Journal of Basics and Applied Sciences Research (JOBASR)
ISSN (print): 3026-9091, ISSN (online): 1597-9962
Volume 3(6) November 2025
DOI: https://dx.doi.org/10.4314/jobasr.v3i6.17

Phytochemicals and Antioxidants analysis of Ethanolic Aerial Parts Extract of *Diodia sarmentosa*

Okoro C. A.[1*], Ezejiofor T.I.N.[2], Mgbemena I.C.[3] & Ezeji E.U.[4]
[1,2,3&4]Department of Biotechnology, Federal University of Technology, Owerri-West L.G.A, 460114, Imo State Nigeria
[*]Corresponding Author Email: chiedu.okoro@gmail.com

ABSTRACT
In drug discovery process, the early characterization of phytochemicals for their chemical composition and bioactivity provides essential data for evaluating their potential therapeutic relevance. This study explored the preliminary phytochemical composition, antioxidant properties, and fourier transform infrared (FTIR) analysis of bioactive compounds in *Diodia sarmentosa* (DS) aerial parts 70% ethanol extract. An extraction yield of 11.04 ± 0.002% was obtained. Qualitative analysis detected phenols, sugars, tannins, alkaloids, flavonoids, and other secondary metabolites. In quantitative analysis, alkaloids (2747.48 ± 0.24 µg AE/mL), tannins (90.33 ± 0.01 µg TAE/mL), flavonoids (306.67 ± 0.66 µg QE/mL), and phenols (218.33 ± 0.01 µg GAE/mL) were present in high concentrations. In comparison to ascorbic acid standard, antioxidant assays showed low activities: 2,2-diphenyl-1-picrylhydrazyl (DPPH) IC_{50} was 868.82 ± 0.02 µg/mL (ascorbic acid standard: 34.07 ± 0.01 µg/mL), Ferric reducing antioxidant power (FRAP) EC_{50} was 789.5 ± 0.01 µg/mL (ascorbic acid standard: 262.08 ± 0.01 µg/mL), nitric oxide (NO) IC_{50} was 911.67 ± 0.01 µg/mL (ascorbic acid standard: 35.48 ± 0.01 µg/mL), and Cupric reducing antioxidant capacity (CUPRAC) value was 1548.56 ± 0.22 AAE. FTIR analysis revealed the presence of characteristic functional groups, including hydroxyl (O–H), carbonyl (C=O), and aromatic (C=C) vibrations, indicative of polyphenolic compounds, alcohols, esters, secondary amides, and related bioactive metabolites. Its extensive phytochemical profile, antioxidant efficacy and accompanying functional groups demonstrate its potential for managing ailments such as oxidative stress related diseases, warranting more research for potential pharmaceutical development and therapeutic applications.

Keywords:
Antioxidant activity
Diodia sarmentosa;
Phytochemical analysis
FTIR; Medicinal plants

INTRODUCTION

Traditional folk medicine practitioners have long relied on medicinal plants as primary therapeutic resources, being proven sources of bioactive compounds, many of which remain unexplored. These plants have been utilized for centuries in the promotion of health, prevention of illness, and treatment of diseases (Jain et al., 2016). Over time, several medicinal plants traditionally employed in healthcare have been scientifically identified, classified, and validated for clinical application. Exemplary bioactive compounds include quinine from *Cinchona* bark, used in malaria therapy; digoxin and digitoxin from *Digitalis purpurea*, employed in the management of cardiac disorders; and zingiberone from *Zingiber officinale*, recognized for its anthelmintic properties (Hardman, 1991).

The continued preference for medicinal plants is attributed to their affordability, established history of use suggesting low toxicity, and their potential to provide viable alternatives to counteract drug resistance associated with conventional pharmaceuticals (Jain et al., 2016).

Medicinal plants are rich sources of antioxidants such as tannins, flavonoids, terpenoids, phenolics and vitamins (C, E). They also have other secondary metabolites that can scavenge free radicals, chelate metal ions (which can catalyze radical formation), reduce oxidants, and modulate endogenous antioxidant enzymes. These non-enzymatic antioxidant compounds often act via multiple mechanisms, providing multi-targeted protection (Ashraf et al. 2024) against oxidative stress.

How to cite this article: Okoro C. A., Ezejiofor T.I.N., Mgbemena I.C. & Ezeji E.U. (2025). Phytochemicals and Antioxidants analysis of Ethanolic Aerial Parts Extract of *Diodia sarmentosa*. *Journal of Basics and Applied Sciences Research*, *3*(6), 159-169. https://dx.doi.org/10.4314/jobasr.v3i6.17

DS is a creeping perennial herbaceous plant which belongs to the Rubiaceae family. It is primarily found in the tropics and in the subtropics zones of the globe and because of its therapeutic qualities, it is frequently utilized in traditional African and Asian folk medicine (Ekpo et al., 2019).

Scientific investigations corroborate these traditional claims. Studies including that of Elechi, et al., (2020) revealed that DS contains tannins, flavonoids, alkaloids and saponins. These are directly responsible for its pharmacological properties which align with its documented traditional uses (Elechi, et al., 2020; Anyanwu-Azuka, et al., 2022). Akah, et al., (1998) reported its anti-ulcer potential, while Umoh, et al., (2016) and Ekpo, et al., (2020) confirmed its anti-inflammatory and analgesic effects. The n-hexane leaf extract of DS has demonstrated anti-diabetic properties (Elechi, et al., 2020). Studies carried out by Ezejiofor and Okoroafor, (2019) and Ezejiofor & Okoroafor, 2022 revealed its efficacy against uterine leiomyoma and hepatocellular carcinoma respectively in albino rats. Two separate studies carried out by Korie, et al., (2022), and Ezejiofor & Korie, (2019) revealed that DS ethanolic leaf extract exerted cardioprotective and lipid-lowering properties in high-fat meal-induced hyperlipidemia respectively in Wistar rats. Ezejiofor, et al., (2020) demonstrated its antioxidant and free radical scavenging activities to further highlight its therapeutic potential particularly in mitigating oxidative stress induced by a high-fat diet.

Despite earlier studies on the antioxidant, qualitative, and quantitative properties of ethanolic and aqueous DS leaf extracts, the phytochemical, antioxidant, and FTIR profiles of the aqueous-ethanol extract made from the aerial portions are not well documented.

The present investigation of DS aerial parts harvested from Owerri thus introduces a novel analytical perspective, bridging this knowledge gap and providing empirical support for its ethnomedicinal relevance. This approach offers the potential to support, refine, and integrate folk medicine practices with modern therapeutic strategies, ultimately contributing to both local healthcare and broader scientific understanding.

MATERIALS AND METHODS

Laboratory materials and equipment: Analytical-grade solvents, reagents, and standards including ethanol, ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, quercetin, tannic acid, and atropine were used in this study. All analyses were performed using standard laboratory apparatus, and functional group characterization was conducted with a Shimadzu IR Affinity-1S Fourier Transform Infrared (FTIR) spectrophotometer.

Plant material collection: The aerial parts of *Capsicum annuum* were freshly collected from Obinze Village, Owerri West Local Government Area, Imo State, Nigeria. Species identification and authentication were conducted by a plant taxonomist in the Department of Biology, Federal University of Technology Owerri with a herbarium number K006212000.

Plant extracts preparation and extraction: Fresh *Diodia sarmentosa* aerial parts were sorted, washed with distilled water. They were allowed for two to four weeks, to air dry to a consistent weight at room temperature. A lab mortar and pestle were used to grind the air-dried plant materials into a fine powder. Extraction was carried out by maceration as described by Erhirhie & Ilodigwe (2019). 92.4 g of DS was dissolved in 554.0 ml of 70% aqueous ethanol. They were agitated continually for 48 hours. After 48 hours, muslin cloth was used to filter the mixture, and the filtrates were recovered and concentrated to a paste-like form using a water bath at 55^0C. A rotary evaporator (Büchi Rotavapor R-200) was used to concentrate the filtrates until they were completely dry. The resulting crude extracts were weighed and kept for phytochemical profiling in opaque, airtight containers. The extract yield (%) was determined according to the formula below:

$$\text{Percentage yield} = \frac{\text{Weight (g) of concentrated extrac}}{Weight\ (g) of\ powdered\ plant\ material} \text{ x } 100$$

Qualitative analysis of phytochemical constituents: Phytochemical screening of the herbal extracts was conducted to detect the presence of proteins, carbohydrates, phenolic compounds, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids. The analytical procedures followed the protocols outlined by Sofowora (1993), Trease and Evans (1989), and Harborne (1973), as referenced in Yadav and Agarwala (2011). To prepare the DS extract, 2 g of the sample was dissolved in 30 mL of 30% aqueous ethanol. For each specific assay, 0.5 mL of this prepared extract was used, while 1 mL of 30% aqueous ethanol served as the control for comparison.

Protein detection (Millon's test):

For protein identification, 0.5 mL of each extract was treated with 1 mL of Millon's reagent. The formation of a white precipitate that turned red upon gentle heating confirmed the presence of proteins.

Carbohydrate detection (Iodine test):

To test for carbohydrates, 0.5 mL of each sample was combined with 1 mL of iodine solution. The appearance of a dark blue, black, green, or purple coloration indicated the presence of carbohydrates.

Phenolic compounds (Ferric chloride test):

Each 0.5 mL sample was mixed with 2 mL of a 2% ferric chloride ($FeCl_3$) solution. The emergence of a blue-green or black coloration signified the presence of phenols.

Tannins (Gelatin test):

To detect tannins, a few drops of 1% gelatin solution were added to 0.5 mL of each Jextract. The formation of a white precipitate confirmed the occurrence of tannins.

Flavonoids (Shinoda test):

Three small fragments of magnesium ribbon were added to 0.5 mL of each sample, followed by the dropwise addition of concentrated hydrochloric acid. The development of a pink to scarlet coloration after a few minutes indicated the presence of flavonoids.

Saponins (Frothing test):

For saponin screening, 0.5 mL of extract was diluted with 5 mL of distilled water in a test tube and shaken vigorously for about one minute. The persistence of stable foam for at least five minutes suggested the presence of saponins.

Glycosides (Keller–Killiani test):

Each 0.5 mL portion of the sample was mixed with 1 mL of acetic acid containing a drop of $FeCl_3$ solution. The appearance of a violet or greenish ring confirmed the existence of cardiac glycosides.

Steroids (Liebermann–Burchard test):

A 0.5 mL aliquot of extract was combined with 2 mL of chloroform, and concentrated sulfuric acid (H_2SO_4) was carefully added along the side of the tube. The development of a red coloration in the lower chloroform layer indicated steroidal compounds.

Terpenoids (Salkowski test):

To 0.5 mL of sample, 1 mL each of chloroform and concentrated H_2SO_4 were added. A reddish-brown interface ring confirmed the presence of terpenoids.

Alkaloids (Tannic acid test):

A few drops of 10% tannic acid solution were introduced into the sample. The formation of a buff-colored precipitate was taken as evidence of alkaloids.

Reducing sugars (Benedict's test):

For detecting reducing sugars, 0.5 mL of sample was treated with 1 mL of Benedict's reagent and heated for two minutes. The production of a green, yellow, or red precipitate indicated the presence of reducing sugars.

Quantitative analysis of phytochemical constituents

Analysis of total phenolic constituents: The total phenolic content of the extracts was quantified using the Folin–Ciocalteu reagent method as outlined by Yadav and Agarwala (2011). The reaction mixture consisted of 0.2 mL of Folin–Ciocalteu reagent (diluted to 20% v/v), 1 mL of 7.5% sodium carbonate (Na_2CO_3) solution, 0.4 mL of the sample extract, and 0.8 mL of distilled water. The prepared mixture was incubated in a water bath at 40°C for 30 minutes and then allowed to cool to room temperature. Absorbance was subsequently measured at 760 nm using a spectrophotometer. All analyses were conducted in triplicate, and phenolic content was determined from a gallic acid calibration curve, expressed as gallic acid equivalents (GAE mg/mL). The standard calibration curve was established using gallic acid concentrations of 7.8, 15.63, 31.25, 62.5, 125, 250, 500, and 1000 µg/mL. A reagent blank containing all components except the sample was prepared using distilled water. The total phenolic content was calculated from the equation of the gallic acid calibration curve ($y = 0.0011x + 0.0071$).

Analysis of total flavonoid constituents: The total flavonoid content of the extracts was determined using the aluminum chloride colorimetric assay as described by Muthukumaran et al. (2016), with quercetin serving as the standard compound. A volume of 125 µL of the sample was mixed with 75 µL of 5% sodium nitrite ($NaNO_2$) solution and allowed to stand for six minutes. Subsequently, 150 µL of 10% aluminum chloride ($AlCl_3$) solution was added, and the reaction mixture was incubated for five minutes. This was followed by the addition of 750 µL of 1 M sodium hydroxide (NaOH), after which the total volume was adjusted to 2.5 mL using distilled water. The mixture was then incubated for 15 minutes, during which it developed a pink coloration. Absorbance was measured at 510 nm using a spectrophotometer. The flavonoid content was expressed as grams of quercetin equivalent per milliliter of sample (g QE/mL). A standard calibration curve was prepared using quercetin concentrations of 15.63, 31.25, 62.5, 125, 250, 500, and 1000 µg/mL. A reagent blank containing all components except the sample was prepared with distilled water. The total flavonoid concentration was determined from the quercetin calibration curve ($y = 1E–04x + 0.0063$).

Analysis of total tannins constituents: The tannin content of the extracts was quantified using a modified Folin–Ciocalteu colorimetric method. A 0.25 mL aliquot of the sample extract (1 mg/mL) was combined with 1.875 mL of distilled water in a 5 mL test tube. Subsequently, 0.125 mL of Folin–Phenol reagent and 0.25 mL of 35% sodium carbonate (Na_2CO_3) solution were added, and the total volume was adjusted to 5 mL

with distilled water. Standard tannic acid solutions at concentrations of 7.8, 15.63, 31.25, 62.5, 125, 250, 500, and 1000 µg/mL were prepared following the same procedure. The mixtures were thoroughly mixed and incubated at 30°C for 30 minutes. Absorbance readings for both standard and test solutions were recorded at 725 nm using a spectrophotometer. The tannin content was expressed as tannic acid equivalents (µg TA/mL). A reagent blank, containing all reagents except the extract, was prepared in parallel. The total tannin concentration of each sample was determined from the tannic acid calibration curve (y = 0.0012x + 0.3098).

Analysis of total alkaloids constituents: The total alkaloid content of the extracts was determined using the bromocresol green colorimetric method. To each test tube containing 0.25 mL of the sample, 1.25 mL of bromocresol green solution and 1.25 mL of phosphate buffer (pH 4.7) were added. The mixture was vigorously shaken with 1 mL of chloroform, after which the chloroform layer was separated into a clean test tube. Additional chloroform was added to bring the volume to the desired level, and absorbance was measured at 470 nm using a spectrophotometer. Standard atropine solutions with concentrations of 7.8, 15.63, 31.25, 62.5, 125, 250, 500, and 1000 µg/mL were prepared in the same manner to generate a calibration curve. A reagent blank containing all components except the DS extract was also prepared. The total alkaloid concentration was calculated using the atropine calibration curve (y = 9E–05x + 0.0157) and expressed as atropine equivalents (µg AE/mg of dried extract).

Antioxidant Assays:

Free radical (DPPH) scavenging test: The free radical scavenging activity of the extracts was evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay, following the method described by Erhirhie et al. (2020). A 0.6 mM DPPH solution was freshly prepared in methanol. For each reaction, 0.25 mL of various dilutions of the sample was mixed with 0.25 mL of the DPPH solution and 2 mL of methanol. For the standard, ascorbic acid was used at concentrations of 7.82, 15.63, 31.25, 62.5, 125, 250, 500, and 1000 µg/mL. The mixtures were incubated in the dark at room temperature for 30 minutes, after which absorbance readings were taken at 517 nm using a spectrophotometer. A control was prepared by mixing 0.25 mL of DPPH solution with 2.25 mL of methanol. All analyses were conducted in triplicate.

The percentage of DPPH radical scavenging activity was calculated using the formula below, where AC is the absorbance of the control and AS is the absorbance of the sample:

$$\text{DPPH scavenging activity} = 100\left(\frac{AC-AS}{AC}\right)$$

A plot of percentage inhibition versus concentration was generated, and the concentration that produced 50% inhibition (IC_{50}) was determined using regression analysis (Microsoft Excel, 2010). The equations derived were y = 0.0179x + 35.23 for DS and y = 0.3854x + 36.871 for ascorbic acid. Lower absorbance values corresponded to higher DPPH radical scavenging potential. Data were expressed as mean ± standard error of mean (SEM).

Ferric reducing antioxidant power (FRAP) assay: The ferric reducing antioxidant power of the extracts was determined following the procedure described by Habibur et al. (2013). A 0.25 mL aliquot of each sample dilution, along with ascorbic acid standards at concentrations of 7.82, 15.63, 31.25, 62.5, 125, 250, 500, and 1000 µg/mL, was mixed with 0.625 mL of phosphate buffer and 0.625 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The resulting mixtures were incubated at 50°C for 20 minutes. After incubation, 0.625 mL of 10% trichloroacetic acid (TCA) was added, and the solutions were centrifuged at 3000 rpm for 5 minutes. From the supernatant, 0.625 mL was transferred to a new test tube, mixed with 0.625 mL of distilled water, and combined with 0.125 mL of 0.1% (w/v) ferric chloride ($FeCl_3$) solution. The absorbance of each mixture was measured at 700 nm using a spectrophotometer, with ascorbic acid serving as the reference standard. All assays were conducted in triplicate. Percentage inhibition was calculated using the formula below.

$$\% \text{ Inhibition} = (\text{Absorbance of sample} - \text{Absorbance of blank}) \times 100$$

The percentage inhibition was calculated using the appropriate formula. For ascorbic acid, a plot of percentage inhibition versus concentration was generated, and the effective concentration required to produce 50% reduction (EC_{50}) was determined from the regression equation. Higher absorbance (optical density) values were indicative of stronger ferric reducing antioxidant activity. The results were presented as mean ± standard error of mean (SEM).

Cupric reducing antioxidant capacity assay: The cupric reducing antioxidant capacity of the extracts was evaluated following the procedure outlined by Alaribe et al. (2020). In this assay, 0.25 mL of ammonium acetate buffer (1.0 M, pH 7.0) was added to 0.25 mL of the sample extract (1000 µg/mL prepared in ethanol). To this mixture, 0.25 mL of 10 mM cupric chloride ($CuCl_2$) solution and 0.25 mL of 7.5 mM neocuproine solution (prepared in ethanol) were added. The total volume was adjusted by adding 1 mL of distilled water, and the mixture was incubated at room temperature for 30 minutes. After incubation, the absorbance was measured at 450 nm against a reagent blank using a

spectrophotometer. All determinations were performed in triplicate.

Ascorbic acid at concentrations of 1.96, 3.91, 7.82, 15.63, 31.25, 62.5, 125, 250, 500, and 1000 µg/mL was used as a positive control. The reagent blank was prepared in the same manner, substituting 0.5 mL of methanol for the sample extract. The cupric reducing antioxidant capacity was expressed as ascorbic acid equivalents (AAE) using the ascorbic acid calibration curve (y = 9E–06x + 0.071). All results were reported as mean ± standard error of mean (SEM).

Nitric oxide radical scavenging assay: The nitric oxide (NO) radical scavenging activity of the extracts was assessed following the procedure described by Atere et al. (2018). In this assay, 0.5 mL of sodium nitroprusside solution (10 mM) prepared in phosphate-buffered saline (0.2 M, pH 7.4) was mixed with 0.25 mL of the sample or ascorbic acid at varying concentrations (7.82, 15.63, 31.25, 62.5, 125, 250, 500, and 1000 µg/mL). The reaction mixtures were incubated under light for 150 minutes.

Following incubation, 0.25 mL of 1% sulphanilamide in 5% phosphoric acid was added and the solution was kept in the dark for 10 minutes. Subsequently, 0.25 mL of 0.1% N-1-naphthyl ethylenediamine dihydrochloride (NED) was added. The absorbance of the resulting pink chromophore was measured at 546 nm after 10 minutes using a spectrophotometer. Ascorbic acid served as the reference antioxidant.

The percentage inhibition of nitric oxide radicals was calculated using the following relationship:

$$(\%)\text{inhibition of NO} = \left[\frac{\text{Absorbance of control} - \text{Absorbance of the sample}}{\text{Absorbance of control}}\right] \text{x}100$$

Fourier Transform Infrared Spectroscopy (FTIR):

FTIR was used to identify the functional groups in the phytochemicals. Each dried extract sample (2mg) was mixed with potassium bromide (1:100) to prepare translucent discs for analysis on a Shimadzu IR Affinity 1 FTIR spectrophotometer, scanning from 400 to 4000 cm^{-1} at a resolution of 4 cm^{-1}.

Statistical Analysis:

Data were analyzed using Microsoft Excel (2016). Results were presented as the mean ± standard error of the mean (SEM), and IC_{50} values were determined through regression analysis conducted within the Excel software environment.

RESULTS AND DISCUSSION

Extraction yield: Based on the initial and final weights recorded after extraction, the percentage yield of the 70% ethanol extract from the aerial parts of DS was 17.02 ± 0.005%. This yield is comparatively higher than those reported for ethanolic (10.92 ± 0.11%) and aqueous (8.13 ± 0.11%) whole-plant extracts in the study by Hariharan et al. (2021). The increased extraction efficiency observed with the hydroethanolic solvent system may result from the combined polarity of ethanol and water, which enhances the dissolution of a wider spectrum of phytochemicals including both polar and moderately non-polar constituents such as flavonoids and phenolic compounds.

Qualitative phytochemical analysis: DS aerial parts extract was screened for eleven phytochemicals (Table 1). Notably, phenols and carbohydrates were detected in abundance. Tannins, alkaloids and flavonoids were detected in moderate levels (++). Glycosides, terpenoids, saponins, proteins, steroids, and reducing sugars were detected in low concentrations (+). This result suggest that the identified phytochemicals may serve as the bioactive constituents contributing to the plant's pharmacological activities. This underscores the relevance of DS and similar plants as valuable sources of biologically active compounds with notable medicinal potential. The abundant presence of phenols suggests the ability of DS aerial parts to exert anti-inflammatory and immune system enhancing effects (Okwu, 2004; Okwu & Omodamino, 2005). The moderate presence of tannins suggests that DS aerial parts have wound healing properties. Flavonoids exert anti-oxidant, immune system enhancing and anti-inflammatory properties (Barakat et al. 1995). The presence of alkaloids indicates that the plant has antitumour properties (Isah, 2016). The presence of saponins suggests that DS aerial parts may have very anti-fungal infections (Sheikh et al. 2013). Steroids have antimicrobial properties (Raquel, 2007) and are very important because they form major part of the sex hormones (Okwu, 2001). Glycosides exert blood pressure lowering properties (Nyarko & Addy 1990).

Quantitative phytochemical composition: Quantitative analysis confirmed the phytochemical abundance of the aqueous–ethanol extract from the aerial parts of DS, as summarized in Table 2. The total phenolic content (TPC) was 218.33 ± 0.01 mg gallic acid equivalents (GAE) per gram of extract, indicating a considerable concentration of phenolic constituents. The total flavonoid content (TFC) reached 306.67 ± 0.66 mg quercetin equivalents (QE) per gram, reflecting a notable accumulation of flavonoids. Similarly, the extract contained 90.33 ± 0.01 mg tannic acid equivalents (TAE) per gram of total tannins and 2747.48 ± 0.24 mg atropine equivalents (AE) per gram of total alkaloids, emphasizing its diverse and complex phytochemical composition.

Comparatively, Okoroafor et al. (2020) reported lower values in ethanolic leaf extracts of DS with total phenolic content of 1121.02 ± 5.67 mg GAE/100 g, total flavonoid content of 320.15 ± 1.83 mg/100 g, and tannin content of

64.68 ± 1.08 mg/100 g. These findings align with the present study, supporting the high phytochemical density of the hydroethanolic extract, which likely contributes to its pronounced biological activities (Salisu et al., 2025).

Table 1: Qualitative Phytochemical Analysis Result for *Diodia sarmentosa* aerial parts aqueous-ethanol extract

Phytochemical class	**Test**	**Level of presence**
Proteins	Millon's test	+
Carbohydrates	Iodine test	+++
Phenols	Ferric chloride test	+++
Tannins	Geletin test	+++
Flavonoids	Shinoda test	++
Saponins	Frothing test	++
Glycoside	Keller-kilani test	++
Steroids	Liebermann-Burchard's test	+
Terpenoids	Salkowski's test	+
Alkaloids	Picric acid test	+
Reducing sugar	Benedict test	+

Key: Legend: - = Absent, + = trace), ++ = moderate, +++ = abundant.

Table: 2: Quantitative Analysis Result for *D. sarmentosa* Aerial Parts Aqueous-Ethanol Extract

Phytochemical Sample	**Quantity (mg/g)**
Total phenolic content	218.33 ± 0.01 mgGAE/g
Total flavonoids content	306.67 ± 0.66 mgQE/g
Total tannins content	90.33 ± 0.01 mgTAE/g
Total alkaloids content	2747.48 ± 0.24 mgAE/g

Mean ± SEM [n=3], GAE = Gallic acid equivalent, QE = Quercetine quivalent. TAE = Tannic acid equivalent, AE = Atropine equivalent.

In-vitro antioxidant activity: Living organisms continuously produce reactive oxygen species (ROS), reactive nitrogen species (RNS), and other free radicals as inevitable by-products of normal cellular metabolism, immune defense mechanisms, exposure to environmental factors such as ultraviolet radiation pollutants and other stresses. When the generation of such radical species overwhelms the body's antioxidant defense systems, a state known as oxidative stress occurs. Oxidative stress induces structural and functional damage to lipids, proteins, nucleic acids, and other vital biomolecules, thereby contributing to the onset and progression of numerous chronic and degenerative conditions such as cancer, cardiovascular disorders, neurodegenerative diseases, diabetes mellitus, inflammatory pathologies, and age-related dysfunctions (Waseem et al., 2017).

The antioxidant potential of the 70% ethanol extract from the aerial parts of *Diodia sarmentosa* was assessed through four complementary assays: DPPH free radical scavenging, ferric reducing antioxidant power (FRAP), nitric oxide inhibition, and cupric reducing antioxidant capacity (CUPRAC). The results demonstrated consistently high IC_{50} and EC_{50} values compared to the ascorbic acid standard, suggesting moderate overall antioxidant potency. The DPPH radical scavenging assay (Fig 1) yielded an IC_{50} of 825.14 ± 0.02 µg/mL, significantly higher than 34.1 ± 0.01 µg/mL for ascorbic acid, indicating weak hydrogen-donating capacity. In contrast, Okoroafor et al. (2020) presented considerably lower IC_{50} values of 10.121 µg/mL and 10.994 µg/mL for the aqueous and ethanolic leaf extracts of DS respectively, suggesting significantly higher antioxidant activity than observed in the present study. Similarly, in the FRAP assay (Fig 2), the extract exhibited an EC_{50} of 781.97 ± 0.01 µg/mL versus 262.08 ± 0.01 µg/mL for ascorbic acid, confirming a lower ferric-reducing ability. The nitric oxide scavenging IC_{50} (911.67 ± 0.01 µg/mL) (Fig 3) also revealed limited radical-neutralizing potential when compared with ascorbic acid (35.48 ± 0.01 µg/mL). Ezejiofor et al. (2020) similarly reported a nitric oxide scavenging IC_{50} of 907.17 ± 45.36 µg/mL for DS leaf extract. In line with the results of this investigation, the CUPRAC value of 1548.15 ± 0.22 AAE (Fig 4) extract further reflected a moderate reducing power. The results suggest a generally modest antioxidant capacity in this particular extract preparation.

Consistent with this study, a high total phenolic content in the 70% ethanol extract of DS does not always correspond to strong antioxidant activity (Rahiman et al., 2012; Bajpai et al., 2005). Variations in solvent type, plant parts, geographic origin, extraction procedures, and the presence of pro-oxidants or interfering compounds may account for these differences (Dai & Mumper, 2010; Iloki-Assanga et al., 2015; Yeo & Shahidi, 2019; Bako et al., 2024; Yamauchi, 2024).

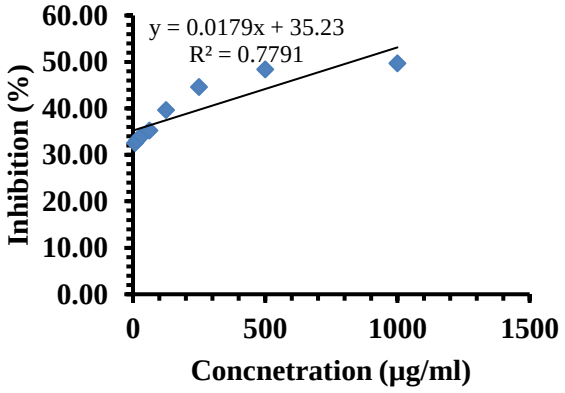

Fig 1: DPPH Scavenging activity of *D. sarmentosa* (DS) aerial parts extract

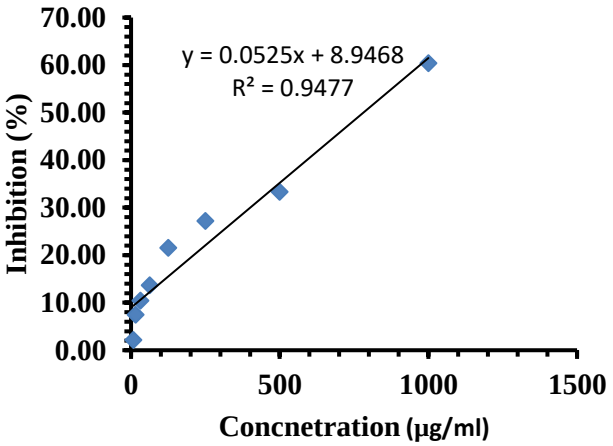

Fig 2: FRAP Scavenging activity of *D. sarmentosa* (DS) aerial parts extract

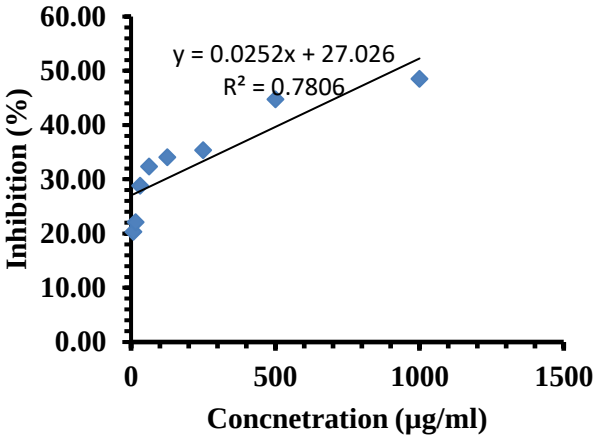

Fig 3: Nitric oxide scavenging activity for *D. sarmentosa* aerial parts

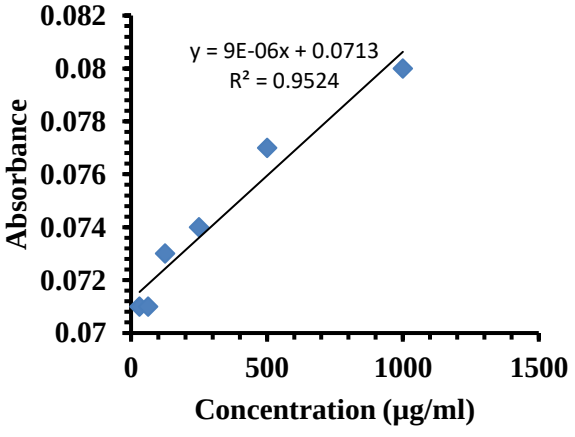

Fig 4: Ascorbic acid calibration curve for *D. sarmentosa* CUPRAC assay

FTIR spectrum of *Diodia sarmentosa* aerial part 70%-ethanol extract: The FTIR spectrum (Fig. 5) displayed multiple absorption peaks indicative of diverse functional groups, as summarized in Table 3. Prominent O–H stretching vibrations at 3748.41 cm^{-1} and 3548.92 cm^{-1} confirm the presence of hydroxyl groups characteristic of alcohols or phenolic compounds. These bands suggest hydrogen-bonded hydroxyl functionalities that influence both bioactivity and solubility. The absorption at 3120.37 cm^{-1} represents O–H stretching typical of alcohols or carboxylic acids, while the band at 3005.34 cm^{-1} corresponds to =C–H stretching, indicative of aromatic or alkene groups. The peak observed at 2832.04 cm^{-1} reflects aliphatic C–H/N–H stretching, possibly arising from amine salts or aldehyde groups, and the broad band near 2592.74 cm^{-1} is attributed to O–H or S–H stretching associated with carboxylic acids or thiols. A minor absorption at 2384.67 cm^{-1} may correspond to nitrile (C≡N) groups or atmospheric CO_2 interference, while the 1923.43 cm^{-1} peak suggests C–H bending or C=C=C stretching related to aromatic compounds or allenes.

In the fingerprint region, a strong absorption at 1597.97 cm^{-1} corresponds to C=C stretching and N–H bending, confirming the presence of alkenes or amines. Peaks at 1240.66 cm^{-1} and 1041.38 cm^{-1} are assigned to C–O and C–O–C stretching vibrations, characteristic of esters, ethers, or polysaccharides. Collectively, the FTIR spectrum reveals hydroxyl, carboxyl, aromatic, aliphatic, and ether/ester functionalities, suggesting the presence of oxygenated aromatic compounds such as phenolics and other bioactive phytoconstituents associated with antioxidant and antimicrobial activity. Minor absorptions near 2384 cm^{-1} and 1923 cm^{-1} are likely due to instrumental or atmospheric interferences.

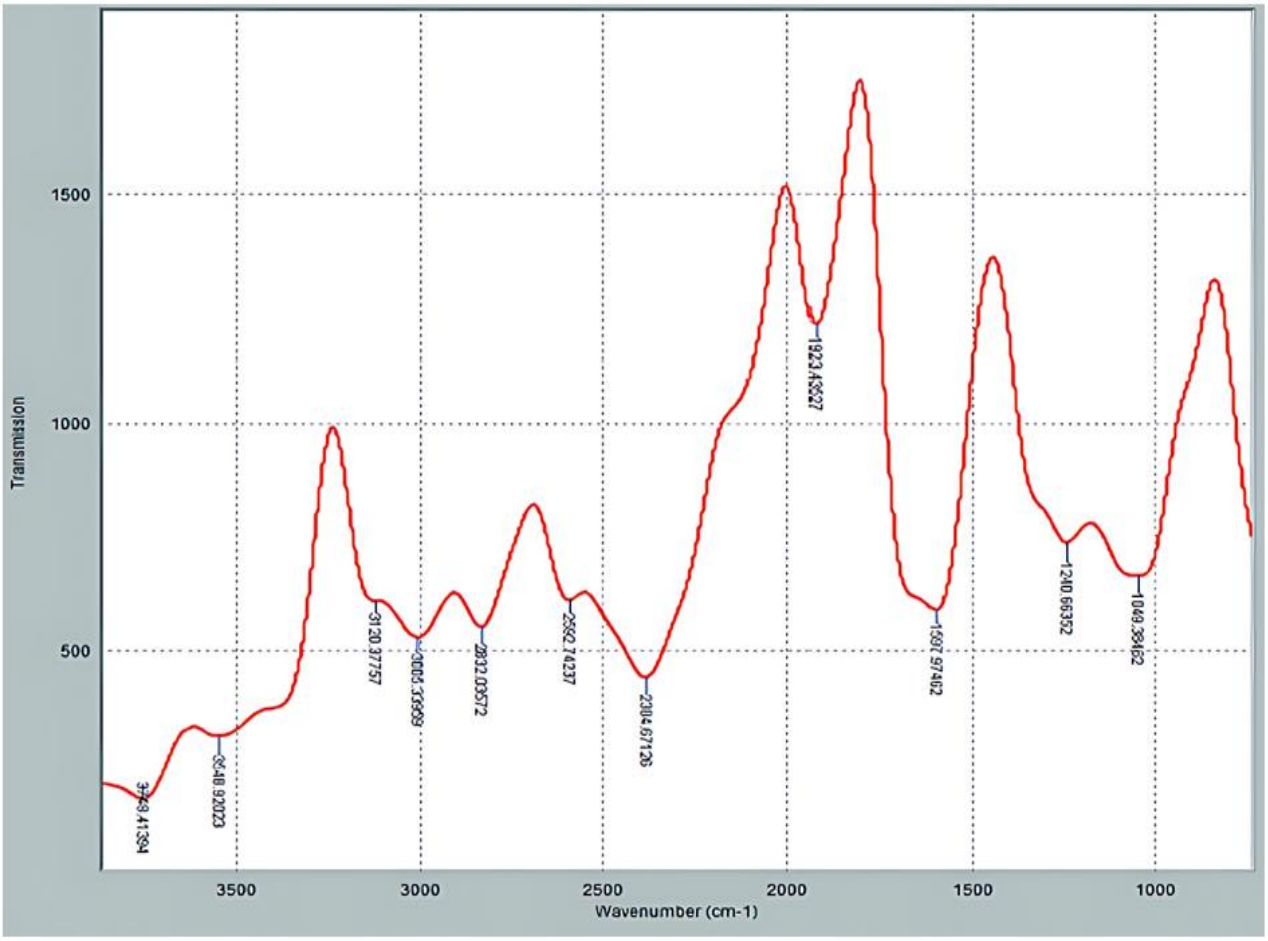

Fig 5: FTIR chromatogram of *D. sarmentosa* aerial part aqueous-ethanol extract

Table 3: FTIR Absorption Bands and Corresponding Functional Group Assignments for *Diodia sarmentosa* Aerial Parts 70% Ethanol Extract

Wave number (cm^{-1})	Probable Functional Group(s)	Possible Compound Class / Interpretation
3748.41 / 3548.92	O–H stretching	Alcohols, phenols
3120.37	O–H stretching	Carboxylic acids
3005.34	=C–H stretching	Aromatic rings, alkenes
2832.04	C–H / N–H stretching	Aldehydes, amines
2592.74	O–H / S–H stretching	Carboxylic acids, thiols
2384.67	C≡N stretching	Nitriles / (possible CO_2 interference)
1923.43	C–H bending / C=C=C stretching	Aromatic compounds
1597.97	C=C stretching / N–H bending	Alkenes, amines, aromatic systems
1240.66	C–O stretching	Esters, polysaccharides
1041.38	C–O–C stretching	Ethers, esters, polysaccharides

CONCLUSION

DS exhibits a rich phytochemical profile characterized by the presence of phenolics, flavonoids, tannins, and alkaloids, supporting its potential as a source of medicinal compounds. FTIR analysis corroborated these findings by revealing prominent hydroxyl, carboxyl, and aromatic functional groups associated with phenolic and flavonoid structures. Despite demonstrating only moderate antioxidant activity, the presence of these bioactive constituents justifies its traditional use and warrants further pharmacological investigation to fully elucidate its therapeutic potential

REFERENCE

Akah, P. A., Orisakwe, O. E., Gamaniel, K. S., & Shittu, A. (1998). Evaluation of Nigerian traditional medicines: II. Effects of some Nigerian folk remedies on peptic ulcer. *Journal of Ethnopharmacology*, 62(2), 123–127. https://doi.org/10.1016/S0378-741(98)00060-9

Alaribe, C. S., Oladipupo, A. R., Ojo-Nosakhare, O., Kehinde, O., & Ogunlaja, A. S. (2020). GC-MS analysis and mitochondrial functionality potential of the fruits of *Tetrapleura tetraptera* by cupric reducing antioxidant capacity assay. *Journal of Phytomedicine and Therapeutics*, 19(1), 338–347.

Anyanwu-Azuka, S. I., Aloh, G. S., Kalu, W. O., & Eleazu, C. (2022). Phytochemical screening and evaluation of the anti-diarrhoea properties of *Diodia sarmentosa* leaves in castor-oil-induced diarrhoea in albino rats. *Nutrition & Food Science*, 52(2), 255–269. https://doi.org/10.1108/NFS-03-2021-0093

Ashraf, M. V., Khan, S., Misri, S., Gaira, K. S., Rawat, S., Rawat, B., Khan, M. A. H., Shah, A. A., Asgher, M., & Ahmad, S. (2024). High-altitude medicinal plants as promising source of phytochemical antioxidants to combat lifestyle-associated oxidative stress-induced disorders. *Pharmaceuticals (Basel, Switzerland)*, 17(8), 975. https://doi.org/10.3390/ph17080975

Atere, T. G., Akinloye, O. A., Ugbaja, R. N., Ojo, D. A., & Dealtry, G. (2018). *In vitro* antioxidant capacity and free-radical-scavenging evaluation of standardized extract of *Costus afer* leaf. *Food Science and Human Wellness*, 7(4), 266–272.

Bajpai, M., Pande, A., Tewari, S. K., & Prakash, D. (2005). Phenolic contents and antioxidant activity of some food and medicinal plants. *International Journal of Food Sciences and Nutrition*, 56(4), 287–291. https://doi.org/10.1080/09637480500146606

Bako, B., Danladi, A. H., Bulus, G. G., & Shinggu, J. P. (2024). A comprehensive review of solvent-induced variability in antioxidant profiling of plant extract: *Justicia secunda*. *Progress in Chemical and Biochemical Research*, 7(1), 1–21. https://doi.org/10.48309/pcbr.2024.417487.1292

Barakat, M. Z., Shahab, S. K., Darwin, N., & Zahemy, E. I. (1993). Determination of ascorbic acid from plants. *Analytical Biochemistry*, *53*, 225–245.

Dai, J., & Mumper, R. J. (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15(10), 7313–7352. https://doi.org/10.3390/molecules15107313

Ekpo, Z., Adamu, A., & Magaji, M. (2020). Pharmacognostic and anti-inflammatory studies on the

aerial parts of *Diodia sarmentosa* Swartz (Rubiaceae). *Journal of Pharmacy Development and Industrial Pharmacy*, 1(3), 1–15.

Ekpo, Z., Ambi, A. A., Magaji, M. G., & Ojeje, S. O. (2019). Pharmacognostic and anti-inflammatory studies on the aerial parts of *Diodia sarmentosa* Swartz (Rubiaceae). *Journal of Pharmacy Development and Industrial Pharmacy*, 1(3), 1–15.

Elechi, N. A., Okezie-Okoye, C., & Abo, K. A. (2020). Antidiabetic potentials of *Diodia sarmentosa* SW (Rubiaceae) leaves on alloxan-induced diabetic rats. *Saudi Journal of Medical and Pharmaceutical Sciences*, 6(9), 622–626. https://doi.org/10.36348/sjmps.2020.v06i09.006

Erhirhie, E. O., & Ilodigwe, E. E. (2019). Sub-chronic toxicity evaluation of *Dryopteris filix-mas* (L.) Schott leaf extract in albino rats. *Brazilian Journal of Pharmaceutical Sciences*, 55, 1–14. https://doi.org/10.1590/s2175-97902019000118107

Erhirhie, E. O., Ilodigwe, E. E., Ajaghaku, D. L., et al. (2020). Antioxidant activities of the leaf extract and fractions of *Dryopteris filix-mas* (L.). *Biological and Medicinal Natural Product Chemistry*, 9(1), 1–6.

Ezejiofor, T. I. N., & Korie, S. C. (2019). In search of natural antihypertensives – Antihyperlipidaemic potentials of ethanolic leaf extract of *Diodia sarmentosa* on high-fat-diet-fed Wistar rats. *Asian Journal of Medical and Pharmaceutical Sciences*, 7(2), 77–85.

Ezejiofor, T. I. N., & Okoroafor, C. H. (2019). Biochemical and histopathological evidence of anticancer potentials of ethanolic leaves extract of *Diodia sarmentosa* (Sw) against diethyl nitrosamine-induced hepatocellular carcinoma in albino rats. *Reviews on Antiviral Therapy and Infectious Diseases*, *8*, 77–78.

Ezejiofor, T. I. N., Korie, S. C., & Peter, C. (2020). Free radical scavenging and antioxidant potential of ethanolic leaf extracts of *Diodia sarmentosa* on high-fat-fed Wistar rats. *Journal of Advances in Biology & Biotechnology*, 23(10), 54–63. https://journaljabb.com/index.php/JABB/article/view/30184

Ezejiofor, T. I. N., & Okoroafor, C. H. (2022). Effects of ethanol extracts of *Diodia sarmentosa* leaves on biochemical and histopathological indices of monosodium glutamate-induced uterine leiomyoma in rats. *Biomedical Research and Therapy*, 9(7), 5140–5148. https://doi.org/10.15419/bmrat.v9i7.749

Habibur, R., Manjula, K., & Anoosha, T. (2013). *In vitro* antioxidant activity of *Citrullus lanatus* seed extracts. *Asian Journal of Pharmaceutical and Clinical Research*, 6(3), 152–157.

Harborne, J. B. (1973). *Phytochemical Methods.* Chapman and Hall.

Hardman, R. (1991). Progress on medicinal plants. *Pharmaceutical Journal*, 247, 540–542.

Hariharan, T., Vasan, P., & Gopalakrishna Murthy, T. R. (2021). Phytochemical analysis of *Diodia sarmentosa* whole-plant powder. *Pharma Innovation Journal*, 10(7), 842–845.

Iloki-Assanga, S. B., Lewis-Luján, L. M., Lara-Espinoza, C. L., Gil-Salido, A. A., Fernandez-Angulo, D., Rubio-Pino, J. L., & Haines, D. D. (2015). Solvent effects on phytochemical constituent profiles and antioxidant activities using four different extraction formulations for analysis of *Bucida buceras* L. and *Phoradendron californicum*. *BMC Research Notes*, 8, 396. https://doi.org/10.1186/s13104-015-1388-1

Isah, T. (2016). Anticancer alkaloids from trees: Development into drugs. *Pharmacognosy Reviews*, 10(20), 90–99. https://doi.org/10.4103/0973-7847.194047

Muthukumaran, P., Saraswathy, N., Aswitha, V., Balan, R., & Gokhul, V. B. (2026). Assessment of total phenolic, flavonoid, tannin content and phytochemical screening of leaf and flower extracts from *Peltophorum pterocarpum* (DC.) Backer ex K. Heyne: A comparative study. *Pharmacognosy Journal*, *8*(2), 140–143.

Nyarko, A. A., & Addy, M. E. (1990). Effects of aqueous extract of *Adenia cissampeloides* on blood pressure and serum analyte of hypertensive patients. *Phytotherapy Research*, *4*(1), 25–28. https://doi.org/10.1002/ptr.2650040107

Okoroafor, H. C., Awagu, F. E., & Azeke, E. A. (2020). Phytochemical and antioxidant properties of *Diodia sarmentosa* Swartz leaves. *Mongolian Journal of Chemistry*, 21(47), 27–32. https://doi.org/10.5564/mjc.v21i47.1430

Okwu, D. E. (2001). Evaluation of chemical composition of indigenous species and flavouring agents. *Global Journal of Pure and Applied Sciences*, 8(2), 203–208.

Okwu, D. E. (2004). Phytochemicals and vitamin contents of indigenous species of South Eastern Nigeria. *Journal of Sustainable Agriculture and the Environment*, 6, 30–37.

Okwu, D. E., & Omodamino, O. D. (2005). Effects of hexane extract and phytochemical content of *Xylopia aethiopica* and *Ocimum gratissimum* on uterus of guinea pig. *Bio Research*, 3(2), 40–44. https://doi.org/10.4314/br.v3i2.28589

Raquel, F. E. (2007). Bacterial lipid composition and antimicrobial efficacy of cationic steroid compounds. *Biochimica et Biophysica Acta*, 2500–2509.

Rahiman, S., Tantry, B. A., & Kumar, A. (2012). Variation of antioxidant activity and phenolic content of some common home remedies with storage time. *African Journal of Traditional, Complementary and Alternative Medicines*, 10(1), 124–127. https://doi.org/10.4314/ajtcam. v10i1.16

Salisu, I., Tijjani, A.& Ikechukwu, O.A. (2025). Comparative Phytochemical Profiling of Terminalia catappa, Azadirachta indica and Stachytarpheta Angustifolia Bridging Traditional use and Scientific Validation. *Journal of Basics and Applied Sciences Research*, 3(5),88-92. https://dx.doi.org/10.4314/jobasr.v3i5.11

Sheikh, N., Kumar, Y., Misra, A. K., & Pfoze, L. (2013). Phytochemical screening to validate the ethnobotanical importance of root tubers of *Dioscorea* species of Meghalaya, North East India. *Journal of Medicinal Plants Studies*, 1(6), 62–69.

Sofowora, A. (1993). *Medicinal Plants and Traditional Medicine in Africa.* Spectrum Books.

Trease, G. E., & Evans, W. C. (1989). *Pharmacognosy* (11th ed.). Bailliere Tindall.

Umoh, U. F., Ajibesin, K. K., & Ubak, N. G. (2016). Preliminary anti-inflammatory and analgesic effects of *Diodia sarmentosa* Sw. leaf in rodents. *World Journal of Pharmacy and Pharmaceutical Sciences*, 5(12), 203–212.

Waseem Hassan, Noreen, H., Rehman, S., Gul, S., Kamal, M. A., Kamdem, J. P., Zaman, B., & da Rocha, J. B. T. (2017). Oxidative stress and antioxidant potential of one hundred medicinal plants. *Current Topics in Medicinal Chemistry*, 17(12). https://doi.org/10.2174/156802661766617 0102125648

Yadav, R. N. S., & Agarwala, M. (2011). Phytochemical analysis of some medicinal plants. *Journal of Phytology*, 3(12), 10–14.

Yamauchi, M., Kitamura, Y., Nagano, H., Kawatsu, J. & Gotoh, H. (2024). DPPH measurements and structure–activity relationship studies on the antioxidant capacity of phenols. *Antioxidants (Basel)*, 13(3), 309. https://doi.org/10.3390/antiox13030309

Yeo, J., & Shahidi, F. (2019). Critical re-evaluation of DPPH assay: Presence of pigments affects the results. *Journal of Agricultural and Food Chemistry*, 67(26), 7526–7529. https://doi.org/10.1021/acs.jafc.9b02462