the leaves in southern Nigeria for culinary purposes. Evidence from the qualitative and quantitative phytochemical evaluation as well as the different *in vitro* antioxidant assessments of *D. sarmentosa* leaves suggest that the plant has useful phytochemicals, indicative of its antioxidant properties.

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