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Banso A
Department of Biological Sciences, Federal Polytechnic, Bida Niger, Nigeria

Dachi S
Department of Agriculture, University of Jos, Jos Plateau, Nigeria

Usman JI
Department of Biological Sciences, Federal Polytechnic, Nasarawa, Nasarawa, Nigeria

Ajewole AE
Department of Biological Sciences, Federal Polytechnic, Bida Niger, Nigeria

Nma Etsu-Musa
Department of Biological Sciences, Federal Polytechnic, Bida Niger, Nigeria

Corresponding Author:
Banso A
Department of Biological Sciences, Federal Polytechnic, Bida Niger, Nigeria

Assessment of bioactive phytochemical and free radical scavenging analysis of leaf extract of *Alchornea cordifolia* (Schumach & Thonn) Mull. Arg.

Banso A, Dachi S, Usman JI, Ajewole AE and Nma Etsu-Musa

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Abstract
Alchornea cordifolia, a plant belonging to the *Ephorbiaceae* family and primarily found in Africa regions, has a rich history of traditional usage for treating various ailments such as fungal, bacterial and inflammatory disorders. The leaves, roots and stem barks are commonly used either individually or in combination to address a wide range of conditions from wounds and diarrhea to coughs and asthmatic attacks. Antioxidants are essential for protecting the body against oxidative damage caused by reactive oxygen species, which are linked to numerous diseases like cardiovascular diseases and cancer. Natural antioxidants are increasingly being acknowledged as potential preventive medicine. The extract of *Alchornea cordifolia* were examined for their secondary metabolites, antioxidant properties and antibacterial effects on *Escherichia coli* (ATCC28923), *Staphylococcus aureus* (ATCC28923), *Enterococcus faecalis* (ATCC29212) and *Bacillus subtilis* (ATCC6051). Chemical analysis of the plant leaves revealed the presence of saponins, steroids, tannins, alkaloids and general glycoside. The quantitative assessment of the secondary metabolites showed specific concentrations of various compounds in the leaf extract of *Alchornea cordifolia*. The study demonstrated that the antioxidant activity demonstrated using DPPH (2, 2-diphenyl-1-1-picrylhydrazyl) scavenging assay increased with higher concentrations of the leaf extract. Although it was lower than that of ascorbic acid. The susceptibility of the test organisms to the plant extract was evidenced by the zones of inhibition they produced. The results indicated that the bacteria were vulnerable to the plant extract. Specifically, the ethanol leaf extract of *Alchornea cordifolia* showed the lowest minimum inhibitory concentration against *Escherichia coli* (ATCC28923) and *B. subtilis* (ATCC6051) but the highest against *Staphylococcus aureus* (ATCC28923). This study suggests that the compounds present in *Alchornea cordifolia* leaf extract have the potential for future use in chemotherapy applications.

Keywords: *Alchonea cordifolia***,** inflammatory, quantitative**,** antioxidant, antibacterial, susceptibility

1. Introduction
Medicinal plants are increasingly relied upon for healthcare in developing countries, highlighting their importance in both traditional and modern medicine worldwide (Gupa *et al*., 2017; WHO, 2002) [1, 2]. The resurgence of interest in plant- based remedies is driven by the rise of chemoresistance, prompting researchers to seek alternative solutions. In Nigeria, the longstanding use of plants for healthcare is undergoing resurgence among the population. The world health organization reports that 80% of Africans still rely on traditional medicinal practices for their primary healthcare needs (Ayena *et al.* 2021) [3]. The significance of medicinal plants is increasing globally, driven by a rising demand for their products and the expansion of their usage across various domains (Brochot *et al*., 2017) [4]. Presently, it is estimated that 25% of modern medicines trace their origins, either directly or indirectly to medicinal plants, often through the integration of modern technologies with traditional knowledge (Saini *et al*., 2018) [5]. *Alchonea cordifolia*, a member of *Euphobiaceae* family found mainly in African regions, has a long history of traditional use for various ailments such as fungal, bacterial, and inflammatory disorders (Manga *et al*., 2004) [6]. Its leaves, roots and stem barks are utilized alone or in combination to treat conditions ranging from wounds and diarrhea to coughs and asthmatic attacks (Anash *et al*., 2011; Bayor *et al*. 2008) [7, 8]. Among the organs cited above, the leaves are frequently the most used Numerous studies have documented the plant's pharmacological activities, including anti-inflammatory, antidiarrheal, hepaprotective, antiviral, and antidiabetic properties.

(Agbor *et al*., 2004; Odimegwu *et al*., 2018) [9, 10]. These actions are attributed to a range of active compounds found in different parts of the plant, such as alkaloids, fatty acids, terpenoids, steroids, flavonoids, and phenolic acids. (Boniface *et al*., 2018) [11]. These compounds exert significant pharmacological effects on human body and are frequently found in varying concentrations within the organs of some plants leading to a range of uses (Velu *et al*., 2018) [12]. Additionally, the plant's efficacy against various pathogens supports its traditional medicinal uses.

Materials and Methods

Collection of plant material

The plant material was collected from the Botanical garden at the University of Ilorin, Nigeria. The leaves of *Alchornea cordifolia* were authenticated at the International Institute of Tropical Agriculture (IITA) in Ibadan according to the guidelines set by the International Committee for Botanical Nomenclature (ICBN). The fresh leaves were carefully collected, placed in polythene bags, labeled and stored for future use.

Extraction of plant material

The plant leaves were dried in the shade at room temperature, and the plant material was extracted using a method similar to that described by Banso *et al*., (2024a) [13] with slight adjustments. To start 10 grams of plant powder was mixed with 200 mls of methanol in a conical flask. The flask was them covered with aluminum foil, sealed with sterile cotton wool, and placed in a rotary shaker at 37 °C for 24 hours to ensure thorough mixing. After the extraction process, the resulting extract was filtered through Whatman no 1 filter paper and stored in the refrigerator until required.

Qualitative phytochemical screening

Quantitative phytochemical screening was performed on the ethanol leaf extract of the plant material to analyze secondary metabolites.

Test for general glycosides

To test for general glycosides, 1g of coarsely powdered leaf sample was divided into two beakers. In one beaker, 5ml dilute sulphuric acid was added. Both beakers were heated for 5 minutes and the contents were then filtered ino test tubes. The resulting filterate was then made alkaline by adding 5% sodium hydroxide and heated with Fehling's solution for 3 minutes. The formation of a reddish-brown precipitate in the aqueous filtrate indicated the presence of general glycoside (Banso *et al*., 2020) [14].

Test for saponins

To test for saponins, a 0.5g sample of the extract was dissolved in 10 ml of distilled water. The solution was then wormed in a water bath for 5minutes. The emergence of foam during this process indicates the presence of saponins

Test for alkaloids

To conduct the alkaloid test, a 15g sample of the leaf extract was dissolved in 6ml 1% hydrochloric acid (HCl) and stirred in a water bath for 5minutes The resulting solution was the divided into three portions for testing:

1. **Dragendroff's test:** 1ml of Dradendroff's reagent (Potassium bismuth iodide solution) was added to 2ml of the solution. The formation of an orange-coloured precipitate indicated the presence of alkaloids
2. **Mayer's test:** 1ml of Mayer's reagent (mixture of Mercuric chloride and Potassium iodide) was added to another 2ml portion of the solution. A cral coloured precipitate indicated the presence of alkaloids.
3. **Wagner's test:** A couple of drops of Wagner's reagent (Mixture of iodine and potassium iodide) were added to the final 2ml portion of the solution. The presence of a brown precipitate indicated the presence of alkaloids

Test for steroids

To test for steroids, a 10ml chloroform extract of the leaf sample was evaporated to dryness, and the resulting mass was dissolved in 0.5ml of chloroform. Subsequently, 0.5ml of acetic anhydride and 2ml of concentrated sulphuric acid were added (Known as Leibermann-Buchard reaction). The presence of blue-green colour or a combination of these hues was interpreted as a positive indication of the presence of steroidal compounds (Banso *et al*., 2021) [15].

Test for terpenoids

To test for terpenoids, the same method as for steroids was used, with the distinction that the appearance of a red, pink or violet colour was interpreted as a positive indication of the presence of terpenoids.

Test for tannins

To test for tannins, dissolve 0.5g of the extract in 10ml of distilled water, and then add a few drops of 5% ferric chloride. A black or blue-green precipitate would indicate the presence of tannins.

Test for sesquiterpenes

To test for sesquiterpenes, 0.5 ml of aqueous leaf extract was mixed with 0.5ml of methanol by shaking. Then0.4ml of 5% sulphuric acid containing 0.5% ferric chloride was added to the mixture and stirred using a glass rod. The mixtures were boiled in water bath (Grant model) for 1 minute. The emergence of green to black colouration after the addition of ferric chloride was regarded as positive for the presence of sesquiterpenes (Banso *et al*., 2024c) [16].

Quantitative analysis

Determination of total phenolic content

The total phenolic content of the ethanolic leaf extract was determined through the Folin-Ciocalteu method using a UV-Vis spectrophotometer. Initially 1ml of methanol leaf extract was combined with 9 ml of distilled water in a 25 ml volumetric flask and mixed thoroughly. After a 5 minutes interval, 10 ml of 7% sodium carbonate was added followed by vigorous shaking of the mixture. To reach a final volume of 25 ml, 4 ml of distilled water was incorporated, and the flask was then placed in the darkness for incubation. Additionally galic acid standard solutions of varying concentrations (20, 40, 60, 80, and 100 µg/ml) were prepared and also incubated for 90 minutes alongside with the test sample. A blank was prepared using 1ml of distilled water instead of the extract. Following the incubation period, the absorbance was measured using UV-Vis spectrophotometer at 550 nm (Chakraborty *et al*., 2002; Geetha *et al*., 2010) [17, 18].

Determination of total tannin content

The total tannin content in the ethanol leaf extract was determined using the Folin-Ciocalteu method and a UV-Vis spectrophotometer. To a 10 ml volumetric flask, 1 ml of 1mg/ml ethanol leaf extract was mixed with 7.5ml of distilled water. Following this, 0.5 ml of Folin-Ciocalteu reagent was added and the solution was well mixed. After 5-minute incubation, 1ml of 35% sodium carbonate was added, and the volume was adjusted to 10
ml. A blank sample was prepared by substituting the extract with 1 ml of distilled water. Standard solution of gallic acid at concentrations of 20, 40, 80, and 100 µg/ml were also prepared. The absorbance of the samples was measured at 725 nm using a UV-Vis spectrophotometer after 30 minutes of incubation (Hullatti and Murthy, 2010) [19].

Determination of total flavonoid content

The total flavonoid content in the leaf extract was determined adding 1ml of a 1mg/ml extract to a 10ml volumetric flask this was followed by addition of 4ml of distilled water, 0.3ml of 5% sodium nitrate and 0.3 ml of 5% aluminum chloride. Next, 2 ml of 1M sodium hydroxide was added to the mixture and shaken well and the final volume was adjusted to 10 ml by adding 2.4 ml of distilled water. A blank solution was prepared using 1ml of distilled water instead of extract. Additionally, standard solutions of quercetin (20, 40, 60, 80, and 100 µg/ml) were prepared by first desolving quercetin in methanol. After a 30-minute incubation period the absorbance of both the standard and test solutions was measured at 510nm using a UV-Vis spectrophotometer (Akhtar and Iqbal, 1991; Malarvili and Ganathi, 2009) [20, 21].

Antioxidant activity

DPPH (2, 2-diphenyl-1-1-picrylhydrazyl) Radical scavenging assay. For the assessment of antioxidant activity using a DPPH (2, 2-diphenyl-1-1-picrylhydrazyl) Radical scavenging assay, a DPPH stock solution was prepared by dissolving 2.4 mg DPPH in 100 ml of methanol. Various concentrations (50, 100, 150, 200, and 250 µg/ml in ethanol) of the plant extract were then prepared. Each concentration (100 µl) of the leaf extract was combined with 3ml of DPPH in a 25ml volumetric flask. Similarly, Standard solution of ascorbic acid (at concentrations of 50, 100, 150, 200, and 250µg/ml in distilled water) were prepared following the same procedure. A control sample containing only DPPH was prepared methanol was used as blank. After a 30-minute incubation period, the absorbance of both the standard and test solutions was measured at 515nm using a UV-Vis spectrophotometer. The following formula adopted from Banso *et al*. (2024b) [22] was utilized to calculate the radical scavenging activity of the leaf extract.

$$\text{DPPH \%} = \frac{\text{Ab(control)} - \text{Ab(sample)}}{\text{Ab(control)}} \text{ X } 100$$

(Ab(control)) = Absorbance of the control sample (DPPH only)
(Ab(sample)) = Absorbance of the test sample (DPPH + leaf extract)

Antimicrobial activity

Test organism

Strains of *Escherichia coli* (ATCC28923), *Staphylococcus aurteus* (ATCC28923), *Enterococcus faecalis* (ATCC29212) and *Bacillus subtilis* (ATCC6051) were utilized in this study. The strains were acquired from National Institute of Pharmaceutical Research, Abuja, Nigeria.

Standardization of bacteria

The bacteria suspension was standardized using a McFarland standard to achieve a turbidity level equivalent to$1X10^6$ bacteria cell/ml (0.5 McFarland standards) and this level was maintained consistently during the entire study.

Antibacterial bioassay

Agar diffusion method adopted by Banso *et al*. (2024a)[13] was utilized to assay for the effect of the leaf extract against *Escherichia coli* (ATCC28923), *Staphylococcus aurteus* (ATCC28923), *Enterococcus faecalis* (ATCC29212) and *Bacillus subtilis* (ATCC6051). The cells of the representative bacteria were inoculated on different nutrient agar plates and spread uniformly using a sterile glass spreader. A sterilized pastures pipette was used to introduce 200µl of 50mg/ml [leaf extract/DMSO] of the extract of the plant material into the wells bored on the surface of the culture (Banso and Banso)[13]. The plates were left at room temperature for an hour to facilitate substance diffusion before the growth of the organisms. Subsequently the organisms were incubated at 37°C for 24hours, and the zones of inhibition were recorded; positive control wells containing antibiotics were also included for comparison. Antibacterial activities were measured as diameter of zone of inhibition and compared with the zone of inhibition produced by the positive control (Amoxillin).

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was assessed following the approved broth microdilution method by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The tested extract dissolved in 10% DMSO was serially ½ diluted directly in a microtiter plate containing Mueller Hibton broth. This led to a concentration of $5x10^6$cfu/ml in each well for assessment. A positive control with amoxillin at final concentration of 10µg/ml was included. The plate was covered with a sterile sealer and incubated for 24 h at 37 °C. The plate was then sealed and incubated with for 24 h at 37 °C. MIC was defined as the lowest extract concentration of the extract that completely hindered bacterial growth, with lower MIC indicating higher extract activity (Banso *et al*., 2021) [15].

Data analysis

The results obtained underwent analysis of variance, and mean comparisons were carried out using Turkey's multiple range tests with SPSS version 20.0 (IBM Corporatio, Armonk, NY, USA). Statistical significance was determined at p-value below 0.05.

Results

Phytochemical study of the plant leaf used in this study showed that *Alchornea cordifolia* contained saponins, steroids, tannins, alkaloids and general glycoside. Terpenoids and sesquiterpens were however absent in the plant leaf (Table 1).

Table 1: Phytochemical constituent of leaf extract of *Alchornea cordifolia*

Active principle	leaf extract
Terpenoids	-
Saponins	+
Steroids	+
Sesquiterpens	-
Tannins	+
Alkaloids	+
General glycoside	+

(+) = Detected (-) = Not detected

Total secondary metabolite in leaf extract of *Alchornea cordifolia*

The total saponins, steroids, tannins, alkaloids and general glycoside detected in the leaf extract of *Alchornea cordifolia* were 11.5±0.4, 12.0±0.1, 13.5±2.0, 9.5.5±2.0 and 19.0±1.0 mg/100g respectively (Table 2)

Table 2: Total secondary metabolites detected in ethanol leaf extract of *Alchornea cordifolia*

Active principle	Quantity (mg/100g) ± SD
Terpenoids	ND
Saponins	11.5±0.4
Steroids	12.0±0.1
Sesquiterpens	ND
Tannins	13.5±2.0
Alkaloids	9.5±2.0
General glycoside	19.0±1.0

ND = Not detected

DPPH (2, 2-diphenyl-1-1-picrylhydraxyl) scavenging activity

The DPPH scavenging activity of ascorbic acid and leaf extract of *Alchornea cordifolia* are shown in Table 3. The results showed that the DPPH scavenging activity is concentration dependent. Values recorded for ascorbic acid at concentrations of 50, 100, 150, 200 and 250µg/ml were 35.00±2.40, 55.00±3.22, 63.46±1.09, and 88.50±3.10 and 96.45±2.60% respectively. However values of 8.46±2.05, 14.90±4.50, 25.20±2.50, and 36.41±3.05 and 45.89±3.40% respectively were recorded for *Alchornea cordifolia.*

Table 3: DPPH Scavenging activity of ascorbic acid and leaf extract of *Alchornea cordifolia.*

Concentration (µg/ml)	Percentage of DPPH scavenged by ascorbic acid ±SD	Percentage of DPPH scavenged by leaf extract ±SD
50	35.00±2.40	8.46±2.05
100	55.00±3.22	14.90±4.50
150	63.46±1.09	25.20±2.50
200	88.50±3.10	36.41±3.05
250	96.45±2.60	45.89±3.40

SD = Standard deviation

Antimicrobial properties of leaf extract of *Alchornea cordifolia*

Diameter of zones of inhibition recorded against the test organisms ranged between 11.5±0.4 and 18.4±0.1mm. Zones of inhibition of 11.5±0.4, 15.5±2.0 and 13.5±0.1mm were recorded against aqueous, methanol and ethanol extracts respectively when assayed against *E. coli* (Table 4) Values of 14.5±0.2, 18.4±0.1 and 16.5±2.0mm were recorded against *E. faecalis* when the extracts were assayed against the organism. Aqueous, methanol and ethanol extracts produced 13.9±2.5, 17.5±0.5 and 16.9±0.6mm respectively when the extracts were assayed against *B. subtilis* (Table 4).

Table 4: Susceptibility pattern of leaf extracts of *Alchornea cordifolia.*

	Leaf extract (50mg/ml)			Standard antibiotic
	Diameter of zone of inhibition (mm) ± SD			
Bacteria strain	Aqueous Ex	Methanol Ex	Ethanol Ex	Amoxillin
E. coli	11.5±0.4	15.5±2.0	13.5±0.1	28.6±2.5
S. aureus	13.6±1.2	16.0±0.2	14.6±0.1	32.7±0.1
E. faecalis	14.5±0.2	18.4±0.1	16.5±2.0	39.0±0.2
B. subtilis	13.9±2.5	17.5±0.5	16.9±0.6	34.0±0.1

SD = Standard deviation, Ex = extract

The result of the minimum inhibitory concentration indicates that the minimum inhibitory concentration of methanol leaf extract of *Alchornea cordifolia* against *E. coli* was 25 mg/ml; however the minimum inhibitory concentration recorded against *S. aureus, E. faecalis, B. subtilis* were 40, 30 and 35 mg/ml respectively (Fig. 1).

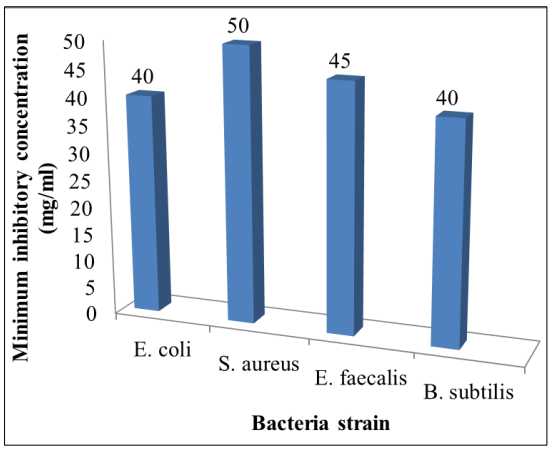

Fig 1: Minimum inhibitory concentration (mg/ml) of ethanol extract of *Alchornea cordifolia* against the test bacteria

Discussions

The leaf extract of *Alchornea cordifolia* contains saponins, steroids, tannins, alkaloids and general glycoside while terpenoids and sesquiterpens were not found. These findings align with those of Yang *et al*. (2018) [23], who suggested that certain secondary metabolites could be present only in specific plant parts, with environmental stress potentially influencing the abundance of different secondary metabolites in plants.

The qualitative and quantitative production of secondary plant metabolites can be affected by various agronomic factors such as developmental stage, plant organs, fertilization and soil pH. Climatic conditions like light intensity and water availability, as well as genetics, can also impact both the quantity and quality of the phytochemicals (Bjorkman *et al.,* 2011) [24]. This study demonstrated that the DPPH (2, 2-diphenyl-1-1-picrylhydrazyl) scavenging activity of leaf extract of *Alchornea cordifolia* extract increased with higher concentrations, albeit wiyth lower antioxidant potential compared to ascorbic acid. Antioxidants inhibit oxidation in molecules, preventing oxidative chain reactions. Oxidative prowess of *Alchornea cordifolia* leaf likely stems from flavonoids phenolics, tannins and glycosides present within the leaf. This compound particularly phenolics, exhibit redox properties, crucial for neutralizing free radicals, oxygen singlets, triplets and decomposing peroxides. Phenolic compounds acting as natural antioxidants, transform DPPH from a purple stable radical to a colourless form, indicating their ability to donate hydrogen and reduce DPPH to DPPH-H. This is observed through the bleaching of purple DPPH solution (Vazqez-Leon *et al.,* 2017) [25].

This study found that leaf extracts of *Alchornea cordifolia* exhibited antibacterial properties against *E. coli* (ATCC28923), *S. aureus* (ATCC28923) *E. faecalis* (ATCC29212)) and *B. subtilis* (ATCC6051). The size of the inhibition zones varied among the tested organisms, suggesting different levels of susceptibility to the plant extracts. Banso et al. (2024c) [16] noted that the effectiveness of an agent can differ depending on the target species. Additionally, Banso *et al*. (2021) [15] emphasized that factors such as initial population density, growth rate and diffusion rate of the antimicrobial agent can influence the position of the zone of inhibition. These variations underscore the potential of the plant extract and support its use in medicinal applications, likely due to the presence of observed phytochemicals, which are significant sources of pharmaceutical compounds (Banso *et al*., 2024b) [22].

In this study, the ethanol leaf extract of *Alchornea cordifolia* displayed the lowest minimum inhibitory concentration against *Escherichia coli* (ATCC28923) and highest against *Staphylococcus aureus.* Banso *et al.* (2024c) [16] proposed that antimicrobial agents with lower activity tend to have highest minimum inhibitory concentration values, while highly effective agents exhibit lower values. This research suggests that the compounds found in *Alchornea cordifolia* leaf extract hold promise for potential application in chemotherapy.

Conclusion

Leaf extracts of *Alchornea cordifolia* contains saponins, steroids, tannins, alkaloids and general glycoside. DPPH (2, 2-diphenyl-1-1-pirylhydrazyl) scavenging activity of leaf extract of the plant increases with higher concentrations of the extracts. The antimicrobial properties of *Alchornea cordifolia* leaf extract is due to the presence of, steroids, tannins, alkaloids and general glycoside The identified components in the extract show promise for use as antimicrobial agents in the development of new drug formulations.

Conflict of interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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