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Phytochemical Profiling and *In vitro* Antioxidant Activity of Methanolic Extracts from *Fadogia cienkowskii* Leaves

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study assessed the phytochemicals in the methanolic extract of *Fadogia cienkowskii* 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. cienkowskii* leaves produced a yield of 4%. The main phytochemical components found in the plant were phenolics (tannins and flavonoids), saponins, protein, and

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carbohydrates. The amount of total phenols in the dried plant extract was 2.085± 0.35 µg garlic acid equivalent (GAE)/mg, tannins in the extract was 0.755±0.17 µg GAE/mg, flavonoid contents in the extract was 3.646± 0.45 µg GAE/mg, and flavonols in the extract was 0.868± 0.20µg quercetin equivalent/mg. At a dosage of 10,000µg/ml, the extract demonstrated a noteworthy dose-dependent ability to scavenge DPPH radicals, effectively blocking 96.56 ± 0.46 % of DPPH, in contrast to ascorbic acid, which inhibited 89.10 ± 3.73 % at the same concentration. At a concentration of 2500µg/ml of extract, the extract demonstrated the highest level of superoxide anion (O_2) inhibitory action, at 71.91±0.66% of the radical, in comparison to quercetin, which exhibited 68.23 ± 0.41% at 250µg/ml. The extract's ability to scavenge Nitric Oxide (NO) was concentration-dependent, with 250µg/ml of it being more effective than α-tocopherol. These results consequently imply that the leaf extract of *F. cienkowskii* 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: Fadogia cienkowskii; 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-}) and hydroxyl radicals (–OH), which combine with crucial biological components such 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].

"Fadogia cenkowskii, 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]. Various types of soil as well as environmental hazards do affects a plant's composition of phytochemical. 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 it's *in vitro* antioxidant characteristics.

2. 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, naphthylethylene diamine dihydrochloride, potassium dihydrogen phosphate (KH_2PO_4), potassium hydroxide (KOH), acetic acid, gallic acid, rutin, ferric chloride ($FeCl^{3+}$), ethylenediaminetetraacetic acid (EDTA), phosphate buffered saline (PBS), sodium carbonate (Na_2CO_3), perchloric acid ($HClO_4$), butyratedhydroxyltoluene (BHT), polyvinylpolypyrrolidone, riboflavin, ferrous sulphate ($FeSO_4.7H_2O$), 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).

2.1.1 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 4oC until needed" [28].

2.1.2 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].

2.1.3 Qualitative phytochemical analysis

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

2.1.4 Quantitative phytochemical analysis

Using the Folin-Ciocalteau reagent (FCR) according to instructions by Odeghe et al. [13], total phenolics were calculated. Insoluble polyvinyl-polypirrolidone (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.

2.1.5 *In vitro* anti-oxidant assays

2.1.5.1 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:

$$\%\ Inhibition\ = 100\ \% \times \left(\frac{A_0 - A_S}{A_0}\right)$$ ------ 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.

2.1.5.2 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 µM riboflavin, 100 µM EDTA, NBT (75 µM) and 1.0 mL of test sample solutions (10–250 µg/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:

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

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

2.1.5.3 Nitric oxide radical ($NO\cdot$) scavenging assay

Sodium nitroprusside (SNP) produced nitric oxide (NO), which was quantified using the Marcocci 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 ^{o}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% naphthyl ethylene diamine dihydrochloride). 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 naphthylethy lenediamin edihy drochloride. 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.

2.2 Statistical Analysis

The means ± 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. RESULTS

3.1 Extract Yield

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

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.

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

Plant species	Extraction yield (%)
Fadogia cienkowskii	4.0%

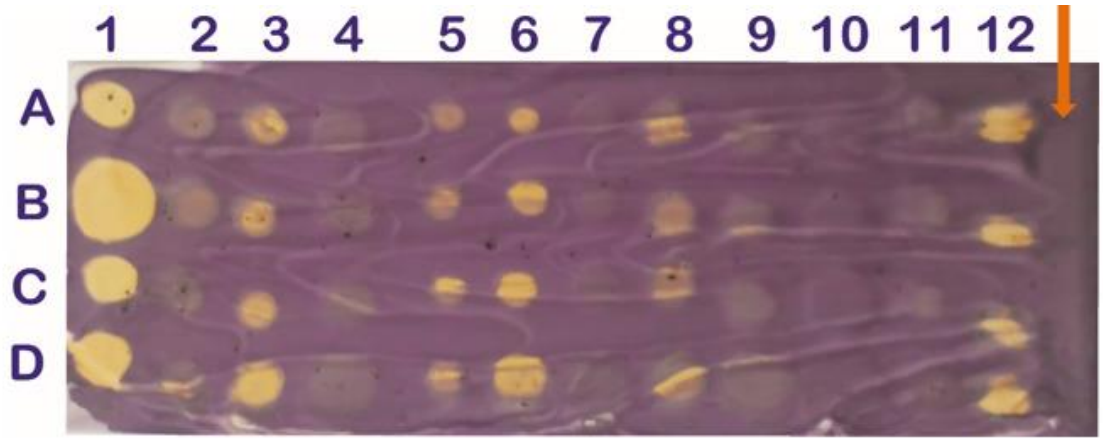

Fig. 1. Dot blot for DPPH radical scavenging capacity of extract. From top to bottom, the dots are; A12, B12, C12 and D12

3.3 Qualitative Analysis on Phytochemical Constituents

Qualitative analysis carried out *Fadiogia cienkowskii* 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.

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± 0.35µg garlic acid equivalent (GAE)/mg of dry plant extract, tannins 0.755±0.17µg garlic acid equivalent (GAE)/mg of dry plant extract, flavonoid contents 3.646 ± 0.45µg garlic acid equivalent (GAE)/mg of dry plant extract and flavonols 0.868 ± 0.20µgquercetin equivalent /mg of dry plant extract (Table 3).

3.5 Free Radical Scavenging Activity

3.5.1 Effect of *Fadogia cienkowskii* 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 ± 0.46 % of DPPH at a concentration of 10000µg/ml compared to ascorbic acid which inhibited 89.10 ± 3.73 % at the same concentration.

3.5.2 Effect of *Fadogia cienkowskii* methanolic leaf extract on superoxide ($O_2^{\cdot-}$) 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 ($O_2^{\cdot-}$) inhibitory activity of 71.91± 0.66 % of the radical at the concentration of 2500µg/ml of extract, compared to Quercetin (68.23 ± 0.41%, at 250µg/ml). The $O^{2\cdot-}$ scavenging effect of the extract could culminate in the prevention of OH radical formation since $O_2^{\cdot-}$ and H_2O_2 are required for OH radical generation.

3.5.3 Effect of *F. cienkowskii* methanolic leaf extract on nitric oxide ($NO^{\cdot}$) radical production

Nitric oxide ($NO^{\cdot}$) 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 $NO^{\cdot}$ liberated from SNP (Fig. 2). The extracts exhibited strong $NO^{\cdot}$ 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 250µg/ml of the extract scavenging most efficiently compared to α-tocopherol (Fig. 2)

Table 2. Phytochemical constituents in *Fadiogia cienkowskii* methanolic leaf extract

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

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

Table 3. Quantitative phytochemical constituents of *Fadiogia cienkowskii* methanolic leaf extract

Extract	Phenolic contents *			Total flavonols‡	Total flavonoids ‡
	Total Phenols	Non-tannins	Tannins		
Fadogia cienkowskii (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 ± 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

Table 4. DPPH radical scavenging activity of *F. cienkowskii* methanolic leaf extract

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

Data represented as mean ± SEM (n = 3)

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)

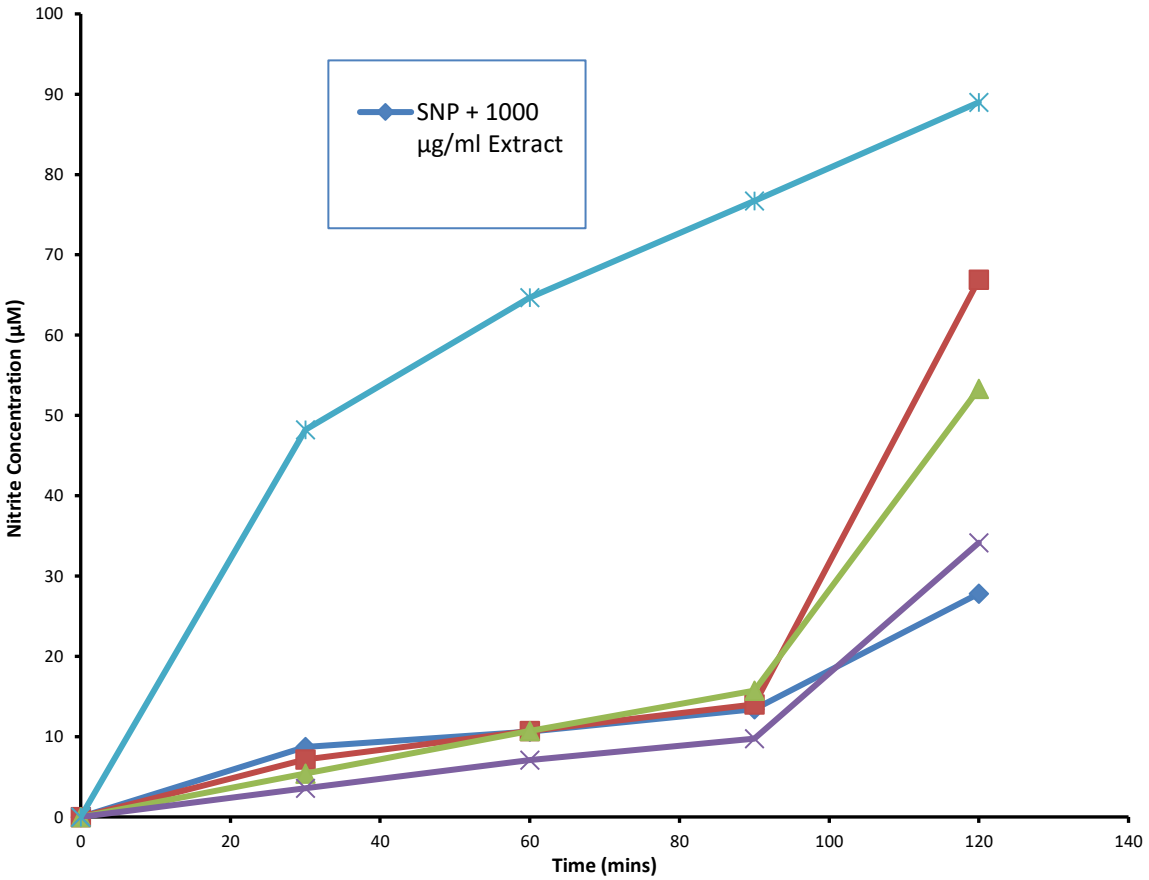

Fig. 2. Effect of methanol extract of *F. cienkowskii* leaves on the accumulation of nitric oxide (NO^-) radical upon decomposition of sodium nitroprusside (SNP) at room temperature

Table 6. Free radical inhibitory potency (IC_{50})

	IC50 value for inhibitory potential (µg/ml)	
Inhibitor	**DPPH radical**	**Superoxide anion ($O_2^{\cdot-}$)**
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*

3.6 IC_{50} for Free Radical Inhibition

The concentration of the extracts that inhibited 50 % of the free radicals and Superoxide anion radicals (IC_{50}) was used to determine the potency of the extracts. The lower the IC_{50} value the better the drug (extract) potency. As shown in Table 4, the plant extracts were efficient inhibitors of different free radicals compared to standard anti-oxidants. The IC_{50} value for DPPH radical inhibition was 151.91µg/ml; while $O^{2.-}$ anion inhibition was 380.19µg/mL (Table 6).

4. 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 ± 0.45 µg of flavonoid content

was found in dried plant extract, but the total amount of phenols was 2.085± 0.35 µg 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. 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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