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Comparative Study of the *In Vitro* Antioxidant Activities of Methanolic Extracts of the Leaves and Stem bark of *Anogeissus leiocarpus* (DC.) Guill & Perr

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Abstract

In this present investigation, the leaves and stem bark extracts of the Anogeissus leiocarpus plant, a family member of the combretaceae, were tested and compared for their antioxidant activities using the radial scavenging effect (DPPH) and ferric-reducing antioxidant power (FRAP). The extraction of the plant materials was conducted with a Soxhlet extractor, and methanol served as the solvent, while standard chemical procedures were used for qualitative and quantitative analysis of phytoconstituents. The results of the phytochemical investigation revealed the presence of alkaloids, flavonoids, saponins, tannins, phenols, and steroids in all the extracts, whereas only stem bark extract consisted of cardiac glycosides. Quantitative estimations of bioactive compounds revealed higher phenolic and flavonoid contents in the stem bark (122.72±0.00 mg/g and 72.16±0.05 mg/g) than the leaves (89.25±0.05 mg/g and 48.28±0.00 mg/g), respectively. Similarly, the result showed that all extracts possessed sufficient antioxidant activity in a dose-dependent trend, and the antioxidative activities were correlated with the total phenolic and flavonol contents; thus, the stem bark demonstrated higher antioxidant activity as compared to the leaves. This suggests the use of the stem bark of the Anogeissus leiocarpus plant for the treatment of oxidative stress-related disorders.

Key words: *Anogeissus leiocarpus*, Methanolic Extracts, Phytoconstituents, Antioxidant

Introduction

In healthy cells as well as aerobic organisms, reactive oxygen and nitrogen species production is unavoidable. But an excess of free radicals sets off a negative chain reaction in the body that can damage the cell membrane, inhibit the main enzymes' ability to function, obstruct cellular processes essential to the body's proper operation, prevent normal cell division, destroy deoxyribonucleic acid (DNA), and prevent the production of energy (Manisha *et al*., 2017; Guchu *et al*., 2020; Oloyede *et al*.,

2022). A multitude of clinical conditions, including diabetes, heart failure, ischemia, carcinogenesis, chronic inflammatory illnesses, cardiovascular conditions, and neurodegenerative disorders, are linked to oxidative damage to these biomolecules. Propyl gallate and butylated hydroxyanisole are two examples of synthetic antioxidant chemicals that are used to treat oxidative stress. Adverse health effects, including cancer and liver damage, have been linked to these substances. It is more likely that naturally occurring antioxidants found in plants will offer effective, secure, readily available, and reasonably priced treatments (Refez *et al*., 2017; Peter *et al*., 2018). Numerous therapeutic plants have been shown in studies to possess antioxidant and antibacterial properties. However, plants have a wide range of qualities due to the presence of several phytochemicals and secondary metabolites (Ogbeba *et al*., 2017; Tauheed *et al*., 2020).). According to several studies (Anas *et al*., 2016; Abdullahi *et al*., 2020; Jacob *et al*., 2021; Joseph *et al*., 2022) certain plant components, including alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, and polyphenols, have healing effects. The World Health Organization (WHO) has made the necessary steps to carry out research in an effort to find novel and potent plant-based medicinal chemicals that can prevent, treat, or mitigate illnesses brought on by oxidative stress and pathogenic microbes (WHO, 2019). *Anogeissus leiocarpus* is one such promising plant that is utilised in Nigeria to cure a variety of illnesses.

Anogeissus leiocarpus (DC.) Guill & Perr, a deciduous tree species that may reach heights of 15–18 m and diameters of up to 1 m, is a significant ethno-medicinal plant in both Africa and Asia (Namadina *et al*., 2021; Mubarak *et al*., 2023). (Udeozo *et al*., 2014; Ali *et al*., 2017; Dayok *et al*., 2018). The plant, also called the African birch or Axle-wood tree, is a member of the *combretaceae* family (Arabab, 2014; Sale *et al*., 2020). It is known as marke (Hausa), ayin (Yoruba), and atera (Igbo) in Nigerian (Stephen *et al*., 2020; Mubarak *et al*., 2023). The leaf, stem bark, twigs, and roots of *Anogeissus leiocarpus* are used to treat a variety of conditions, including gonorrhea, wounds, fever, stomach infections, acute respiratory tract infections, and tuberculosis. Bioactive substances such terpenoids, alkaloids, flavonoids, and tannins are thought to be responsible for the plant's medicinal qualities (Arbab, 2014; Chidozie & Adoga, 2020; Valsan *et al*., 2021). There are numerous documented traditional uses for the plant; bronchitis, hay fever, and cough are treated with the infusion of leaves (Mann *et al*., 2014; Mubarak *et al*., 2023). The roots are utilized as chewing sticks because they are effective against fungal infections like dermatitis and mycosis, are thought to prevent oral infections, and are used to treat typhoid fever and malaria (Temitope *et al*., 2017). Stem bark decoction and maceration are used to treat a variety of illnesses, including helmintosis, schistosomiasis, leprosy, amoebic dysentery, and sexually transmitted infections, as well as anorexia, constipation, fatigue, itching, eczema, psoriasis, carbuncle sores, boils, and the removal of parasite cysts in the host (Mann *et al*., 2014; Sale *et al*., 2020; Usman *et al*., 2020). In a related finding, male Wister rats that were rendered sexually impaired by paroxetine showed reduced erectile dysfunction when exposed to stem bark from *Anogeissus leiocarpus* (Ademosun *et al*., 2019).

Studies have shown that the aqueous extract of the leaves possesses pharmacological properties that include antibacterial, anti-inflammatory, anti-diabetic, and wound-healing

properties (Chidozie & Adoga, 2020). Comparably, the application of the plant in the traditional treatment of *candidiasis* was linked to the antifungal activity of the stem bark ethanol extract (Temitope *et al*., 2017). Additionally, pharmacological activities were observed against *Klebsiella* spp., *Escherichia coli*, and *Pantoea agglomerana* by butanol, hexane, and an aqueous extract of stem bark. The plant's phytochemical components were credited with this, and it was proposed that they could provide treatments for treating bacteria that are resistant to drugs (Stephen *et al*., 2020). It is in view of the numerous benefits of the plant mentioned above that the objectives of this research was to evaluate and compare the antioxidant activities of the leaves and stem bark extract of *Anogeissus leiocarpus* plant. However, in this research the antioxidant activities was established based on two experimental model, namely Radical scavenging effects (DPPH) and Ferric Reducing Antioxidant Power (FRAP).

MATERIALS AND METHODS

Collection of plant sample

The leaves and stem bark of the *Anogeissus leiocarpus* plant were collected from Uttachu village in Kontagora Local Government Area of Niger State (situated between latitude $39^{0}19'$N and $13^{0}32'$N and longitude $03^{0}31'$N and $08^{0}22'$E), located about 30km away from Kontagora. Samples collected were authenticated by a plant taxonomist at the Faculty of Life Sciences, Kebbi State University of Science and Technology, Aleru, Nigeria. A voucher specimen (KSUSTA/PSB/H/84DC.) was deposited in the herbarium of the institute.

Plant processing and extraction

The leaves and stem bark of the *Anogeissus leiocarpus* plant collected were air dried under shade for a period of one month and subsequently pulverised using electrical blander to form fine powders. About 500 g of each plant powder was used for the extraction using a soxhlet extractor (Abdullahi & Mainul, 2020).

Procedure

The powdered materials were exhaustively extracted with methanol under reflux in 200-ml conical flasks. The finely ground powder was placed in porous bags made of whatman fitter paper, which were placed in the chamber of the Soxhlet apparatus. The extracting solvent (100% methanol) in the flask was heated, and its vapours were condensed in a condenser. The condensed solvent was dropped into the thimble containing the plant material and extracted. When the level of liquid in the chamber reached the top of the syphon tube, the liquid content of the chamber was syphoned into the flask. The process was continued until a drop of solvent from the syphon tube did not leave residue when evaporated. Each of the extracts was concentrated *in vacuo* using a rotary evaporator, which ensured the evaporation of bulky solutions to a small volume concentration. The resultant concentrations were weighed and kept in the refrigerator prior to usage (Abdullahi & Mainul, 2020).

Qualitative Phytochemical Screening of the Crude Extract

Preliminary phytochemical screening, which involves performing simple chemical tests to detect the presence of secondary metabolites such as tannins, flavonoids, phenols, phenolic

compounds, saponins, and glycosides, was carried out in accordance with the methods of Mercy et al. (2017), Huma *et al*. (2021), and Yusuf *et al*. (2022).

Screening for Alkaloids
One (1) ml of the extract was measured in a watch glass, and a small amount of dilute hydrochloric acid and Mayer's reagent were added to the solution. The formation of white precipitation indicates the presence of alkaloids.

Screening for Tannin
A few drops of basic lead acetate solution were added to 1.6 ml of the plant extract; the appearance of a white precipitate indicates the presence of tannin.

Screening of Flavonoids
One point three (1.3) m1 of the extract was mixed with 5 g of magnesium and boiled for 5 minutes; the appearance of an orange to red colour indicates the presence of flavonoids.

Screening for Saponins
Two point five (2.5) ml of the extract were mixed with a few drops of distillation water, and the mixture was shaken vigorously; a copious lather formation indicates the presence of saponins.

Screening for Phenol
A few drops of ferric chloride solution were added to 2 mL of the extract in the watch glass; the appearance of a bluish green colour indicates the presence of phenol.

Screening for Cardiac glycosides
Two point five (2.5) ml of the extract was mixed with a small quantity of anthrone in a watch glass, and one drop of concentrated sulfuric acid was added to make a paste. Heat gently over a water bath; a dark green colour indicates the presence of glycosides.

Screening for Terpenoids
One (1) ml of the extract was added to 0.5 ml of chloroform, followed by a few drops of concentrated sulfuric acids. The formation of a reddish brown precipitate indicates the presence of terpenoids.

Screening for Steroids
One (1) ml of the extract was added to 0.5 ml of chloroform, followed by a few drops of concentrated sulfuric acids. The formation of a reddish brown precipitate indicates the presence of terpenoids.

Determination of Antioxidant Properties of *Anogeissus leiocarpus*
The radial scavenging effect (DPPH) and the ferric reducing power assay (FRAP), as reported by Beatrice *et al*. (2020) and Jane *et al*. (2021), were used to assess the antioxidant activity of the leaves and stem bark extracts of *Anogeissus leiocarpus* plants.

Radical Scavenging Effect (DPPH) Assay

The 2, 2, diphenyl-1-picrylhydrazyl (DPPH) radical scavenging mechanisms reported by Beatrice et al. (2020) and Jane et al. (2021) were used to assess the antioxidant properties of leaves and stem bark extracts of the *A*. *leiocarpus* plant. Approximately 0.5 milliliters of every extract at varying concentrations (0.0625, 0.125, 0.25, 0.5, and 1.0 mg/ml) in methanol were combined with 0.5 milliliters of a DPPH 0.3 mM methanolic solution. At 300 °C, the mixture was mixed and allowed to incubate for 30 minutes. Using methanol as a blank, the absorbance was measured at 517 nm. The plant extracts' antioxidant properties were determined and expressed as percentages of DPPH free radical inhibition.

$$\% \text{ scavenging} = \frac{\text{Absorbance of control - Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

Ferric Reducing Power Assay (FRAP)

With minor adjustments, the reducing power assay of *A*. *leiocarpus* leaves and stem bark extracts was calculated in accordance with a publication by Jane *et al*. (2021). Two milliliters of potassium ferricyacinide [K3Fe (CN)6] and 2.5 milliliters of phosphate buffer (0.2 milliliter, PH 6.6) were combined with one milliliter of each extract solution at various concentrations (0.2, 0.4, 0.6, 0.8, and 1.0 mg/ml). After 20 minutes of incubation at 50 °C, 2 ml of 10% w/v trichloroacetic acid was added, and the mixture was centrifuged at 1000 revolutions per minute (rpm). Two milliliters of the supernatant were aspirated, and one milliliter of 0.1% ferric chloride (Fecl3) and two milliliters of distilled water were combined. Afterwards, at 700 nm, the absorbance was calculated using a UV spectrophotometer and recorded. An increase in the absorbance value of the reacting mixture indicates an increase in reducing power. Three replicates were met for each test sample, and average data was recorded. Ascorbic acid was used as a positive control standard.

Estimation of Total Phenolic Contents of the extracts

The Folin-Ciocalteus reagent (FCR) was used to calculate the fractions' total phenolic content. 0.4 ml of diluted 1:10 v/v FCR was combined with various concentrations of the fractions during the operation. 4 ml of the sodium carbonate solution was added after 5 minutes. After adding distilled water to the tubes to reach a final capacity of 10 ml, they were left to remain at room temperature for 90 minutes. Using a spectrophotometer, the sample's absorbance was calculated at 750 nm in relation to the blank. The standard for estimating phenolic compounds was gallic acid.

Estimation of Total flavonoids contents of the extracts

By employing the aluminium chloride method with catechin as a reference, the total flavonoid concentration was ascertained. A 10-milliliter volumetric flask was filled with 1 milliliter of the test fraction and 4 milliliters of water. Three milliliters of 10% aluminium chloride and three milliliters of 5% sodium nitrite were added after five minutes. 2 milliliters of 1M sodium hydroxide were added to the reaction mixture following a roughly 6-minute room temperature incubation period. At once, distilled water was added to bring the final volume to 10 ml. A

blank spectrophotometer was used to test to the reaction mixture. Immediately, the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometer. Results were expressed as catechin equivalent (mg catechin/g dried fraction).

Data Analysis

Every experiment was carried out in triplicate, and the mean ± SD (standard deviation) was used to express the findings. Using SPSS version 22, a one-way analysis of variance (ANOVA) was performed on the collected data.

Results

Qualitative phytochemical screening

Qualitative phytochemical analysis of the extracts of the various plant components showed that only the stem bark contained cardiac glycosides, whereas all of the extracts contained alkaloids, flavonoids, tannins, saponins, phenols, and steroids. Terpenoids, however, were discovered to be lacking in both (table 1).

Table 1: Qualitative phytochemical screening

Phytochemical compound	Leaves	Stem bark
Alkaloids	+	+
Flavonoids	+	+
Tannins	+	+
Saponins	+	+
Terpenoids	-	-
Phenols	+	+
Cardiac glycosides	-	+
Steroids	+	+
Coumarins	-	-
Anthraquinones	-	-

(+) = presence; (-) = absence

DDPH Radical Scavenging Activities of Methanolic Extracts of the Leaves and stem bark of *Anogeissus leiocarpus*

Table 2 and Figure I show that the methanolic extracts of the stem and leaves bark extracts exhibited outstanding in-vitro DPPH radical scavenging capabilities in a dose-dependent trend. The DPPH radical scavenging abilities of the investigated methanolic plant extracts were substantially lower than those of the standard, ascorbic acid ($P<0.05$). The methanolic extract of the stem bark generated notably more DPPH radical activity than the leaf extract at all doses examined. At all concentrations, there was a significant difference ($P<0.05$) in the percentage of DPPH radical scavenging activities between the stem bark extract and the leaves. This investigation additionally established the concentrations of the examined plant extracts needed to scavenge 50% of the DPPH radical (IC50). The extracts of stem bark and

leaves have IC50s of 0.31 and 0.46 mg/mL, respectively. Conversely, 0.18 mg/mL was the IC50 value for standard ascorbic acid.

Table 2: *In vitro* DPPH Scavenging activities of methanol extracts of the leaves and stem bark of *A. leiocarpus*

Concentration (mg/ml)	Control (ascorbic acid)	Stem bark	Leaves
0.0625	38.92±0.50^{d}	20.81±1.75^{b}	11.86±1.78^{a}
0.125	57.15±0.20^{a}	35.75±132^{b}	20.05±0.75^{c}
0.25	69.13±0.75^{b}	42.10±0.17^{a}	29.35±0.30^{c}
0.5	76.46±0.37^{c}	53.56±1.69^{d}	38.65±1.73^{b}
1.0	85.63±0.16^{a}	64.33±0.33^{c}	46.25±0.45^{d}
IC_{50} mg/ml	0.18	0.31	0.46

Values are expressed as mean±SD, values with same superscript within the same raw are not significantly different (p>0.05) using one way analysis of variance (ANOVA)

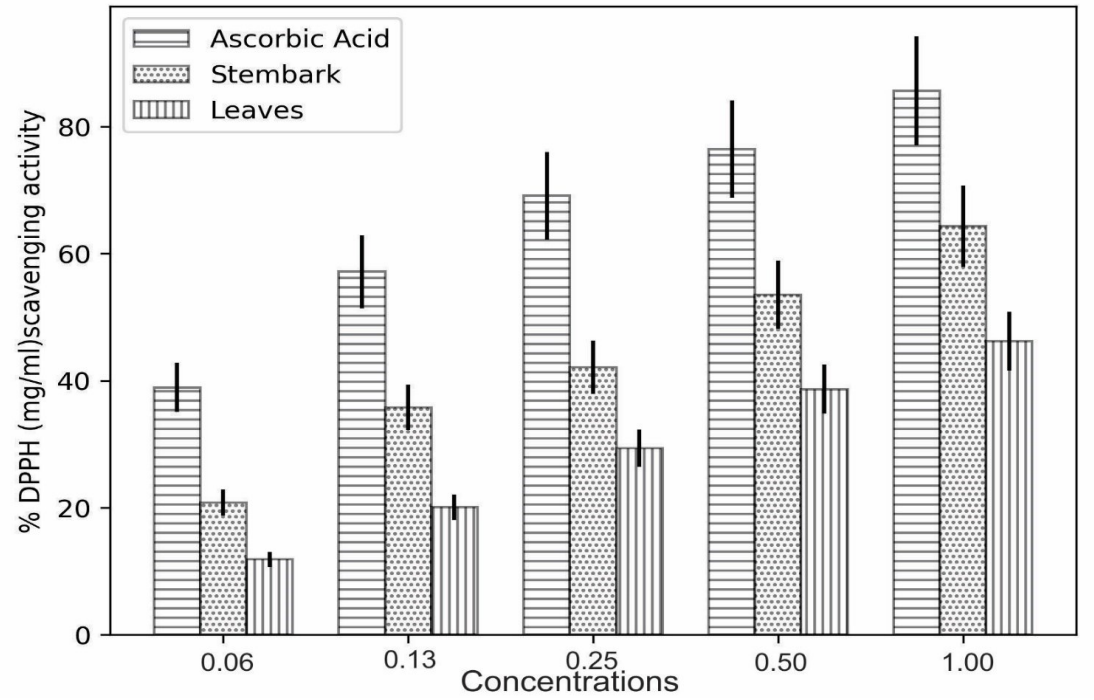

Fig: 1. DPPH scavenging activities of methanolic extract of the leaves and stembark of *A. leiocarpus*

Ferric Reducing Antioxidant Power (FRAP)

According to this investigation, the methanolic extract of the *Anogeissus leiocarpus* plant's leaves and stem bark showed notable concentration-dependent increases in absorbance value at a wavelength of 700 nm (table 3 and figure II). The stem bark extract exhibited considerably greater absorbance values (P<0.05) than the leaves extract at all tested doses. Although the

absorbance of the standard (ascorbic acid) was much higher than that of the plant extracts under study (P<0.05), additionally, the half-effective concentration (EC50) of the plant extracts under investigation needed to result in an absorbance value of 0.5 was found. It was shown in this study that ascorbic acid had a lower EC50 value than the plant extracts under investigation. When compared to the EC50 values of the leaves extract, the methanolic extract of the stem bark, however, exhibited the lowest value.

Table 3: *In vitro* ferric reducing antioxidant power activities of methanolic extracts of *A. leiocarpus* leaves and stem bark

Concentration (mg/ml)	Control (ascorbic acid)	Stem bark	Leaves
0.2	1.80±0.02^{a}	1.26±0.04^{c}	0.60±0.01^{b}
0.4	2.35±0.00^{b}	1.65±0.00^{a}	1.00±0.00^{d}
0.6	2.83±0.01^{c}	1.92±0.01^{b}	1.40±1.64^{a}
0.8	3.15±0.01^{d}	2.30±0.02^{a}	1.64±0.01^{c}
1.0	3.85±0.00^{ab}	2.82±0.00^{c}	1.91±0.02^{b}
EC_{50} mg/ml	0.24	0.42	0.56

Values are expressed as mean±SD, values with same superscript within the same raw are not significantly different (p>0.05) using one way analysis of variance (ANOVA)

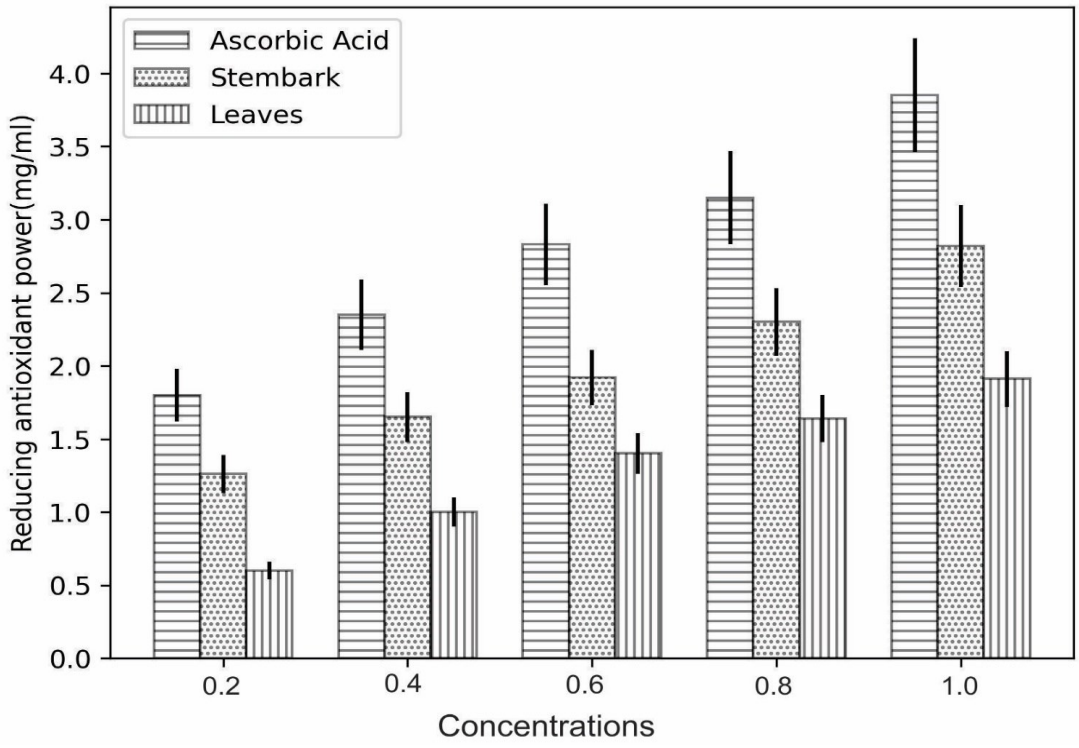

Fig: II. Ferric reducing antioxidant power of methanolic extracts of *A. leiocarpus* leaves and stembark

Estimation of total phenols and flavonoids contents of the extracts

Tables 4 and 5, respectively, provide the estimated total phenols and flavonoids. The leaves and stem bark extracts have total phenolic contents of 89.25±0.05 mg/g and 122.72±0.00 mg/g, respectively. On the other hand, the plant's leaf and stem bark had total flavonoid contents of 48.28±0.00 mg/g and 72.16±0.05 mg/g, respectively.

Table 4: estimation of total phenolic contents of the extract

Sample	TPC (mgGAE/g)
Leaves	89.25 ± 0.05^{b}
Stem bark	122.72 ± 0.00^{a}

TPC; total phenolic content, mgGAE/g; milligram gallic aid equivalent per gram of sample, values are expressed as mean±SD means with different superscript letters are significantly different by one way analysis of variance (ANOVA).

Table 5: estimation of total flavonoids contents of the extracts

Sample	TFC (mgQE/g)
Leaves	48.28 ± 0.00^{a}
Stem bark	72.16 ± 0.05^{b}

TFC; total flavonoids contents, mgQE/g; milligrams quercetin equivalent per gram of sample, values are expressed as mean±SD, mean with different superscript letters are significantly different by one way analysis of variance (ANOVA)

DISCUSSION

Antioxidants are essential compounds that can shield the body from harm caused by free radicals. Antioxidants, which can be used to remove excess free radicals from the body, are found in large quantities in many plants. These antioxidants include carotenoids, phenols, flavonoids, and tannins. The present investigation and comparison focused on the in vitro DPPH scavenging ability of methanolic extracts obtained from the leaves and stem bark of the *Anogeissus leiocarpus* plant. It is clear that the plant's stem bark extract has greater antioxidant activity than the leaves extract, and the overall findings demonstrated that the extracts showed a concentration-dependent relationship, in line with earlier findings from other researchers (Abubakar *et al*., 2018; Temitope *et al.*, 2021; and Joseph *et al.*, 2022). According to reports from Brand-Williams (1995), Fidrianny *et al*. (2015), and Beatrice *et al*. (2020), phytochemical compounds like phenolics have gained a lot of attention recently as potential natural antioxidants because of their potent ability to act as metal chelators and radical scavengers. These compounds' combined activities may be reflected in the extracts' capacity to scavenge. According to studies, singlet oxygen quenches, hydrogen donors, and redox characteristics of phenolic compounds are mostly responsible for their antioxidant action (Sharma *et al*., 2017; Oloyede *et al*., 2022). In this investigation, the two extracts under investigation all had IC50 values less than 1 mg/mL. This implies that the plant extracts have

a high concentration of phytochemical components that can scavenge potential harm by giving free radical hydrogen. The results were similar to those of the Beatrice *et al*. (2020) in vitro investigation, who produced evidence of the antioxidant properties of methanolic extracts from *Vernonia lasiopusa* (Hoffoni), *Acacia hockii* (Dewild), and *Caesalpinia volkensy* (Haima). The antioxidant activity, GC-MS analysis, and phytochemical screening of three Nigerian medicinal herbs were examined by Ezekwe *et al*. (2020), and their findings are consistent with our result.

Comparably, the ability of the plant extracts to convert the ferric ion (Fe3+) to the ferrous ion (Fe2+) is the basis for the Ferric Reducing Antioxidant Power (FRAP) assay used in this investigation. Accordingly, the absorbance capacity at 700 nm can be used to analyse the Fe2+ formations (Adebegha *et al*., 2016; Arika *et al*., 2019; Oloyede *et al*., 2022). According to Ezekwe *et al*. (2021), a rise in absorbance at this wavelength corresponds to a rise in reducing power. The results of this investigation also showed that the methanolic leaves and stem bark extracts of *A*. *leiocarpus* increased in absorbance values in a concentration-dependent manner, indicating a sizable ferric-reducing antioxidant. This study can be compared to that of Shago *et al*. (2021), who found that the peels and leaves of *Citros sinesis* and Mongnifera *indica* have dose-dependent ferric-reducing action. Since the EC50 values for each of the investigated plant extracts were less than 1 mg/mL, they were all regarded as potent antioxidants. This study supported that of Jacob *et al*. (2021), who found comparable results in the *Moringa* plant's leaves, seeds, pods, and coats.

The study makes a compelling case for the presence of phenolic chemicals in this plant, which are thus thought to be responsible for its biological effects. According to Joseph *et al*. (2022), the primary cause of phenolic compounds' antioxidant potential is their redox characteristics, which enable them to function as metal chelators, hydrogen donors, reducing agents, or singlet oxygen quenchers. As one of the phenolic compounds present in medicinal plants, flavonoids have been linked to a number of pharmacological activities, such as anti-inflammatory and anti-tumor effects. They can also function as antioxidants, shielding cells from the damaging effects of free radicals (Ukon *et al*., 2014; Shago *et al*., 2021). Reactive species neutralization capacity and antioxidant capacity are influenced by flavonoids' structure, hydroxyl atom location, and other characteristics, according to Joseph *et al*. (2022). Oluwafunke *et al*. (2014) and Beatrice *et al*. (2020) reported that these compounds exhibited strong scavenging capabilities against harmful radicals, which are linked to several health conditions.

In this investigation, the plant's stem bark extract had greater antioxidant activity than its leaves, while the reference antioxidant vitamin C showed the highest activity. This indicates that there is a significant difference ($p<0.05$) in the antioxidant activity of the plant's leaf and stem in each of the two experimental models. The outcome agrees with the total amount of flavonoids and phenols found in each extract. The stem bark extract had higher levels of flavonoids and flavonoids than the leaf extract, indicating that a variation in the concentration of the bioactive components was the cause of the observed variation in the extracts' activity. Based on the study, methanolic extracts of the *Anogeissus leiocarpus* plant's leave and stem bark showed oxidant activities, suggesting the presence of valuable bioactive compounds in varying amounts of the plant parts. This could lead to the development of powerful, effective,

safe, and reasonably priced antioxidants to reduce oxidative stress. The study provides additional evidence in favour of the plant's usage in traditional medicine to treat illnesses linked to oxidative stress.

CONCLUSION AND RECOMMENDETIONS

This study found that the *Anogeissus leiocarpus* plant's stem bark methanolic extract has strong antioxidant-associated phytochemicals and high antioxidant capacities, which may help prevent or treat illnesses linked to oxidative stress. It is advised to conduct additional research targeted at separating and characterizing the pure bioactive chemicals. To ascertain their safety, toxicity tests on the plants stem bark extracts ought to be carried out.

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