ANTIOXIDANT CAPACITY, TOTAL PHENOLIC AND TOTAL FLAVONOID CONTENT OF THE METHANOL EXTRACT OF *ALCHORNEA CORDIFOLIA* (CHRISTMAS BUSH) LEAVES

Supported by tetfund

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Received: September 14, 2023 Accepted: November 28, 2023

Abstract: *Alchornea cordifolia* is a widely recognized traditional African medicinal plant with a history of diverse therapeutic applications. It is employed in ethnomedicine for the treatment of wounds, gonorrhoea, conjunctivitis, diarrhoea, urinary problems, and gastrointestinal disorders. This study aimed to evaluate the antioxidant properties, total phenolic content (TPC), and total flavonoid content (TFC) of the methanol extract of *Alchornea cordifolia* leaves. TPC was estimated by using the Folin-Ciocalteu colourimetric method with gallic acid as the standard, and the absorbance was measured at 750 nm. The quercetin spectrophotometric method was used to determine the TFC while the radical scavenging activity of the extract was assessed by using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay as well as Total Antioxidant Capacity (TAC). Preliminary phytochemical screening of the leaves revealed the presence of phenols, flavonoids, alkaloids, glycosides, eugenols, steroids, terpenoids, and carbohydrates, but tannin was absent. The TPC was 106.10 ± 1.98 mg GAE/g while the TFC was determined to be 84.55 ± 3.34 mg QE/g. The IC_{50} values obtained in the DPPH assay were 2.99 µg/mLh for the extract and 1.32 µg/mL for ascorbic acid (the standard). In the total antioxidant capacity (TAC) analysis, the IC_{50} was 221.6 µg/mL for ascorbic acid and 246.8 µg/mL for the plant extract. These results underscore the potential of *Alchornea cordifolia* leaves as a valuable source of natural antioxidants, suggesting its significance in ethnomedicine.

Keywords: Antioxidant, gallic acid, total phenolic, total flavonoid, *Alchornea cordifolia*, phytochemical

Introduction

The power of plants to regulate biological processes and ward off illness and disease is just one of several essential qualities that make them valuable for pharmacological research and drug development. The majority of plant-derived medicines have their roots in traditional medicine (Osho *et al.*, 2007). Despite recent emphasis on the study of synthetic medications, interest in medicinal plants has resurged. This is because, unlike therapeutic herbs, several chemically synthesized medicines are not without side effects. They are also expensive unlike medicinal plants, which are crucial for those living in rural areas who cannot afford the cost of modern health care systems. All plant structures include active components that have the potential to be effective medicinal agents, but the concentration is frequently higher in one portion, and this part is favoured. Examples include stem bark, seeds, fruits, leaves, and flowers (Smith-Hall *et al.*, 2012).

The plant *Alchornea cordifolia*, which belongs to the family of Euphorbiaceae, is generally found in all regions of Africa and is often used traditionally for treating a variety of fungal, bacterial, inflammatory and parasitic disorders. A decoction of the plant leaves has also been used in the treatment of colds, coughs, and headached and even for the prevention of spontaneous abortion (Anash *et al.*, 2011). Extracts of *A. cordifolia* leaves have been used to cure sores, cuts, and wounds. The root and stem bark have been utilized in the management of jaundice and the fruits have been utilized in eye treatment and pigmentation problems.

A. cordifolia has been the subject of numerous investigations on its biological activities, and the results have revealed its antiviral, anti-inflammatory, antidiabetic, and hepaprotective qualities (Mavar-Manga *et al.*, 2008). Several antimicrobial screenings of the extract of *A. cordifolia* leaves have shown its ability to combat a variety of harmful bacteria, thereby lending credence to the utilization of the herb in conventional medicine to treat illnesses. These important pharmacological effects have been traced to various concepts derived from various parts of *A. cordifolia* plant. Also, previous research on the phytochemical study showed that the plant is high in fatty acids, flavonoids, alkaloids, phenolic acids, terpenoids and steroids (Bonniface *et al.*, 2016).

Generally, organic products have made immense contribution to the development of contemporary medicine. Despite the popularity of synthesized products being on the increase due to their time efficiency, production cost, better quality assurance, rapid effects and rigorous regulations, their efficacy and safety have been a major concern, resulting in about 80% of the populace in the developing world to be dependent on natural or organic. Hence, with the large range of traditional uses, administration and several pharmaceutical properties associated with *A. cordifolia* as a therapeutic plant, the current study aims to evaluate the antioxidant activity of its leaves extract to give a scientific basis for its traditional uses.

Materials and Methods

Chemicals and reagents

All the chemicals used for the study were of analytical grade and were directly utilized without further purification. The reagents were prepared and used according to standard procedures.

Collection and identification of the plant sample

Alchornea cordifolia leaves were obtained from the natural habitat (N5°8′5″ E7°20′7″) in Osisioma-Ngwa Local Government Area, Aba, Abia State, Nigeria.

The leaves were identified by Dr Akinnibosun Henry Adewale of the Department of Plant Biology and Biotechnology (PBB), Herbarium Unit, Faculty of Life Sciences, University of Benin, Benin City, Nigeria. A specimen number (UBH-A502) was assigned to the leaves.

Preparation of sample

The leaves of *A. cordifolia* were harvested fresh and air-dried under shade for two weeks. They were subsequently pulverized into powder using a stainless steel blender. The powder was transferred into a polyethene bag and kept for subsequent analysis.

Preparation of extract

A weighing balance was used to measure 150g of the powdered plant sample, and transferred into a glass jar. 1000 mL (1L) of methanol was added into it, covered and allowed to extract. The jar was agitated at intervals for 6 hours for proper dissolution of soluble matter. After 72 hours, the mixture was filtered and filtrate was allowed to gently evaporate over a water bath (set at 60°C) until a slurry was obtained. The percentage yield of the extract was calculated to be 12.8%. Thereafter, it was transferred into a sample bottle and kept in a refrigerator at 4°C.

Determination of total phenolic content (TPC)

The total phenol content of the extract was determined by the method described by Kim *et al*, (2003). The extract solution (0.5 mL) with a concentration of 1000 mg/mL was added to 4.5 mL of deionized distilled water and 0.5 mL of Folin Ciocalteu's reagent (previously diluted with water 1:10, v/v) was then added to the solution. After mixing thoroughly in a tube, it was maintained at room temperature for 5 minutes followed by the addition of 5 mL of 7% sodium carbonate and 2 mL of deionized distilled water. It was then mixed again with the samples, the mixtures formed were incubated for 90 minutes at room temperature. The absorbance was measured with a spectrophotometer at 750 nm. The total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per gram of extract (mg GAE/g extract). All experiments were carried out in triplicates and the standard curve was prepared by using gallic acid in six different concentrations (12.5, 25, 50, 75, 100 and 150 mg/L).

Determination of total flavonoid content (TFC)

Total flavonoid contents were estimated using the method described by Ebrahimzadeh *et al*, (2008). Briefly, 0.5 mL of extract (1 mg/mL) was mixed with 1.5 mL of methanol and then, 0.1 mL of 10 % aluminium chloride was added, followed by 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The mixture was incubated at room temperature for 30 minutes. The absorbance was measured by using a spectrophotometer at 415 nm. The results were expressed as milligrams of quercetin equivalents (QE) per gram of extract (mg QE/g extract). The standard curve was prepared by using quercetin in four different concentrations (12.5, 25, 50 and 75 mg/L).

Determination of antioxidant activity

(i) DPPH Radical Scavenging Assay

The radical scavenging ability of the crude methanol extract of *A. cordifolia* leaves was estimated by using the DPPH radical assay as described by Jain *et al*, (2008). A solution of 0.1 mM DPPH in methanol was prepared, and 1.0 mL of this solution was mixed with 3.0 mL of extract in methanol containing 0.001 - 0.05 mg/mL of the extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 minutes. The absorbance of the mixture was measured with a UV spectrophotometer at 517 nm. Ascorbic acid was used as the reference standard. The ability to scavenge DPPH radical was calculated by using equation (i).

DPPH radical scavenging activity (%) = $[\frac{(A_0-A_1)}{(A_0)}]$ x 100 ……..……(i)

Where:

A_0 is the absorbance of DPPH radical + methanol

A_1 is the absorbance of DPPH radical + sample extract /standard.

The 50% inhibitory concentration value (IC_{50}) was calculated as the effective concentration of the sample that is required to scavenge 50% of the DPPH free radicals.

(ii)Total Antioxidant Capacity (TAC)

The assessment of the total antioxidant capability (TAC) of the methanol extract of *Alchornea cordifolia* leaves was carried out using ascorbic acid as the standard reference. The methodology closely aligns with the protocol delineated by Moonmun *et al*, (2017), with slight modifications by Ojo and Christopher, (2014) tailored to the unique parameters of this research.

Phosphomolybdate reagent was prepared by mixing 30 mL of 28 mM sodium phosphate, 30 mL of 0.6 M sulfuric acid, and 30 mL of 4 mM ammonium molybdate solutions. Subsequently, 0.3 mL of different concentrations (ranging from 20, 50, 100, 150, 200 and 250 µg/mL) of the extract solution were introduced to 3 mL of the phosphomolybdate reagent. This amalgam was then incubated in a dark environment for a duration of 90 minutes. The subsequent evaluation involved the measurement of absorbance at 765 nm. Ascorbic acid was used as standard in the same varying concentrations, for comparison.

The percentage (%) TAC was calculated by using equation (ii):

$[\frac{(A_0-A_1)}{(A_0)}]$ x 100……..(ii)

Where:

A_0 is the absorbance of the phosphomolybdate reagent (blank),

A_1 is the absorbance of phosphomolybdate reagent + sample extract/standard.

The concentration of extract at which 50% inhibition is observed (IC_{50}) is calculated in µg/mL and compared with the standard.

Results and Discussion

Phytochemical screening

The result of the phytochemical screening of *A. cordifolia* leaves is shown in Table 1. Saponins, glycosides, flavonoids, steroids, phenolic compounds, eugenols,

terpenoids, alkaloids and carbohydrates were present while tannins were not detected.

Table 1: Phytochemical constituents of the methanol extract of *A. cordifolia* leaves

Phytochemical constituents	Inference
Glycosides	+
Saponins	+
Flavonoids	+
Tannins	-
Phenolic compounds	+
Eugenols	+
Steroids	+
Terpenoids	+
Alkaloids	+
Carbohydrates	+

Phytochemicals are natural compounds found in plants that are believed to have numerous health benefits for humans. These compounds are responsible for the colours, flavours, and aromas of many plants and are thought to contribute to the protection of the plant against environmental stressors such as diseases, pests, and UV radiation (Dangoggo *et al*., 2012). Phytochemicals have been shown to own a range of biological processes, which may include anti-inflammatory, anticancer, and antioxidant characteristics (Dwivedi *et al*., 2020). Research has also shown that certain phytochemicals can assist in lowering the risk of chronic illnesses including cancer, diabetes, and heart disease (Scalbert *et al.*, 2005).

Total Phenolic Content (TPC)

The total phenolic content of the extract was calculated from the regression equation of the calibration curve ($y = 0.0065x + 0.1137$, $R^2 = 0.9702$) and expressed as mg gallic acid equivalents (GAE) per gram of sample. The value obtained was 106.10 ± 1.98 mg GAE/g. Phenols are well-known for their remarkable ability to combat free radicals, as they can effectively chelate metals both in vitro and in vivo. Consequently, phenols and most of its derivatives primarily function as antioxidants. Phenolic compounds have demonstrated anti-inflammatory properties that are valuable for managing conditions like inflammatory bowel disease, rheumatoid arthritis, and skin conditions (Christman and Gu, 2020). Phenolic compounds also offer protection against oxidative stress and inflammation caused by air-borne particulate matter. Plant-based diets, which are often rich in polyphenols, provide nutritional benefits and help guard against the development of chronic illnesses (Liu *et al*., 2023). Polyphenols have been associated with various health advantages, including improvements in cardiovascular well-being. (Minatel *et al* 2017). The value obtained as the TPC in this study is similar to (120.38 ± 9.31 mgGAE/g) that obtained by Sinan *et al.,* 2015 from the ethyl acetate extracts of *A. cordifolia* leaves. However, a comparatively higher value (239.66 mg GAE/g of extract) was obtained by Olayemi *et al*, (2017), in a study on the methanol extract of *A. cordifolia* leaves.

Total flavonoid content (TFC)

The regression equation of the calibration curve ($y = 0.0196x + 0.0393$, $R^2 = 0.9702$) was used to estimate the TFC of the methanol extract of *A. cordifolia* leaves. It was calculated to be 84.55 ± 3.34 mg QE/g of sample.

Flavonoids are phytochemical compounds present in numerous plants, including vegetables, stems, leaves, flowers, nuts, vegetables, cereals and fruits, with potential applications in medicinal chemistry. A diet rich in flavonoid-containing foods can provide various health benefits (Bondonno *et al.,* 2019). Flavonoids have found applications in skin care and cosmetic products, analso as anticancer, antimicrobial, antimalarial, antioxidant, anti-tumor, antiviral, and anti-proliferative agents. Moreover, flavonoid has been demonstrated to contribute to the prevention of metabolic disorders and the maintenance of cognitive function as individuals age (Asad *et al.,* 2020). In a previous study by Sinan *et al*, (2021), the TFC of the methanol extract of *A. cordifolia* leaves was measured at 57.18 ± 0.94 mg QE/g, which was slightly lower than the value reported in this study. Koffi *et a.,* (2021), estimated the total flavonoid content of the methanol extract of *A. cordifolia* leaves to be 92.75 mg QE/g, which is marginally higher than the value reported in this study. These findings suggest that the leaf extract of *A. cordifolia* has a higher amount of phenolic content than flavonoid content.

Anti-Oxidant Activities

DPPH percentage radical scavenging activity and total antioxidant capacity

The percentage radical scavenging activities (RSA) of the methanol extract of *A. cordifolia* leaves are respectively displayed in Figure 1, for DPPH assay and Figure 2 for the total capacity (TAC), at varying concentrations. In the two *in vitro* antioxidant models used, the RSA of the plant extract compared favourably with that of ascorbic acid (standard). As shown in Figure 1, within a concentration range of 1 - 50 µg/mL, the extract of *A. cordifolia* leaves displayed RSA of between 44.31 - 64.68% for the DPPH assay. A similar trend was observed for the RSA of ascorbic acid (42.16 - 66.16%)

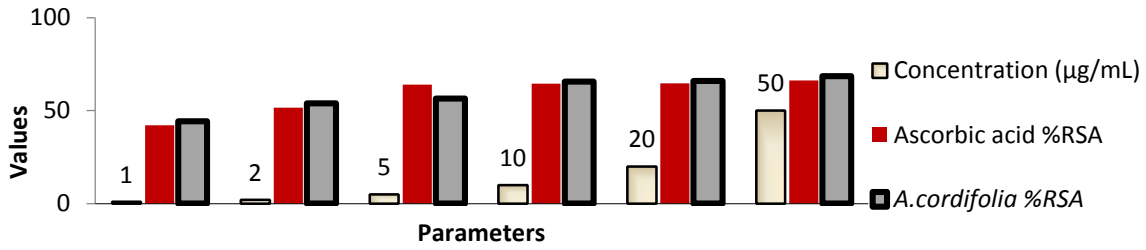

Figure 1: DPPH % Radical scavenging activity (RSA) values for ascorbic acid and *A. cordifolia* at different concentrations

Antioxidants are molecules that scavenge and neutralise free radicals in the body. They are found in a wide range of plant medicines and foods. Free radicals are compounds that can potentially harm the body when their levels are elevated and have been linked to various diseases, including diabetes, cancer, and heart disease (Lobo *et al*., 2010). The free radicals are generated as a consequence of compromised or disturbed mitochondrial respiratory processes. The radical scavengers work by inhibiting lipid peroxidation, effectively neutralizing the highly reactive free radicals. Among the most frequently used antioxidant medications in stroke therapy, as well as in both clinical and preclinical studies, are those that can scavenge free radicals (Shirley *et al.*, 2014). The free radical scavenging activity of the methanol extract of *A. cordifolia* leaves, in the DPPH assay, followed a concentration-dependent pattern. This implies that its antioxidant principles are most effective at high concentrations.

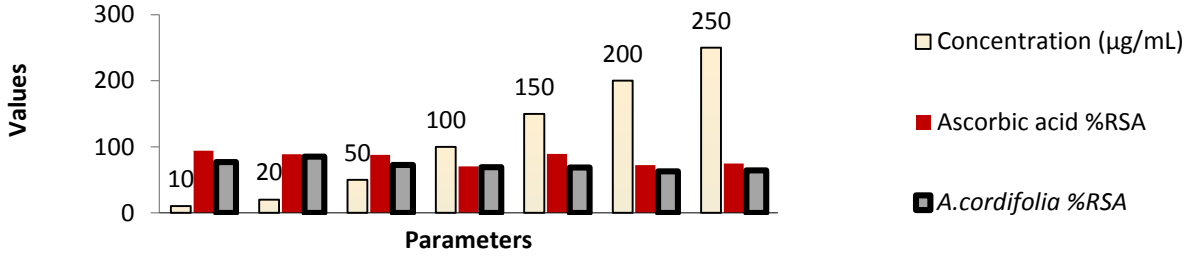

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Figure 2: TAC % Radical scavenging activity (RSA) values for ascorbic acid and *A. cordifolia* at different concentrations

Figure 2 shows the percentage RSA values for ascorbic acid and the methanol extract of *A. cordifolia* leaves at different concentrations for the total antioxidant capacity (TAC), determined using the phosphomolybdate method. The results show that the TAC is independent of the concentrations of the ascorbic acid and the plant extract. Fairly constant values of percentage RSA were obtained for the entire concentration range (10 - 250 µg/mL). Therefore, the least concentration of the plant extract (and ascorbic acid) can exact a maximum TAC. These findings confirm that the methanol extract of *A. cordifolia* possesses substantial antioxidant properties. Previous studies by other researchers (Oruka *et al*., 2023; Eboh *et al*., 2022 Koffi *et al*., 2021) also affirm the above claim for the plant extract.

The IC_{50} values for ascorbic acid and the plant extract are shown in Table 2. In the DPPH assay, the IC_{50} was 1.32 µg/mL for ascorbic acid and 2.99 µg/mL for the extract. Similarly, in the TAC experiment, IC_{50} was 221.6 µg/mL ascorbic acid and 246.8 µg/mL for the methanol extract of *A. cordifolia* leaves. This implies that the anti-oxidative ability of the plant extract is highly similar to that of the standard, ascorbic acid.

TABLE 2: 50% Inhibitory concentration of and *A.cordifolia*

Samples	IC_{50}(µg/mL)	
	DPPH assay	TAC
Ascorbic acid	1.32	221.60
A. cordifolia	2.99	246.80

Generally, higher a IC_{50} value indicates a lower antioxidant or radical scavenging capacity. In DPPH assay, a substance having IC_{50} of 50 µg/mL (or less), is said to be a highly potent antioxidant (Jain and Jain 2011). The 50% inhibitory concentration (IC_{50}) assesses the capacity of a substance to effectively impede a certain biological function. When in contrast to control, the IC_{50} is the concentration needed to produce 50% of an antioxidant's activity, such as 50% of the ability to reduce or scavenge free radicals (DPPH). A lower IC_{50} suggest greater antioxidant activity. In this case, the extract exhibited a higher IC_{50} value than the standard (ascorbic acid), which suggests that the antioxidant potential of the methanol extract from *A. cordifolia* is greater.

Conclusion

The overall findings in this study suggest that the methanol extract of *Alchornea cordifolia* leaves possesses robust antioxidant properties, which are comparable to the antioxidant activity of ascorbic acid. Therefore, *Alchornea cordifolia* leaves could serve as a potent natural source of antioxidants, potentially playing a crucial role in preventing various harmful human diseases. However, it's essential to determine the optimal concentration at which this antioxidant activity is most effective for practical applications.

Further research may be necessary to delve into the underlying mechanisms and assess the extract's effectiveness and safety for potential therapeutic uses in addressing issues related to oxidative stress.

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