Walisongo Journal of Chemistry
Walisongo Journal of Chemistry Vol. 7 Issue 1 (2024), 057-069
ISSN: 2621-5985 (online); 2549-385X (print)
DOI: https://doi.org/10.21580/wjc.v7i1.20667

Comparative Studies of the Phytochemical Analysis, Mineral Content, Antibacterial Activity and Antioxidant Potential of Ethyl Acetate Extract of *Alstonia Boonei* Leaves and Stem Bark

Jeremiah Ogboma Uadia[1], Osahon Kennedy Ogbeide [1]*

[1]Department of Chemistry, Faculty of Physical Sciences, University of Benin, P.M.B. 1154, Benin City, Nigeria
Email : kennedy.ogbeide@uniben.edu

Abstract

Folkloric medicine has reported the effectiveness of Alstonia boonei stem bark and leaf extracts in treating and managing different ailments. Hence, this research is a comparative evaluation of the phytochemicals; antioxidant potential, mineral content, and antibacterial capacity of ethyl acetate extract of Alstonia boonei leaves and stem bark. Phytochemical analysis was determined using standard methods; mineral composition was determined using a flame photometer and an atomic absorption spectrophotometer; antibacterial capacity was analysed by agar-well diffusion method; and antioxidant potential was examined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method. The phytochemicals detected were phenolics, eugenols, steroids, flavonoids, alkaloids, and reducing sugars in both extracts. Mineral content for sodium, iron, potassium, zinc, copper, magnesium and calcium was found to be 5.61 ± 1.33, 7.25 ± 1.00, 120.63 ± 1.39, 1.62 ± 0.30, 1.01 ± 0.01, 2.36 ± 0.10 and 15.61 ± 1.20 mg/kg respectively for the leaf extract, and 9.36 ± 0.35, 6.36 ± 0.55, 113.64 ± 0.45, 2.40 ± 0.32, 0.02 ± 0.00, 2.10 ± 0.50 and 28.30 ± 0.22 mg/kg respectively for the stem bark extract. The antioxidant potential at 250, 200, 150, 100, and 50 g/mL gave an IC_{50} of 2.89 ± 0.51, 3.90 ± 0.50, and 2.52 ± 0.22 µg/mL for leaf extract, stem bark extract and standard, respectively. The minimum inhibitory concentration (MIC) outcome was identical for both extracts except for P. aeruginosa. Both extracts' minimum bactericidal concentration (MBC) values were 12.5 to 50 mg/mL, except B. subtilis. Hence, this result provides valuable insight into the comparative usefulness of the plant in phytomedicine.

Keywords: *Alstonia boonei; Antioxidants potential; Mineral composition; Phytochemical; Antibacterial activity*

Introduction

For many years, traditional herbal remedies have been used as medication for different human ailments (Ogbeide *et al*., 2020). The active phytochemicals in various plant sections give various plants their therapeutic qualities (Ogbeide *et al*., 2022; Ogbeide & Akhigbe, 2019). Due to their excessive pharmacological ability, plants continue to be used in traditional drugs to treat different ailments (Ogbeide *et al*., 2018). The compounds that exhibit therapeutic actions against numerous diseases in man are secondary metabolites, which consequently clarify the practice of traditional healing herbs to treat various ailments (Ogbeide *et al*., 2019). Hence, plants have been employed as anti-inflammatory, anti-mutagenic, anti-carcinogenic, antimicrobial and antioxidant drugs (Ogbeide *et al*., 2022; Aghedo & Ogbeide, 2022).

Antioxidants work to combat free radicals and the oxidative reactions they start, protecting cells from potential oxidative reactions-related harm. Reactive species of nitrogen and oxygen, often formed during the metabolic process of cells and by external causes, are examples of free radicals that can cause oxidative mutilations and can be avoided by consuming plants (Ighodaro & Ogbeide, 2020). Depending on where they come from, antioxidants can be subdivided into two categories: natural and synthetic. Today, it is generally accepted that natural antioxidants are pretty safe and help increase the nutritional quality of our meals, leading to good health (Ogbeide *et al*., 2022; Oluwafemi *et al.,* 2015). Antibiotics can occasionally have adverse effects on the body, including hypersensitivity, the loss of beneficial microbes in the gut and mucosa, immunosuppression, and allergic responses (Ogbeide *et al*., 2023). In order to cure infectious disorders, there is a greater need for the research and advancement of novel antibacterial and antifungal medications (Uadia *et al.,* 2023).

Alstonia boonei (Apocynaceae) is a deciduous plant common in rainforests in Uganda, Senegal, Egypt, western Cameroon, Sudan, and Zaire (Okoye *et al*., 2022). According to Oppong et al. (2020), several local names have been assigned, including Emien (Ivory Coast), Awun (Nigeria), Kaini (Sierra Leone), Sinupo (Ghana), Mujina (Uganda), and Botuk (Cameroon). *Alstonia boonei* is used as a remedy for strophanthus poison and for treating digestive diseases, fever, discomfort, chronic diarrhoea and dysentery. The stem bark of *A. boonei* has been utilized to heal fever, malaria, sleeplessness, rheumatic pain, chronic diarrhoea, arrow poisoning and anti-venom for snake bites. According to Uzor *et al*. (2017), the stem bark extract of *A. boonei* can be used to control post-partum haemorrhage, induce labour, and remove retained placenta.

Figure 1: An overview of *A. boonei* plant

A. Boonei leaves are ground up and administered topically in several regions of central and West Africa to heal ulcers, rheumatic aches, muscle pains, and hypertension and reduce swelling. In order to treat resistant malaria, an extract of the leaves is also employed (Omoya & Oyebola, 2019). Most recent compounds of *A. Boonei* isolated were: isobutyryl acetate and tetrahydro-4-(7-hydroxy-10-methoxy-6, 14-dimethyl-15-m-tolylpentadec 13-enyl) pyran-2-one (Olanlokun *et al.,* 2020).

However, this study stands to inform the various information and studies on the scientific investigation and evaluation of *Alstonia boonei's* leaf and stem bark extracts for the management of various ailments, as there seem to be derisory scientific reports on the comparative studies of the phytochemical analysis, mineral content, antibacterial activity and antioxidant potential of the extracts. In light of this, it became imperative to undertake this current study aimed to examine the phytochemicals, antioxidant potential, mineral composition, and antibacterial activities of *Alstonia boonei's* ethyl acetate leaf and stem bark extracts for comparative studies.

Methods

Plant samples collection

The fresh *A. boonei* leaves and stem bark were obtained in May 2023 from the University of Benin's botanical garden, Benin City, Nigeria. Prof. Akinnibosun

identified and authenticated them in the herbarium section of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Nigeria, where a voucher specimen number UBH-A591 was deposited.

Sample preparation and extraction

The fresh leaves and stem bark were cleaned, cut into pieces, and air-dried for three weeks before ground into a coarse powder via the British milling machine (AJT 200 turret mill). With occasional manual stirring and shaking, ethyl acetate (1.5 L) was macerated with the powdered samples (500 g). The mixtures were filtered via Whatman grade 1 qualitative filter paper after 72 hours, and the residues in both extractions were then macerated for an additional 72 hours with the solvent (750 mL) and filtered. The filtrates were concentrated to dryness with the help of a rotary evaporator at 40°C. The weights of the extracts were taken and then stored in the fridge freezer at four °C until analysis. Equation 1 was used to compute the percent yield.

$$\% \text{ yield} = \frac{\text{weight of extract}}{\text{weight of sample}} \times 100 \ldots\ldots\ldots (1)$$

Qualitative phytochemical screening

An established approach described by Trease and Evans (2002) and revised by Uadia et al. (2023) was used to screen the bioactive components of ethyl acetate leaves and stem bark extracts of *A. boonei.*

Determination of Mineral Content

1 g of the extracts was added to a Kjeldahl flask that had been treated with 10 mL of a 3:1 mixture of nitric and perchloric acid. A temperature of 40°C was used to gently heat the flask and its contents for about 20 minutes, and the temperature was raised to 100°C for an additional 20 minutes. After cooling, distilled water (20 mL) was added and filtered into a standard flask (100 mL). AAS measured calcium, magnesium, iron, copper, and zinc, while a flame photometer measured sodium and potassium.

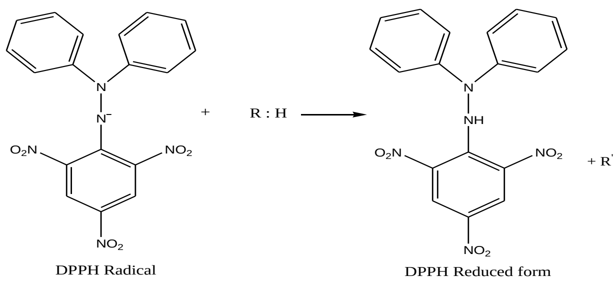
Determination of Antioxidant Potential

The technique published by Kim *et al.* (2003) was employed to evaluate the DPPH radical-scavenging capacity of the leaf and stem bark extracts. The extract (3.0 mL) in methanol comprising 0.001-0.1 mg/mL of the extract was combined with 0.1 mM DPPH (1.0 mL) in methanol solution. After complete vortexing, the mixture was kept at room temperature in the dark for 30 minutes. A visible spectrophotometer (Spectrum Lab 23A, China) was used to test the absorbance at 517 nm. The reference standard utilised was ascorbic acid. The equation below was used to determine DPPH radical scavenging capacity.

$$\text{DPPH radical scavenging activity (\%)} = \frac{A0 - A0}{A1} \ldots\ldots\ldots (2)$$

Where: A_0 = DPPH radical absorbance in methanol; A_1 = DPPH radical absorbance + sample extract/standard in methanol.

The IC_{50} value, the 50% inhibitory concentration, was determined by an exponential equation to match data into the concentration response. The reaction of DPPH with antioxidant species is shown in Scheme 1 below.



Scheme 1: The reaction of DPPH with an antioxidant species (R : H).

Preparation of stock solution

The extracts were synthesised in two-fold dilutions, producing increasing concentrations of 50, 100, 150, 200, and 250 mg/mL. Methanol (20 mL) dissolved 0.045g

of DPPH powder before filling the standard flask to the 100 mL mark. To produce a decreasing concentration similar to those indicated above, 0.25 g of ascorbic acid powder was dissolved in distilled water (40 mL) and made up to 100 mL with distilled water. This study dissolved 1,1-diphenyl-2-picrylhydrazyl (DPPH) in a 0.1 mM methanol solution. The extracts (2 mL) were mixed with DPPH solution (2 mL) at 50, 100, 150, 200, and 250 mg/mL concentrations. At each of the five stated concentrations, 2 mL of the DPPH/methanol solution (2 mL) was mixed with the ascorbic acid/water solution (2 mL) in a test tube. The DPPH/methanol solution (2 mL) was poured into a test tube. The test tubes were vigorously shaken and placed in the dark for 30 minutes. Then, at 517 nm, each absorbance was measured. The 50% inhibition (IC_{50}) was determined from the calibration curve by plotting the percentage of DPPH scavenging activity against the sample concentration. Equation 2 was used to calculate the extracts' DPPH radical scavenging potential.

Determination of antibacterial activity

Sourcing of microorganisms

Clinical isolates of *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus* and *Bacillus substilis* were obtained from University of Benin Teaching Hospital (UBTH), Benin City, Nigeria.

Preparation of bacterial Isolates

A loopful of every strain of bacteria was immunised into 50 mL of sterile nutritional broth in a conical flask (100 mL) to sustain the bacterial strains on a Mueller-Hinton agar medium at 400°C. To activate the strain, the flask of culture was incubated on a rotating shaker for 24 hours.

Preparation of the stock solution

Tween 20 dissolved 1g of the leaves and stem bark extract (3 mL), producing a stock solution with a 250 mg/mL concentration. After that, 0.2 mL of the stock solution's 50 mg/mL concentration was collected.

Determination of zone of inhibition

The antibacterial activity of ethyl acetate leaf and stem bark extracts was tested using the agar-well diffusion method. Cotton swabs were used to distribute 0.2 mL of germs aseptically added to sterile Muller-Hinton agar plates. On each plate, a diameter (6.0 mm) well was aseptically pierced using a disinfected cock borer. After that, the extract (0.2 mL) was added to the wells of the plates using a tiny pipette. A positive control well was also created using 0.2 mL of the antibiotic Gentamycin, followed by incubation of plates for 24 hours at 370 °C. The dimension of the inhibition zone encircling each well was assessed for antibacterial activity using a graduated metre rule. The relative effectiveness of extracts against the different test microbes is indicated by the breadth of the inhibition zone (Iyasele *et al.,* 2022; Opoku & Akoto, 2014).

Minimum inhibitory concentration (MIC)

The lowermost concentration that prevented germs from growing visibly for 24 hours after incubation was called the MIC. The agar-well diffusion technique was engaged to determine minimal inhibitory concentration. A 1000 mg/mL concentration was obtained by diluting 1g of the extract with 1 mL of tween 20. To create a 1 in 20 dilution, this was added to 19 mL of molten Muller-Hinton agar, yielding a final concentration of 6.25, 12.5, 25 and 50 mg/mL (Afolayan & Meyer, 1997). The culture bacteria were streaked over the Muller-Hinton agar-filled petri plates that had previously hardened using a sterilised wire loop. All agar plates were incubated for 18–24 hours at 37°C (Afolayan & Meyer, 1997). The lowermost

 | ISSN 2621-5985 (online) | ISSN 2549-385X (print)
Volume 7, Nomor 1, 2024

concentration at which a distinct inhibition zone was noticed was used to compute the MIC.

Minimum bactericidal Concentration (MBC)

Re-culturing (sub-culturing) broth dilutions that hindered the proliferation of isolated bacteria (i.e., after MIC determination) allowed for the measurement of the MBC, which is referred to as the lowest concentration at which a bacterium isolate may be destroyed. All plates were incubated for 18 to 24 hours at 370°C (Iyasele *et al.,* 2022).

Statistical analysis

The obtained values were analysed using the triplicate determination's mean and standard deviation.

Results and Discussion

The percentage yield of extract

The percentage yields for the leaves and stem bark extracts were 4.58 % and 4.20 %, respectively. The leaf extract has a higher yield, which agrees with Okorie *et al*. (2022).

Qualitative phytochemical screening

Table 1: Qualitative phytochemical results of ethyl acetate leaf and stem bark extract of *A. boonei.*

Phytochemicals	Leaf extract	Stem bark extract
Alkaloids	+	+
Phenolics	+	+
Eugenols	+	+
Steroids	+	+
Terpenoids	-	-
Glycosides	-	-
Tannins	-	-
Saponins	-	-
Flavonoids	+	+
Reducing Sugar	+	+

(+) indicates present and (-) indicates absent

Phytochemical screening of the ethyl acetate leaves and stem bark extract of *A. boonei* indicated the presence of therapeutically active components such as alkaloids, phenols, eugenols, steroids, flavonoids, and reducing sugar (Table 1). This outcome agrees with existing literature by Mollica *et al*. (2022). These phytochemicals have been linked to antibacterial action in the past. These biologically active components are known to exhibit antibacterial activity through various mechanisms. Alkaloids are helpful in medicine because they have antibacterial, antispasmodic, and analgesic properties. It should not be surprising that flavonoids, which are hydroxylated phenolic chemicals identified to be produced by plants as a response to microbial contamination, have been demonstrated to be efficient antibacterial agents in vitro against various pathogens. Their capacity to interact with solvable extracellular proteins and bacteriological cell walls is likely what causes them to be active (Uadia *et al.,* 2023). The association between membrane lipids and steroid sensitivity suggests the mechanism by which steroids selectively bind with membrane lipids and exert their effect by generating liposome leakages. Steroids have also been demonstrated to have antimicrobial characteristics (Alagbe, 2019).

Mineral content

Table 2: Mineral content of ethyl acetate leaf extract of *A. boonei*

Elements	Leaf Extract (mg/kg)	FAO/WHO Permissible limit (mg/kg)
Zinc	1.62 ± 0.30	10
Calcium	15.61 ± 1.20	-
Potassium	120.63 ± 1.39	-
Copper	1.01 ± 0.01	10
Magnesium	2.36 ± 0.10	-
Iron	7.25 ± 1.00	20
Sodium	5.61 ± 1.33	-

Values are mean ± standard error of the mean of triplicate analysis.

Table 3: Mineral content of ethyl acetate stem bark extract of *A. boonei.*

Elements	Stem bark extract (mg/kg)	FAO/WHO Permissible limit (mg/kg)
Zinc	2.40 ± 0.32	10
Calcium	28.30 ± 0.22	-
Potassium	113.64 ± 0.45	-
Copper	0.02 ± 0.00	10
Magnesium	2.10 ± 0.50	-
Iron	6.36 ± 0.55	20
Sodium	9.36 ± 0.35	-

Values are mean ± standard error of the mean of triplicate analysis.

A comparative evaluation of the mineral content of the leaves and stem bark showed that of the seven elements investigated, potassium was found to have the highest concentration in both extracts but was relatively high in the leaf extract, while copper was the lowest in both extracts but had a relatively higher concentration in the stem bark extract compared to the leaves (Figure 2). The increase in the relative abundance of elements in *A. boonei* leaf extract was as follows: Cu, Zn, Mg, Na, Fe, Ca, and K; while for the stem bark extract, it is Cu, Zn, Mg, Fe, Na, Ca, and K. The stem bark has a relatively high concentration of Cu, Zn, Na, and Ca, while the leaf extract has a relatively high concentration of Mg, Fe, and K. This result agrees with Oppong *et al*. (2020).

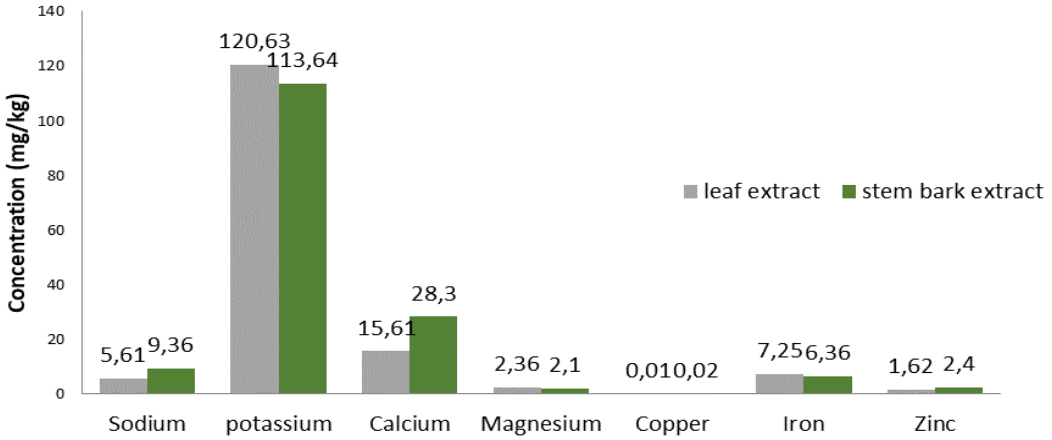


Figure 2. Comparative mineral content of leaf and stem bark extract

Copper and iron minerals are necessary for healthy development, cellular function, and oxygen transport. Also, calcium is required for proper growth and muscular function, while sodium and potassium are for skeletal development, fluid balance, and nerve transmission (Alagbe, 2019). Furthermore, iron helps prevent anaemia and other associated disorders, while sodium contributes to the ionic equilibrium of the human system and preserves tissue excitability. Sodium has also been proven to be crucial in the conveyance of metabolites due to the solubility of salts. Hence, these minerals and nutrients have been proven to impact humans' and animals' health and performance when deficient (Oppong *et al*., 2020; Alagbe, 2019).

Antioxidant potential

Table 4. The antioxidant ability of ethyl acetate leaves extract of A. boonei.

Concentration (µg/mL)	Leaf extract (%)	Ascorbic acid (%)
50	3.48 ± 0.25	13.61 ± 0.05
100	6.39 ± 0.54	30.77 ± 0.50
150	22.23 ± 1.45	31.81 ± 0.05
200	43.42 ± 1.25	33.16 ± 0.02
250	48.32 ± 2.45	49.87 ± 0.32

Values are mean ± standard error of the mean of triplicate analysis.

Table 5. The antioxidant ability of ethyl acetate stem bark extract of A. boonei

Concentration (µg/mL)	Stem bark extract (%)	Ascorbic acid (%)
50	26.50 ± 0.22	13.61 ± 0.75
100	28.64 ± 0.40	30.77 ± 0.58
150	29.03 ± 0.01	31.81 ± 0.09
200	29.29 ± 1.25	33.16 ± 0.02
250	35.16 ± 0.25	48.32 ± 0.66

Values are mean ± standard error of the mean of triplicate analysis.

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical is a stable radical that can easily undergo reduction in the presence of an antioxidant (Scheme 1) and its maximum absorption occurs at 517 nm (purple colour) in the UV spectrum. The level of discoloration reveals the antioxidant's capacity for scavenging. This reaction is now widely used in the evaluation of free radical-scavenging activity investigation due to its simplicity and convenience (Uadia *et al*., 2023; Ogbeide *et al*., 2022).

Using the DPPH assay, a biological test frequently used to assess the antioxidant capability of natural plants and chemicals (Kim *et al*., 2003); a thorough investigation of the antioxidant ability of *A. boonei* leaves and stem bark extracts was conducted. The *A. boonei* leaf and stem bark extract had intense DPPH free radical scavenging activity at 200 and 250 µg/mL doses, mild at 150 µg/mL, and weak at 100 and 50 µg/mL. At 250 µg/mL, being the highest concentration considered, the scavenging effect of the leaf extract was found to be 48.32 ± 2.45%, which was close to the value 49.87 ± 0.32% obtained for the standard, while that obtained for the stem bark extract was 35.16 ± 0.25%. The free radical scavenging activity of the extracts was remarkable, although concentration-dependent.

Table 6: IC_{50} values of Leaf and stem bark extracts of *A. boonei* and standard (ascorbic acid)

Sample	IC_{50} (µg/mL)
Ascorbic acid	2.52 ± 0.22
Leaf extract	2.89 ± 0.51
Stem bark	3.90 ± 0.50

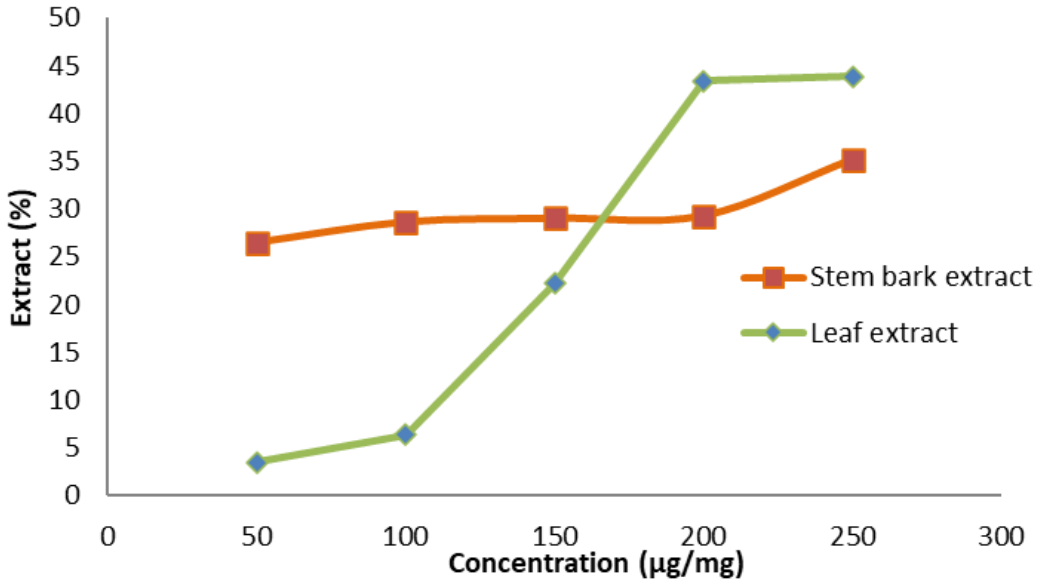


Figure 3. Comparative antioxidant potential of leaf and stem bark extract

The extracts of the leaves and stem bark were found to have IC_{50} values of 2.89 ± 0.51 and 3.90 ± 0.50 µg/mL, respectively, while the reference value was 2.52 ± 0.22 µg/mL. These values demonstrated that the standard was more active than the leaves and stem bark extract since activity increases as the IC_{50} value decreases (Uadia *et al.,* 2023; Oluwafemi *et al.,* 2015). Like stem bark extracts, leaf extracts had more robust antioxidant activity (Figure 4). This result is consistent with prior research by Mollica *et al*. (2022).

Table 7. Antibacterial activity of leaf extract

Bacterial Isolates	50 mg/mL	Gen 80 mg/mL
Pseudomonas aeruginosa	35.0	18.0
Staphylococcus aureus	27.0	25.0
Klebsiella pneumonia	30.0	20.0
Bacillus subtilis	26.5	25.0
Escherichia coli	29.0	24.0

Antibacterial activity

A comparative evaluation of the antibacterial activity of the ethyl acetate leaves and stem bark extract of *A. boonei* at a concentration of 50 mg/mL (Figure 4) displayed significant activity against *P. aeruginosa, K. pneumoniae, E. coli, B. subtilis* and *S. aureus*. This means that all the bacteria isolates were sensitive, although to varying degrees, to the leaf and stem extract as per the National Committee for Clinical Laboratory Standards (NCCLS) guidelines (NCCLS, 2021). *P. aeruginosa* and *S. aureus* are more sensitive to the leaf extract than the stem bark extract, while *E. coli* and *B. subtilis* are more sensitive to the stem bark extract than the leaf extract. Meanwhile, *K. pneumonia* exhibits the same sensitivity to both extracts. This finding agrees with Opoku and Akoto (2014).

Determination of minimum inhibitory concentration (MIC)

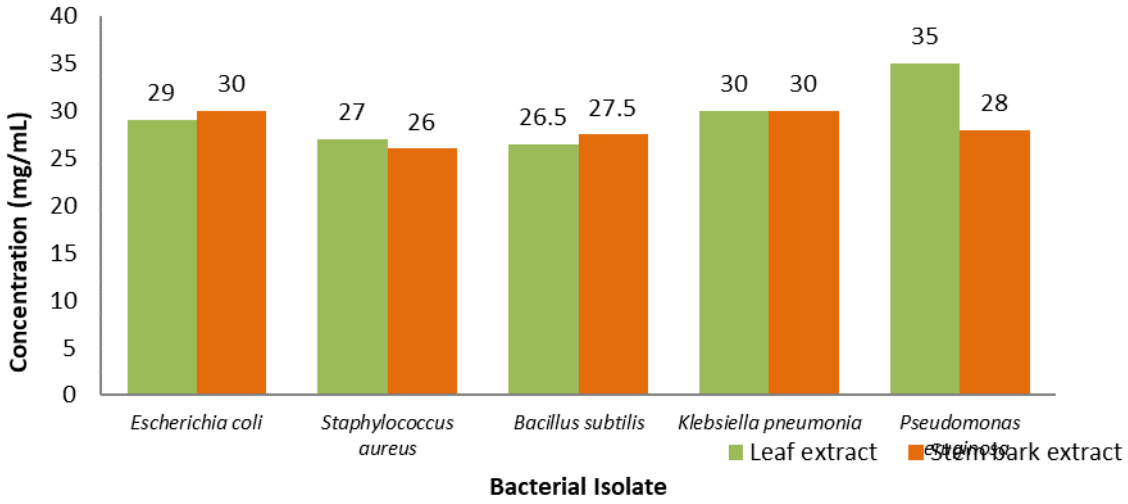


Figure 4. Comparative antibacterial activity of leaf and stem bark extract

Table 8. Antibacterial activity of stem bark extract

Bacterial Isolates	50 mg/mL	Gen 80 mg/mL
Pseudomonas aeruginosa	28.0	18.0
Staphylococcus aureus	26.0	25.0
Klebsiella pneumonia	30.0	20.0
Bacillus subtilis	27.5	25.0
Escherichia coli	30.0	24.0

Table 9. Minimum Inhibitory Concentration of ethyl acetate leaves extract of *Alstonia boonei*

Bacterial Isolates	6.25(mg/mL)	12.5 (mg/mL)	25 (mg/mL)	50 (mg/mL
	Zones of Inhibition			
Pseudomonas aeruginosa	G	G	NG	NG
Staphylococcus aureus	G	NG	NG	NG
Klebsiella pneumonia	G	NG	NG	NG
Bacillus subtilis	G	NG	NG	NG
Escherichia coli	G	NG	NG	NG

Key: G= Growth NG= No Growth

Table 10. Minimum Inhibitory Concentration of ethyl acetate stem bark extract of *Alstonia boonei.*

Bacterial Isolates	6.25 (mg/mL)	12.5 (mg/mL)	25 (mg/mL)	50 (mg/mL)
	Zones of Inhibition			
Pseudomonas aeruginosa	G	G	G	G
Staphylococcus aureus	G	NG	NG	NG
Klebsiella pneumonia	G	NG	NG	NG
Bacillus subtilis	G	NG	NG	NG
Escherichia coli	G	NG	NG	NG

The results of the comparative study of the Minimum Inhibitory Concentration of ethyl acetate extract of *A. boonei* leaves and stem bark (Tables 9 and 10) showed that for both extracts, *E. coli, B. subtilis*, *S. aureus* and *K. pnuemoniae* were all inhibited by the extract at 12.5 mg/mL, and *P. aeruginosa* was inhibited at 25 mg/mL. This is in line with Kokkaiah *et al*. (2017).

Determination of minimum bactericidal concentration

Table 11. Minimum Bactericidal Concentration of ethyl acetate leaf extract of *Alstonia boonei*

Bacterial Isolate	6.25 (mg/mL)	12.5 (mg/mL)	25 (mg/mL)	50 (mg/mL)
Pseudomonas aeruginosa	G	G	NG	NG
Staphylococcus aureus	G	G	G	NG
Klebsiella pneumonia	G	NG	NG	NG
Bacillus subtilis	G	G	G	G
Escherichia coli	G	G	G	NG

Key: G= Growth NG= No Growth

Table 12. Minimum Bactericidal Concentration of ethyl acetate stem bark extract of *Alstonia boonei*

Bacterial Isolate	6.25 (mg/mL)	12.5 (mg/mL)	25 (mg/mL)	50 (mg/mL)
Pseudomonas aeruginosa	G	G	G	NG
Staphylococcus aureus	G	NG	NG	NG
Klebsiella pneumonia	G	NG	NG	NG
Bacillus subtilis	G	NG	NG	NG
Escherichia coli	G	NG	NG	NG

Key: G= Growth NG= No Growth

The results for the minimum bactericidal concentration (MBC) of the ethyl acetate leaves and stem bark extracts of *A.* are presented in Tables 11 and 12. The MBC of the ethyl acetate leaf extract of *A. boonei* was detected at 50 mg/mL with *E. coli* and *S. aureus*, 12.5 mg/mL *with K. pneumoniae* and 25 mg/mL with *P. aeruginosa*. Meanwhile, *B. subtilis* was not determined because it continues to grow even at 50 mg/mL. For the stem bark (Table 12), *E. coli*, *K. pneumonia*, *B. subtilis*, and *S. aureus* displayed minimum bactericidal concentrations at 12.5 mg/mL and *P. aeruginosa* at 50 mg/mL. These results show that the stem bark extract is a more effective antibacterial agent than the leaf extract.

Conclusion

Qualitative phytochemical analysis of ethyl acetate leaves and stem bark extracts of *Alstonia boonei* was investigated and indicated the presence of phenolics, eugenols, steroids, alkaloids, flavonoids and reducing sugars for both extracts. Analysis of the mineral content of the extracts established that the essential minerals were present within the permissible limit. Evaluation of the antioxidant potential of both extracts using a DPPH free radical scavenging assay revealed that the leaf extract is a better antioxidant under the conditions employed, and the activity is concentration-dependent. The antibacterial study of the extracts illustrates that the leaf has better activity regarding *S. aureus* and *P. aeruginosa*. While the stem bark extract showed better activity regarding *E. coli* and *B. subtilis*; however, both extracts had the same activity for *K. pnuemoniae.* The minimum inhibitory concentration for leaf and stem bark extract was the same for all

the bacterial isolates at 12.5 mg/mL, except for *P. aeruginosa* at 25 mg/mL. Minimum bactericidal concentration showed that the stem bark extract is a better antibacterial agent for *E. coli, S. aureus* and *B. subtilis. In contrast,* the leaf extract is better for *P. aeruginosa*. Meanwhile, both extracts had the same effect on *K. pneumoniae*. Hence, this study guides the development of *A. boonei* into novel drugs and food and medicinal flavouring agents.

Acknowledgements

The authors are sincerely indebted to the laboratory technologists at the Department of Chemistry, University of Benin, for their invaluable assistance to the authors as they conducted their experimental work.

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