

Original Research Article

Phytochemical Profiling and In Vitro Antioxidant Activity of Methanolic Extracts from *Fadogiacienkowski* Leaves

ABSTRACT

This study assessed the phytochemicals in the methanolic extract of *Fadogiacienkowski* leaves and ascertained their antioxidant qualities *in vitro*, taking into account the plant's extensive usage in traditional medicine. Standard procedures were followed in the qualitative and quantitative phytochemical examination as well as the *in vitro* antioxidant activities of the extracts. The methanolic extracts of *F. cienkowski* leaves produced a yield of 4%. The main phytochemical components found in the plant were phenolics (tannins and flavonoids), saponins, protein, and carbohydrates. The amount of total phenols in the dried plant extract was $2.085 \pm 0.35 \mu\text{g}$ garlic acid equivalent (GAE)/mg, tannins in the extract was $0.755 \pm 0.17 \mu\text{g}$ GAE/mg, flavonoid contents in the extract was $3.646 \pm 0.45 \mu\text{g}$ GAE/mg, and flavonols in the extract was $0.868 \pm 0.20 \mu\text{g}$ quercetin equivalent/mg. At a dosage of $10,000 \mu\text{g/ml}$, the extract demonstrated a noteworthy dose-dependent ability to scavenge DPPH radicals, effectively blocking $96.56 \pm 0.46 \%$ of DPPH, in contrast to ascorbic acid, which inhibited $89.10 \pm 3.73 \%$ at the same concentration. At a concentration of $2500 \mu\text{g/ml}$ of extract, the extract demonstrated the highest level of superoxide anion (O_2^-) inhibitory action, at $71.91 \pm 0.66\%$ of the radical, in comparison to quercetin, which exhibited $68.23 \pm 0.41\%$ at $250 \mu\text{g/ml}$. The extract's ability to scavenge Nitric Oxide (NO) was concentration-dependent, with $250 \mu\text{g/ml}$ of it being more effective than α -tocopherol. These results consequently imply that the leaf extract of *F. cienkowski* likely mediates its medicinal potentials through the efficient scavenging of free radicals. Therefore, the plant can be used to create safe and natural antioxidant compounds because it has the potential to scavenge free radicals and contains sufficient concentrations of phytochemicals.

KEYWORDS: *Fadogiacienkowski*; Oxidative Stress, Antioxidant, Phytochemical

1. INTRODUCTION

Every plant that has ingredients that can be applied medicinally is considered a medicinal plant, according to the WHO (World Health Organization). Many plant parts, such as flowers, roots, leaves, fruits, rhizomes, stems, barks, etc., have chemical components that are medically active because they are used in the management or treatment of a disease state [1]. Due to their shown and successful therapeutic qualities, medicinal plants have grown in significance in the global healthcare system. Herbalists have long been captivated by the study of medicinal plants used in traditional medicine [2]. According to Nirmala et al. [3] and Odeghe et al. [4], they are the most abundant source of medications for traditional and modern medicine, food supplements, nutraceuticals, pharmaceutical intermediates, and

synthetic drugs [3,4]. Because herbal products have been demonstrated to be safe, affordable, easily accessible, and ecologically friendly, almost 80% of the world's population utilizes medications that contain components derived from herbs [5-8].

Phytochemicals found in medicinal plants, such as ascorbic acid, anthocyanins, carotenoids, phenolics, and flavonoids, diminish the risk of diabetes, hypertension, heart disease, and several types of cancer [9, 10]. The type and color of food affects how phytochemicals work. According to Kasote et al. [11], they could reduce oxidative stress by serving as antioxidants or nutrient protectors. According to Lee et al. [12], Odeghe et al. [13], and Odeghe et al. [14], oxidative stress is a condition of imbalance in which the production of reactive oxygen species exceeds the antioxidant capacity of cells. This is a key concern in the field of food and nutrition sciences.

Free radicals are very reactive chemical entities, particularly superoxide ions ($O_2^{\cdot-}$) and hydroxyl radicals ($\cdot OH$), which combine with crucial biological components such as proteins, phospholipids, and nucleic acids to inflict oxidative damage on healthy human cells [15]. According to Odeghe et al. [16] and Martemucci et al. [17], oxidative stress is key for cellular damage and the emergence of numerous illnesses. Natural antioxidants have been linked to a lower risk of a number of illnesses, including diabetes, cancer, and neurological diseases [18]. Numerous investigations have verified that natural antioxidants present in plant extracts can prevent the deleterious effects of free radicals when subjected to *in vitro* conditions [19-21].

Only 5–10% of the 250,000 species of higher plants have been phytochemically studied, and only a small portion of them have undergone biological or pharmacological screening [22, 23]. With the rising acceptance of herbal medicine, there is a growing demand for comprehensive information regarding the effectiveness and potential toxicity of different plant preparations traditionally employed in disease treatment [24]. This is a major concern of

scientists studying herbal treatments because the chemical composition of the plants contributing to their biological effects is mostly unknown [25].

"*Fadogiacenkowskii*, a member of the Rubiaceae family, is locally known as "Ogwu-agu" in Igbo and "Ufuewureje" in the Igede tribe of Nigeria's Benue State. The leaves were widely recognized for their broad therapeutic efficacy in treating a variety of conditions, including typhoid fever, malaria, diarrhea, general body debility, inflammation, and headaches, particularly in young children"[26, 27]. Leaf extract of *F.cienkowskii* is widely used in traditional medicine, hence it is critical to analyse its phytochemical content and the need provide to data on its *in vitro* antioxidant properties cannot be overemphasized. As a result, this study assessed the phytochemicals in the methanolic extract of leaves from *F.cienkowskii* and identified its *in vitro* antioxidant characteristics.

2.0 MATERIALS AND METHODS

2.1 Chemicals

All the chemicals used in this study were of analytical grade. Methanol was procured from EMD Biosciences (Gibbstown, NJ). L-ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, sulphuric acid, sodium nitroprusside (SNP), sodium nitrite, sulpanilamide, phosphoric acid, naphthylethylenediaminedihydrochloride, potassium dihydrogen phosphate (KH_2PO_4), potassium hydroxide (KOH), acetic acid, gallic acid, rutin, ferric chloride (FeCl_3^{3+}), ethylenediaminetetraacetic acid (EDTA), phosphate buffered saline (PBS), sodium carbonate (Na_2CO_3), perchloric acid (HClO_4), butyrate hydroxyltoluene (BHT), polyvinylpyrrolidone, riboflavin, ferrous sulphate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), hydrogen peroxide (H_2O_2), thiobarbituric acid (TBA), Folin-Ciocalteu's reagent (FCR) and trichloroacetic acid (TCA) were all purchased from Sigma Chemical Co. (St. Louis, MO).

2.2.1 Preparation of extracts

“The leaves of the plant were identified at the University of Nigeria, Nsukka Herbarium, air-dried at room temperature, and ground into a fine powder using a mill. Using 80% methanol, the powdered plant components were extracted **given the fact that methanol is a good solvent for extraction of plant materials**. Using a Büchi Rotavapor R-200 rotary evaporator, the hydromethanolic extracts were concentrated and then kept at 4°C until needed” [28].

2.2.2 Rapid DPPH radical scavenging assay using dot-blot

The method of Soler-Rivas et al. [28] was slightly modified to perform a qualitative screening of the plant extract for anti-oxidant activity (representative of the phenolic content) using DPPH radical. In summary, a portion of thin layer chromatography (TLC) plates (silica gel 60 F254, Merck) were meticulously loaded with an aliquot (5 μL) of each plant extract dilution and standard antioxidant, and left to air dry. After that, the sheets were sprayed with DPPH (0.2% (w/v)) in methanol to demonstrate the extract's antioxidant properties. The extract's phenolic content and scavenging capability were indicated by the intensity of the yellow color and the rate at which the extract spots' color changed from purple to yellow [29].

2.2.4 Qualitative phytochemical analysis

Standard laboratory processes and procedures were used to test for carbohydrates, glycosides, protein, flavonoids, resin, saponins, alkaloids and tannins [30,31].

2.2.5 Quantitative phytochemical analysis

Using the Folin-Ciocalteu reagent (FCR) according to instructions by Odeghe et al. [13], total phenolics were calculated. Insoluble polyvinyl-polyrrolidone (PVPP), which binds tannins according to Makkar et al.'s [32] description, was used to measure the amount of tannin. The method outlined by Odeghe et al. [13] was used to determine the flavonoid content.

2.2.6 *In vitro* Anti-oxidant Assays

2.2.6.1 DPPH radical-scavenging assay

With a few minor adjustments, the extract's scavenging activity against DPPH free radicals was evaluated using the methodology described by Odeghe et al. [13]. In summary, 1.0 ml of 0.3 mM DPPH in methanol was combined with a 2.0 ml solution of the extract diluted two times (2–250 µg/mL) in methanol. After giving the mixture a good shake, it was left to stand at room temperature in the dark for twenty-five minutes. The negative control consisted of 1.0 mL of 0.3 mM DPPH solution plus 2.0 ml of methanol, whereas blank solutions were made using 2.0 mL of each test sample solution and 1.0 mL of methanol. L-ascorbic acid was employed as the positive control. Thereafter, the absorbance of the assay mixture was measured at 518 nm against each blank with an Agilent 8453E UV-visible spectrophotometer. Lower absorbance of the reaction mixture indicated higher radical scavenging activity. DPPH radical scavenging activity was calculated using the equation:

$$\% \text{ Inhibition} = 100 \% \times \left(\frac{A_0 - A_s}{A_0} \right) \text{ ----- Equation 1}$$

where A_0 is the absorbance of the control, and A_s is the absorbance of the tested sample. The IC_{50} value represented the concentration of the extract that caused 50 % inhibition of DPPH radical and was calculated by linear regression of plots, where the abscissa represented the concentration of tested sample and the ordinate the average percent of inhibitory activity from three replicates.

2.2.6.2 Superoxide radical ($O_2^{\cdot-}$)-scavenging assay

This assay was based on the capacity of the extract to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) [33]. Briefly, each 3.0 ml reaction mixture contained 0.05 M phosphate buffered saline (PBS) (pH 7.8), 13 mM methionine, 2 μ M riboflavin, 100 μ M EDTA, NBT (75 μ M) and 1.0 mL of test sample solutions (10–250 μ g/mL). The tubes were kept in front of a fluorescent light (725 lumens, 34 watts) and absorbance was read at 560 nm after 20 min. A box covered in aluminum foil encased the entire reaction assembly. As blanks, identical tubes with reaction mixtures were stored in the dark. By comparing the absorbance of the reaction mixture containing the test sample with that of the control, the percentage inhibition of superoxide formation was calculated using the following equation:

$$\% \text{ Inhibition} = 100 \% \times \left(\frac{A_0 - A_s}{A_0} \right) \text{----- Equation 2}$$

where A_0 is the absorbance of the control, and A_s is the absorbance of the tested sample.

2.2.6.3 Nitric oxide radical (NO^{\cdot}) scavenging assay

Sodium nitroprusside (SNP) produced nitric oxide (NO), which was quantified using the Marocci et al.'s [34] technique. In short, a visible polychromatic light source (a 25 W tungsten lamp) was used to illuminate the reaction mixture (5.0 ml) containing SNP (5 mM) in phosphate buffered saline (pH 7.3), either with or without the plant extract at varying concentrations, for 180 minutes at 25 °C. At 30-minute intervals, the nitrite ion (NO_2^-) produced by the reaction of the NO radical with oxygen was measured by combining 1.0 milliliter of the incubation mixture with the same volume of Griess reagent (1% sulphanilamide in 5% phosphoric acid and 0.1% naphthylethylenediaminedihydrochloride). At 546 nm, the absorbance of the purple azo dye chromophore was measured. This dye was created when nitrite ions were diazotized with sulphanilamide and then coupled with

naphthylethylenediaminedihydrochloride. A standard curve based on sodium nitrite solutions with known concentrations was used to quantify the amount of nitrite produced in the presence or absence of the plant extract. Every experiment was run through at least three times, and the results were shown as the mean of three separate analyses.

2.3 Statistical Analysis

The means \pm standard deviation (SD) pattern was used in presenting data. Differences between the means and the significant levels of the data were calculated by T- Test and analysis of variance according to Steels and Torrie [35].

3.0 RESULTS

3.1 Extract Yield

The yield of the methanolic extracts of *Fadiogiacienkowski* leaf was 4% as shown in Table 1

Table 1: Percent (w/w) extraction yield of investigated extract

Plant species	Extraction yield (%)
<i>Fadiogiacienkowski</i>	4.0%

3.2 Dot Blot for DPPH Radical Scavenging Capacity

As shown in Fig. 1, the plant extracts investigated significantly scavenged DPPH radical by changing the extract spots from purple to yellow. The increased rate of spot colour change to yellow and intensity of the yellow spot indicates higher anti-oxidant activity.

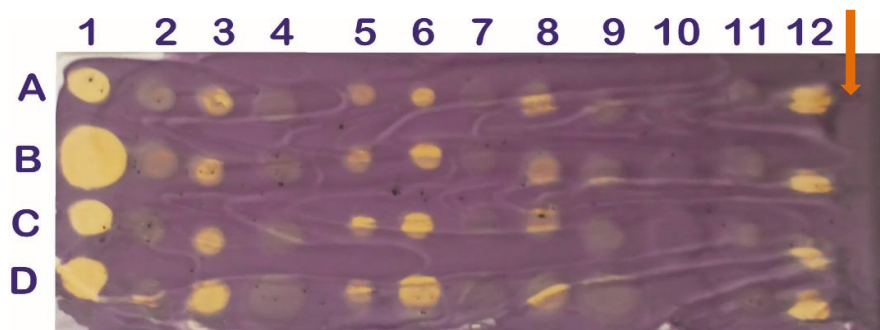


Figure 1: Dot blot for DPPH radical scavenging capacity of extract. From top to bottom, the dots are; A12, B12, C12 and D12.

3.3 Qualitative Analysis on Phytochemical Constituents

Qualitative analysis carried out *Fadiogiacienkowskii* methanolic leaf extract showed the presence of important phytochemical constituents as summarized in Table 2. Phenolics (tannins and flavonoids), Saponins, protein and carbohydrates were the major phytochemical constituents present in the plant in relatively high amounts.

Table 2: Phytochemical constituents in *Fadiogiacienkowskii* methanolic leaf extract

Phytochemical constituents	Relative amount
Flavonoids	+
Tannins	+++
Alkaloids	+
Steroids	+
Glycoside	-
Saponins	++
Resin	-
Protein	++
Carbohydrates	++

KEY

Relative amount: +++ = High; ++ = Medium; + = Low; - = Not present

3.4 Quantitative Analysis on Phytochemical Constituents

Phenolic compounds were a major class of bioactive components in the extract. The amount of total phenols was $2.085 \pm 0.35 \mu\text{g}$ garlic acid equivalent (GAE)/mg of dry plant extract, tannins $0.755 \pm 0.17 \mu\text{g}$ garlic acid equivalent (GAE)/mg of dry plant extract, flavonoid contents $3.646 \pm 0.45 \mu\text{g}$ garlic acid equivalent (GAE)/mg of dry plant extract and flavonols $0.868 \pm 0.20 \mu\text{g}$ quercetin equivalent /mg of dry plant extract (Table 3)

Table 3: Quantitative phytochemical constituents of *Fadiogiencenkowskii* methanolic leaf extract

Extract	Phenolic contents *			Total flavonols [‡]	Total flavonoids [‡]
	Total Phenols	Non-tannins	Tannins		
<i>Fadiogiencenkowskii</i> (leaves)	2.085 ± 0.35	0.338 ± 0.097	0.755 ± 0.17	0.868 ± 0.20	3.646 ± 0.45

Data represented as Mean \pm SD (n = 3)

* Expressed as mg garlic acid equivalents (GAE) / mg dry weight plant extract

‡ Expressed as mg quercetin equivalents (QE) /mg dry weight plant extract

3.5 Free Radical Scavenging Activity

3.5.1 Effect of *Fadiogiencenkowskii* methanolic leaf extract on DPPH radicals

The extract showed significant dose-dependent DPPH radical scavenging capacity (Table 4). *F. cienkowskii* was very efficient, inhibiting $96.56 \pm 0.46 \%$ of DPPH at a concentration of $10000 \mu\text{g/ml}$ compared to ascorbic acid which inhibited $89.10 \pm 3.73 \%$ at the same concentration.

Table4: DPPH radical scavenging activity of *F.cienkowskii* methanolic leaf extract

Extract Concentration ($\mu\text{g/mL}$)	Inhibition (%)	SD	Ascorbate Concentration ($\mu\text{g/mL}$)	Inhibition (%)	SD
10000	96.56 \pm 0.46	0.46	1000	89.10 \pm 3.73	3.73
5000	94.97 \pm 1.10	1.10	500	88.88 \pm 2.66	2.67
2500	85.07 \pm 6.74	6.74	250	84.68 \pm 10.4	10.43
1250	75.84 \pm 1.72	1.72	125	80.74 \pm 4.14	4.14
625	69.95 \pm 6.62	6.62	62.5	65.56 \pm 10.93	10.93
312.5	64.17 \pm 9.22	9.22	31.25	59.76 \pm 8.57	8.57
156.25	52.25 \pm 9.10	9.10	15.625	53.26 \pm 10.6	10.65
78.125	49.66 \pm 9.35	9.35	7.8125	42.65 \pm 1.92	1.92
39.0625	37.26 \pm 0.15	0.15	3.90625	38.66 \pm 1.53	1.54
19.53125	11.08 \pm 4.94	4.94	1.953125	35.41 \pm 4.30	4.31
IC ₅₀	151.91		IC ₅₀	11.41	

Data represented as mean \pm SEM (n = 3)

3.5.2 Effect of *Fadogiacienkowskii* methanolic leaf extract on superoxide ($\text{O}_2^{\cdot-}$) anion radical

The methanolic extract of *F.cienkowskii* inhibited the formation of reduced NBT in a dose-related manner. As shown in Table 5, *F.cienkowskii* showed maximal superoxide anion ($\text{O}_2^{\cdot-}$) inhibitory activity of 71.91 \pm 0.66 % of the radical at the concentration of 2500 $\mu\text{g/ml}$ of extract, compared to Quercetin (68.23 \pm 0.41%, at 250 $\mu\text{g/ml}$). The $\text{O}_2^{\cdot-}$ scavenging effect of the extract could culminate in the prevention of OH radical formation since $\text{O}_2^{\cdot-}$ and H_2O_2 are required for OH radical generation.

Table 5: Superoxide anion radical scavenging activity of *F. cienkowskii*

Extract Concentration (µg/mL)	% Inhibition	SD	Quercetin Concentration (µg/mL)	% Inhibition	SD
5000	71.53 ±	1.001	500	68.25 ± 4.78	4.78
2500	71.91±0.66	0.661	250	68.23 ± 0.41	0.41
1250	71.44±0.68	0.676	125	66.40 ± 4.42	4.42
625	60.61±7.23	7.228	62.5	60.31 ± 1.21	1.21
312.5	56.94±4.44	4.442	31.25	55.04 ± 4.33	4.33
156.25	47.45±3.49	3.488	15.625	52.89 ± 1.71	1.71
78.125	44.54±3.80	3.802	7.8125	47.03 ± 0.04	0.04
39.0625	34.05±5.01	5.011	3.90625	39.37 ± 2.90	2.90
IC50	380.19		IC50	17.018	

Data represented as mean ± SEM (n = 3)

3.5.3 Effect of *F. cienkowskii* methanolic leaf extract on nitric oxide (NO[•]) radical production

Nitric oxide (NO[•]) released from sodium nitroprusside (SNP) has a strong NO⁺ character which can alter the structure and function of many cellular components. This study showed that the phenol rich extracts in SNP solution decreased levels of nitrite, a stable oxidation product of NO[•] liberated from SNP (Fig. 2). The extracts exhibited strong NO[•] radical scavenging activity leading to the reduction of the nitrite concentration in the assay medium, a possible protective effect against oxidative damage. The NO[•] scavenging capacity was concentration dependent with 250 µg/ml of the extract scavenging most efficiently compared to α-tocopherol (Fig. 2)

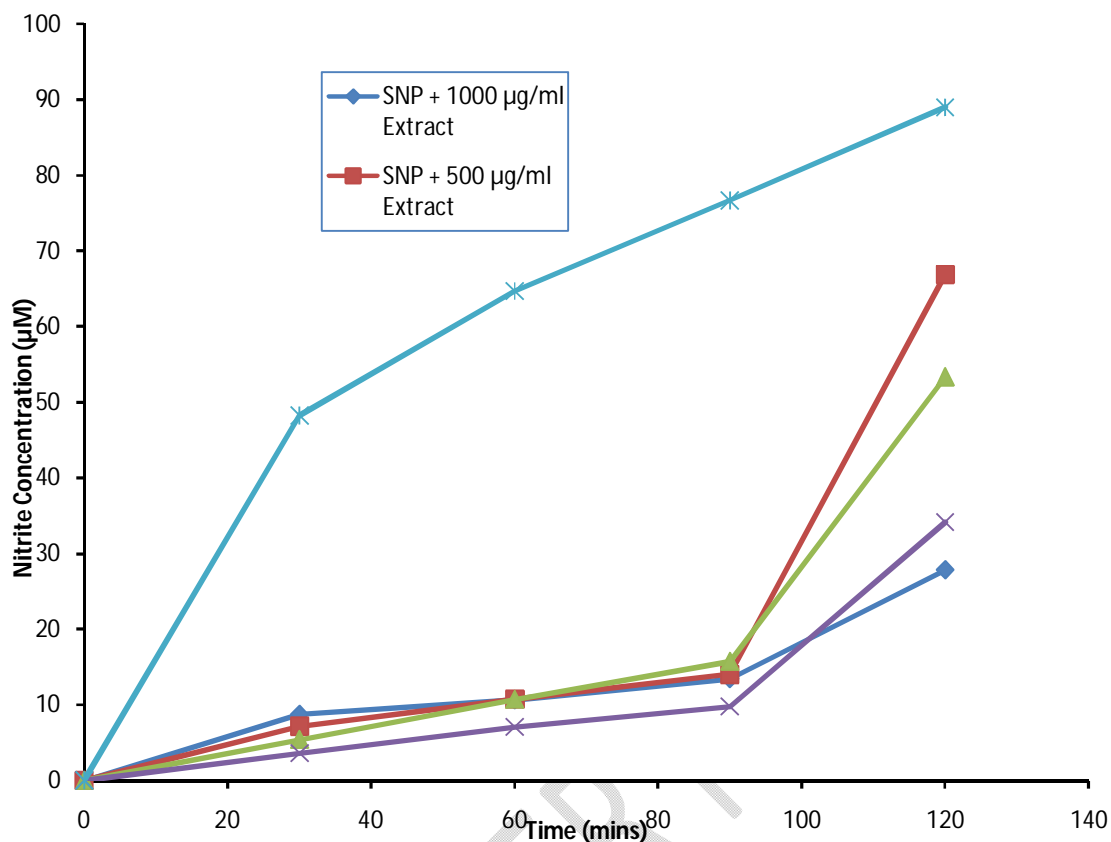


Figure 2: Effect of methanol extract of *F. cienkowskii* leaves on the accumulation of nitric oxide (NO[•]) radical upon decomposition of sodium nitroprusside (SNP) at room temperature.

3.6 IC₅₀ for free radical inhibition

The concentration of the extracts that inhibited 50 % of the free radicals and Superoxide anion radicals (IC₅₀) was used to determine the potency of the extracts. The lower the IC₅₀ value the better the drug (extract) potency. As shown in Table 4 below, the plant extracts were efficient inhibitors of different free radicals compared to standard anti-oxidants. The IC₅₀ value for DPPH radical inhibition was 151.91µg/ml; while O²⁻ anion inhibition was 380.19µg/mL (Table 6).

Table 6: Free radical inhibitory potency (IC₅₀)

Inhibitor	IC ₅₀ value for inhibitory potential (µg/ml)	
	DPPH radical	Superoxide anion (O ₂ ⁻)
Extract	151.91	380.19
Standard anti-oxidant	11.41 *	17.018 #

Data represented as mean ± SEM (n = 3). KEY: * compared to ascorbic acid; # compared to α-Tocopherol

4.0 DISCUSSION

Given its widespread use in traditional medicine, this study evaluated the phytochemicals present in *F.cienkowskii* leaf methanolic extract and determined its antioxidant properties *in vitro*.

An examination of the methanol-pulverized leaf of *F.cienkowskii* using qualitative phytochemical analysis revealed that the plant contains significant secondary metabolites in relatively high concentrations, including tannins, flavonoids, alkaloids, saponins, steroids, protein, and carbohydrates. This outcome conflicts with the studies of Bruce et al. [26] and Chukwube et al. [36]. Additionally, the methanolic leaf extract of *F. cienkowskii*'s quantitative phytochemical test showed that phenolic compounds constituted a significant class of the extract's bioactive ingredients. $3.646 \pm 0.45\mu\text{g}$ of flavonoid content was found in dried plant extract, but the total amount of phenols was $2.085 \pm 0.35\mu\text{g}$ of garlic acid equivalent (GAE)/mg of the extract.

These values, however, are lower than those Chukwube et al. [36] found for the ethanolic leaf extract of *F. cienkowskii*. One possible explanation for the discrepancy could be the solvents' differing polarities. Phytochemicals that make up plant leaves have a bearing on their therapeutic qualities [37]. "Secondary metabolites found in plants, phenols have a variety of therapeutic applications, including the reduction of cardiovascular problems and the actions of antioxidants, mutagenics, carcinogens, and free radical scavengers"[38]. certain

phytochemicals lower the risk of certain diseases and exhibit antioxidant action. Because of their phenolic hydroxyl group, flavonoids are shown to be potent antioxidants that may effectively scavenge reactive oxygen species [39-40]. It is well known that the majority of secondary metabolites in plants are called alkaloids. They can be utilized as analgesics because they are said to have potent effects on both people and animals [41]. Additionally, the extract possesses anti-malaria qualities, which accounts for its historic use in the treatment of malaria. Tannins have anticancer properties and lower the risk of coronary heart disease [42]. There has been talk of saponins as potential anti-carcinogens [43]. “Flavonoids and phenols are notable natural antioxidants. Steroids are used to treat congestive heart failure and have been shown in clinical trials to have anti-inflammatory and analgesic properties” [44].

Free radicals are chemical molecules implicated in the normal physiology of living things, despite being the cause of oxidative stress-related diseases, aging, and tissue damage. When the production of reactive oxygen species (ROS) exceeds the ability of a biological system to remove them, oxidative stress results. Numerous illnesses, including diabetes, heart disease, cancer, and malaria, are influenced by oxidative stress. Such oxidative stress-mediated disorders can be effectively treated using the natural antioxidants present in medicinal plants. In this study, DPPH, superoxide anion, and nitric oxide radicals were used to examine the antioxidant properties of the methanolic extracts of *F. cienkowskii*. The plant extract dramatically reduced the amount of DPPH radical by turning the purple extract spots into yellow ones, as seen in Fig. 1. High antioxidant activity was indicated by the spot's intensity. At a dosage of 2500µg/ml, the plant effectively scavenged DPPH, inhibiting 96.55 ± 0.46 % of the enzyme, while ascorbic acid inhibited 89.10 ± 3.73 % of the enzyme at the same dose. Similarly, the plant's ability to scavenge nitric oxide and superoxide anion was demonstrated by its ability to suppress the generation of reduced NBT and sodium nitroprusside (SNP) in a

concentration-dependent and dose-related manner, respectively (Table 5 and table 2). Chukwube et al. [36] obtained results that were similar.

Numerous processes, including hydrogen donation, stopping a chain reaction mediated by free radicals, preventing hydrogen abstraction, chelating catalytic ions, and getting rid of peroxides and super oxides, have all been suggested to be involved in the antioxidant activity [45]. The observed scavenging effect can be attributed to the presence of active phytoconstituents as the leaf extract contained a high level of phenolic and flavonoid content that may have resulted in the strong antioxidant activity observed against the free radicals [46]. The observed antioxidant of extracts is due to either transfer of hydrogen atoms or by transfer of electrons, which results in the neutralization of free radicals (DPPH). According to a study by Zulfiqar et al. [47], flavonoids and catechins are two examples of phenolic components that have the capacity to efficiently scavenge superoxide anion radicals. This result is in line with other research showing that, depending on the amount and location of hydroxyl groups in the relevant active component, plants high in phenolic compounds, such as quercetin, can scavenge superoxide anion radicals more successfully [48].

5.0 CONCLUSION

These results consequently imply that the therapeutic potentials of *F. cienkowskii* leaf extract are likely mediated by the extract's ability to scavenge free radicals. Therefore, the plant can be used to create natural and safe antioxidant compounds because it has the potential to scavenge free radicals and contains sufficient concentrations of phytochemicals. To isolate and identify the active components in the extracts that may be used pharmacologically, more investigation is required.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

REFERENCES

1. Helmstädter A, Staiger C. Traditional use of medicinal agents: a valid source of evidence. *Drug Discov Today*. 2014; 19(1):4-7.
2. Efekemo O, Orororo OC. Effect of mixture of leaf extract of *Ocimum gratissimum* and *Vernonia amygdalina* on cadmium induced hepatotoxicity in wistar rats. *JBiochem Int*. 2022; 9(4):73-81.
3. Nirmala MJ, Samundeeswari A, Sankar PD. Natural plant resources in anti- cancer therapy-A review. *Res. Plant Biol*. 2011; 1: 1-14.
4. Odeghe OB, Orororo CO, Egbune EO, Onobrudu DA, Nwiloh BI, Bassey UE. Phytochemical analysis, antioxidant capability, and inhibition studies of the aqueous extract of *Phyllanthus discoideus* root. *Biomed Biotechnol Res J*. 2023;7:411-9.
5. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol Ethnopharmacol*. 2014; 4(177):177.
6. Chen SL, Yu H, Luo HM, Wu Q, Li CF, Steinmetz A. Conservation and sustainable use of medicinal plants: problems, progress, and prospects. *Chin Med*. 2016; 11:31-38.
7. Izzo AA, Hoon-Kim, Radhakrishnan R, Williamson EM. A critical approach to evaluating clinical efficacy, adverse events and drug interactions of herbal remedies. *Phytother Res*. 2016; 30(5):691-700.
8. Ahn K. The worldwide trend of using botanical drugs and strategies for developing global drugs. *BMB Reports*. 2017; 50(3):111–116.
9. Njoya EM. Medicinal plants, antioxidant potential, and cancer. In: *Cancer*. Academic Press; 2021. p. 349–57.
10. Orororo OC, Asagba SO, Tonukari NJ, Okandeji OJ, Mbanugo JJ. Comparative Assessment of the Antioxidant Properties of *Hibiscus sabdariffa* L Anthocyanins and its Aqueous Extract in Cadmium-exposed Rats. *Res J Pharma Biol Chem Sci*. 2018.9(3): 836-843
11. Kasote DM, Katyare SS, Hegde MV, Bae H. Significance of Antioxidant Potential of Plants and its Relevance to Therapeutic Applications. *Int J Biol Sci*. 2015; 11(8):982-991.
12. Lee H, Choue R, Lim H. Effect of soy isoflavones supplement on climacteric symptoms, bone biomarkers, and quality of life in Korean postmenopausal women: a randomized clinical trial. *Nutr Res Pract*. 2017; 11:223-231.
13. Odeghe OB, Monanu MO, Anacletus FC. In vitro antioxidant abilities and inhibition of rabbit muscle lactate dehydrogenase by aqueous extract of *Hannoaklaineana* stem bark. *Int J Adv Res* 2016;4:509–15.
14. Odeghe OB, Adikwu E, Ojiego CC. Phytochemical and antioxidant assessments of *Dioscorea bulbifera* stem tuber. *Biomed Biotechnol Res J* 2020;4:305-11.
15. Ekakitie LI, Orororo OC, Okpoghono J. Changes in haematological parameters of aluminium-exposed rats treated with natural bee honey. *IOSR J Environ Sci Toxicol Food Technol*; 2021; 15(5):22-25

16. Odeghe OB, Opajobi AO, Ajala MO, Orororo CO (2023). Enzyme inhibitory properties, free radical scavenging activity and phytochemical profile of *Vitex simplicifolia* leaf. Eur Chem Bull. 2023,12 (8):8666-8682
17. Martemucci G, Costagliola C, Mariano M, D'Andrea L, Napolitano P, D'Alessandro AG. Free radical properties, source and targets, antioxidant consumption and health. Oxygen 2022;2:48-78.
18. Orororo OC, Asagba SO. Treatment with *Hibiscus sabdarrifa* L Anthocyanins Improve Hematological Parameters in Rats Exposed to Cadmium. J Expl Res Pharmaco. 2022; 3:1-10
19. Izzo S, Naponelli V, Bettuzzi S. Flavonoids as Epigenetic Modulators for Prostate Cancer Prevention. Nutrients 2020, 12, 1010.
20. Carrera I, Martínez O, Cacabelos R. Neuroprotection with Natural Antioxidants and Nutraceuticals in the Context of Brain Cell Degeneration: The Epigenetic Connection. Curr. Top Med Chem. 2019, 19: 2999–3011.
21. Orororo OC, Asagba SO, Tonukari NJ, Okandeji OJ, Mbanugo JJ. *Hibiscus sabdarrifa* L. Anthocyanins-Induced Changes in Reproductive Hormones of Cadmium-Exposed Rats. Intl J Sci Res Pub. 2018;12(4):308-311.
22. Ajayi OA. Phytochemical and GC-MS analysis of bioactive components in ethanolic extract of *Rauvolfia vomitoria* Leaves. J Chem Soc Nig. 2021; 46 (4): 0656 – 0660
23. Onobrudu DA, Nzebude CP, Orororo OC. Comparative analysis of the Phytochemicals, Minerals, Antioxidant and Antimicrobial activities of Aqueous and Ethanolic extracts of *Rauvolfia vomitoria* leaves. Asian Plant Res J. 2024;12(5):67-81.
24. Giannenas I, Sidiropoulou E, Bonos E, Christaki E, Florou-Paneri P. The history of herbs, medicinal and aromatic plants, and their extracts: Past, current situation and future perspectives. In: Feed Additives. Academic Press; 2020. p. 1-18.
25. Ali M, Afzal S, Zia A, Hassan A, Khalil AT, Ovais M, Idrees MA. Systematic review of treatment response rates in Pakistani hepatitis C virus patients; current prospects and future challenges. Med. 2016; 95(50):e5327.
26. Bruce SO, Onyegbule FA, Ezugwu CO, Nweke ID, Ezenwelu CR, Nwafor FI. Chemical Composition, Hepatoprotective and Antioxidant Activity of the Crude Extract and Fractions of the Leaves of *Fadogia Cienkowskii* Schweinf (Rubiaceae). Trop J Nat Prod Res. 2021; 5(4):720-731
27. Emeline AV, Kuznetsov VN, Ryabchuk VK, Serpone N. On the way to the creation of next generation photoactive materials. ESPR. 2012; 19:3666-3675.
28. Soler-Rivas C, Espin J, Wichers H. An easy and fast test to compare total free Radical scavenger capacity of foodstuffs. Phytochemical Anal. 2000;11: 330-338.
29. Mosquera OM, Correa YM, Buitrago DC, Niño J. Antioxidant activity of twenty-five plants from Colombian biodiversity. Memórias do Instituto Oswaldo Cruz 2007;102:631-4.
30. Harborne JD (1973) Phytochemical methods: A guide to modern techniques of plant analysis. Chapman and Hall London pp: 279.
31. Trease, G.E. and Evans, W.C. (1989). Pharmacognosy. 11th Edition, Bailliere Tindall, London. 45 - 50
32. Makker MPS, Bluemmel M, Borrowy NK, Becker K. Gravimetric determination of tannin and their correlations with chemical and protein precipitation methods. Sci Agric. 1993; 61: 161-165
33. Beauchamp C, Fridovich I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Analytical Biochem. 1971; 44(1), 276-287.

34. Marcocci, L., Maguire, J. J., Droy-Lefaix, M. T., Packer, L. (2005). The nitric oxide Scavenging properties of *Ginkgo biloba* extract EGb761. *BiochemBiophys. Res. Commun* ;201:748-755.
35. Steel RG, Torrie JH (1981). Principles and Procedures of Statistics: A Biometrical Approach. 2nd Edn., McGraw Hill Book Co., New York.
36. Chukwube VO, Elejeje OO, EzugwuCO, Uchenna EO. Evaluation of In vitro Antioxidant and Qualitative Phytochemical Analysis of Methanol Leaf Extract of *Fadogiacienkowskii*Scheinf Fam. Rubiaceae. *Asian J Res Med Pharma Sci.* 2000; 10(3): 17-23, 2021
37. Orororo OC, Asagba SO, Oghri E,Egbune EO. Comparative Effect Of Garden Egg, Carrot And Oat On Biochemical Parameters In Cadmium Exposed Rats. *Afr JBiochem Res.*2017; 12(3): 28-34.
38. Busari AA,Obadiah CC, Orororo OC,Efejene IO, Aisuodionoe ME, Esthinsheen O, Samantha D, Collins IB, Abdullah AA, Kabir B. Assessment of Cardiovascular function in Periodontitis and Diabetes Mellitus induced Rats Treated with Black Seed (*Nigella sativa*) Oil. *Asian J Cardio Res.* 2024, *in press.*
39. Orororo OC, Efekemo O, Egbune EO, Awhin EP, Odeghe OB, Okoro EO. Amelioration of Cadmium toxicity in the liver and kidney of Wistar rats by combined *Citrus sinensis* and *Manihot esculenta* Leaf extract. *Int J Biochem Res Rev.* 2024; 23 (6): 71-79
40. Orororo OC, Efekemo O, Odeghe OB, Clark PD, Awhin EP, Egbune EO, Efejene IO. Phytochemical Screening, in vitro antioxidant capacity and Nephro-protective effects of combined extract of *Psidium guajava* and *Carica papaya* leaves in rats intoxicated with cadmium. *Asian JMed Health;* 2024; 22 (8): 86-97.
41. Idenyi OM, Orororo OC,Efejene, IO, AisuodionoeME, Uboho FU. Evaluation of Anti-inflammatory and Analgesic Effects of *Allophylusspicatus* Leaf Extract in Mice. *J Com Alt Med Res.* 2024; 9:59-68.
42. Onyegbule FA, Bruce SO, Onyekwe ON, Onyealisi OL, Okoye PC. Evaluation of the in vivo antiplasmodial activity of ethanol leaf extract and fractions of *Jatropha gossypifolia* in *Plasmodium berghei* infected mice. *J Med Plants Res.* 2019;13(11):269-279.
43. Ashour AS, El Aziz MMA, Melad ASG. A review on saponins from medicinal plants: chemistry, isolation, and determination. *J Nanomed Res.* 2019; 8(1): 6–12.
44. Saidu AN, Mann A, Onuegbe CD. Phytochemical screening and hypoglycemic effect of aqueous *Blighiasapida* root bark extract on normoglycemic albino rats. *Bri J Pharm Res.* 2012; 2(2):89-97.
45. Ekakitie LI, Okpoghono J, Orororo OC,Ekakitie OA.Ameliorative prowess of bee honey in the tissues of rats administered aluminium nitrate. *Scientific Afri;* 2021;12 (2021) e00782:1-9
46. Onyegbule FA, Ezenwa CJ, Bruce SO, Umeokoli BO. Standardization, chemical composition and antipyretic evaluation of methanol leaf extract and fractions of *Chrysophyllumalbidum* (Sapotaceae). *Trop J Natural Product Res.* 2020; 4(6): 216-222.
47. Zulfiqar F, Casadesús A, Brockman H, Munné-Bosch S. An overview of plant-based natural biostimulants for sustainable horticulture with a particular focus on *Moringa* leaf extracts. *Plant Sci.* 2020;295:110194.
48. Cizmarova B, Hubkova B, Birkova A. Quercetin as an effective antioxidant against superoxide radical. *Funct Food Sci.* 2023;3:15–25.