Antioxidant and Antimicrobial Potentials of the Ethanol Extract of *Berlinia grandiflora* (Vahl) Hutch. & Dalziel (Leguminosae) Stem Bark

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ABSTRACT

Oxidative stress and antimicrobial resistance are interrelated threats to health that sustain interest in plant-derived therapeutic agents. *Berlinia grandiflora* (Vahl) Hutch. & Dalziel is used in Nigerian traditional medicine for infections, wounds, and several other ailments, but the antioxidant and antimicrobial properties of its stem bark have been incompletely characterised. This study determined the physicochemical parameters, phytochemical constituents, free radical scavenging capacity, and in vitro antimicrobial activity of the ethanol extract of the stem bark.

Authenticated stem bark powder was macerated in absolute ethanol, and phytochemicals were detected by standard qualitative tests. Antioxidant activity was assessed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, with ascorbic acid as the reference. Antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Aspergillus niger,* and *Candida albicans* was evaluated by agar well diffusion, and the minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) were determined by agar dilution, with ofloxacin and fluconazole as reference drugs.

Moisture, total, acid-insoluble, and water-soluble ash were 8.48, 10.72, 1.19, and 5.72%, respectively, and the extract yield was 7.62%. Saponins, phenols, tannins, glycosides, terpenoids, alkaloids, flavonoids, and steroids were present, whereas anthraquinones and cyanogenic glycosides were absent. The extract scavenged DPPH radicals in a concentration-dependent manner (IC_{50} 10.48 µg/mL; ascorbic acid, 2.71 µg/mL). It inhibited all test organisms in a dose-dependent manner; zones of inhibition at 200 mg/mL were 7.3–11.7 mm for the bacteria, 17.0 mm for *A. niger,* and 28.0 mm for *C. albicans*. MICs were 156.2 (*E. coli*), 78.1 (*S. aureus*), 156.2 (*K. pneumoniae*), 39.06 (*A. niger*), and 19.53 µg/mL (*C. albicans*). MBC/MIC ratios of 1–2 for the bacteria and 8 for the fungi indicate bactericidal and fungistatic action, respectively.

These findings provide preliminary scientific support for the traditional use of *B. grandiflora* stem bark and justify bioassay-guided isolation of its active constituents.

Keywords: *Berlinia grandiflora;* stem bark ethanol extract; phytochemicals; antioxidant; antimicrobial.

1. INTRODUCTION

Reactive oxygen species (ROS) are generated continuously during aerobic metabolism and are normally controlled by enzymatic and non-enzymatic antioxidant defences [1,2]. Oxidative stress is implicated in the pathogenesis of cardiovascular disease, diabetes, cancer, neurodegenerative disorders and ageing [3]. Phenolic compounds and flavonoids are particularly important in this regard, as they donate hydrogen atoms or electrons to free radicals and can chelate pro-oxidant metal ions [4]. Oxidative stress and infectious disease are often interrelated, so extracts that combine antioxidant and antimicrobial activity are particularly interesting. At the same time, resistance is eroding the effectiveness of existing antimicrobials, affecting clinically important organisms such as Escherichia coli, Staphylococcus aureus, and *Candida* species [5,6].

Presently, antimicrobial resistance (AMR) is considered a global challenge and a silent pandemic, the third leading cause of death globally, with associated mortality of 4.71 million in 2021 and a projected 8.22 million associated annual mortality by 2050, with most of these burdens borne by Asia and African continents, including Nigeria [7]. A large proportion of the population in developing countries relies on traditional medicine for primary health care [8], and medicinal plants have long been a source of antimicrobial chemotherapy [9,10]. Numerous Nigerian plant species have been documented to possess antimicrobial activity [11]; examples from elsewhere include neem, *Azadirachta indica* [12], and the essential oils of several aromatic species [13]. Plant extracts contain many constituents that may act on multiple targets, enhance one another's bioavailability, or suppress resistance mechanisms, and these properties may reduce the likelihood of resistance developing relative to single-target drugs [14,15].

Berlinia grandiflora (Vahl) Hutch. & Dalziel (Leguminosae) is a medium to large tree distributed across tropical West and Central Africa [16,17]. The Yoruba call it apado, and the Igbo call it ububa [18]. Herbalists use preparations of the plant to enhance fertility [19], treat skin infections, haemorrhoids, liver complaints, cancer, diarrhoea and diabetes [20, 21]; bark sap and decoctions are applied to sores and wounds, haemorrhoids and microbial infections [22]. The stem bark has shown anthelmintic activity attributed in part to betulinic acid and tannins [23], and leaf and stem bark extracts have shown activity against pathogenic bacteria [22,24]. The ethanol leaf extract is rich in phenolic constituents and has antioxidant activity [16].

Despite this ethnomedicinal profile, to our knowledge, the stem bark has not been evaluated in a single framework that links its pharmacognostic quality, phytochemistry, free radical scavenging capacity, and inhibitory and cidal activity against both bacteria and fungi. Ethanol was chosen as the extraction solvent because it dissolves a wide range of polar and moderately non-polar constituents, including phenolics and flavonoids [25]. The present study therefore determined the physicochemical parameters and phytochemical profile of *B. grandiflora* stem bark and evaluated the antioxidant activity and the antibacterial and antifungal activity (zone of inhibition, MIC and MBC/MFC) of its ethanol extract, in order to provide a scientific basis for its traditional use.

2. MATERIALS AND METHODS

2.1 Plant Material

Dried stem bark and voucher specimens of *B. grandiflora* were collected from Iseyin LGA, Oyo State, Nigeria, on a piece of land belonging to the Okegbola family with approval from Mr. A.O. Okegbola, the family head, in 2021. The plant was authenticated at the Forestry Herbarium, Ibadan (FHI), by Egunjobi A.J & Adeyemo A., and a voucher specimen (FHI 113219) was deposited. The bark was cut into pieces, washed and air-dried in the shade, then ground to a powder with a hammer mill driven by a 5 kVA motor [26]. The powder was weighed and stored in an air-tight container until extraction.

2.2 Physicochemical Analysis

All determinations were performed in triplicate on the powdered stem bark.

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2.2.1 Moisture content. A preheated porcelain crucible was weighed (W_1), 2 g of the powder was weighed into it, and the crucible was reweighed (W_2). The sample was heated gradually in an oven to 105 °C and held at that temperature for about 4 h until a constant weight was obtained. The crucible was cooled in a desiccator and weighed (W_3). Moisture content (%) = $[(W_2 - W_3)/(W_2 - W_1)] \times 100$.

2.2.2 Total ash value. A porcelain crucible was placed in a hot-air oven at 105 °C for about 15 min, cooled in a desiccator for about 20 min, and weighed (W_1). About 2 g of the powder was accurately weighed into the crucible, which was reweighed (W_2). The sample was ashed in a muffle furnace at above 600 °C for about 6 h until the residue turned grey. The crucible was removed with tongs, cooled in a desiccator, and reweighed (W_3). Total ash (%) = $[(W_3 - W_1)/(W_2 - W_1)] \times 100$, following WHO [27] with modifications [19].

2.2.3 Acid-insoluble and Water-soluble ash values. For acid-insoluble ash, the total ash obtained by incineration at above 600 °C was transferred into a beaker containing 15 mL of 10% hydrochloric acid, boiled in a water bath for about 5 min, and filtered through ashless filter paper. The beaker and crucible were washed repeatedly through the filter paper with hot water until the washings were free from acid (neutral to litmus paper). The insoluble matter and filter paper were dried in an oven and ignited in the muffle furnace at above 600 °C, and the weight of acid-insoluble ash per gram of powder was calculated. For water-soluble ash, about 15 mL of distilled water was added to the total ash as against acid, and the other procedure for acid-insoluble ash was followed [28].

2.2.4 Extractive values. For the water extractive value, 20 mL of distilled water was added to 5 g of the powder in a beaker and macerated for 24 h with occasional shaking. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was dried to obtain the extractive. For the alcohol extractive value, the procedure was repeated with 20 mL of absolute ethanol. Each extractive value was expressed as a percentage of the 5 g of powder used [28].

2.3 Extraction

The powdered stem bark (463.4 g) was cold-macerated in absolute ethanol (1,600 mL) for 72 h with constant agitation. The mixture was filtered, and the filtrate was concentrated in a water bath at 40 °C. The dried extract was weighed and stored in a glass bottle until required for analysis. The percentage yield was calculated as: yield (%) = (weight of extract/weight of powdered sample) × 100 [29].

2.4 Phytochemical Screening

The ethanol extract was screened for saponins, phenols, tannins, flavonoids, glycosides (general, anthraquinone and cyanogenic), steroids, terpenoids and alkaloids by standard qualitative methods [30,31]. The procedures were as follows.

2.4.1 Saponins (frothing test). About 0.5 g of the extract was added to 20 mL of warm water in a beaker and shaken; the mixture was transferred to a test tube, shaken vigorously for 1 min, and allowed to stand for about 10 min. Froth that did not break readily indicated saponins.

2.4.2 Phenols (ferric chloride test). Two millilitres of the extract were treated with a few drops of 10% ferric chloride solution. A green or bluish-black colour indicated phenols.

2.4.3 Tannins. About 0.5 g of the extract was dissolved in 2 mL of warm water and stirred. 1 mL of this solution was added to 10 mL of distilled water in a test tube, and 4 drops of 1% lead acetate solution were added and shaken; a precipitate indicated possible tannins. The remaining 1 mL of the stock solution was treated with 2% ferric chloride solution; a deep blue colouration indicated tannins.

2.4.4 Flavonoids (alkaline reagent test). About 0.5 g of the extract was added to 10 mL of ethyl acetate in a conical flask and heated for 2 min in a water bath, and the mixture was filtered. To 2 mL of the filtrate, 2–5 drops of 10% sodium hydroxide solution were added. An increase in the intensity of the yellow colour in the lower alkaline layer indicated flavonoids.

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2.4.5 Glycosides. *General test:* 5 mL of 1% sulphuric acid was added to 0.5 g of the extract in a beaker, heated for about 2 min, and filtered. To 1 mL of the filtrate in a test tube, 2–5 drops of 2% sodium hydroxide were added to make it slightly alkaline (red litmus paper turned blue), 1 mL of Fehling's solution was added, and the mixture was boiled for 2 min; a reddish-brown precipitate indicated glycosides. To a further 1 mL of the filtrate, 2–5 drops of 2% sodium hydroxide were added (alkalinity confirmed with red litmus paper), followed by 2 drops of Benedict's solution, and the mixture was boiled for about 2 min; a brown or red precipitate indicated glycosides. *Anthraquinone glycosides (Bornträger's test):* about 0.1 g of the extract was boiled with 5% dilute sulphuric acid for 5 min and filtered while hot, and the filtrate was shaken with an equal volume of chloroform. A rose-pink or purple ring at the interface of the two liquids indicated anthraquinones. *Cyanogenic glycosides (sodium picrate test):* a small amount of the powder was placed in each of three test tubes (A, B and C). The powder in A and B was moistened with a little water, a strip of sodium picrate paper was inserted into each of the three tubes, and the tubes were stoppered immediately. Tube B was placed in a boiling water bath for 5 min, while tubes A and C were kept at room temperature. After about 30 min, any colour change of the sodium picrate paper was recorded; a brick-red colour in tube B indicated the evolution of hydrogen cyanide.

2.4.6 Steroids and terpenoids. *Steroids (Liebermann–Burchard test):* 2 mL of acetic anhydride was added to

2 mL of the extract, and 2 mL of concentrated sulphuric acid was added gradually down the side of the test tube; a red to brown ring indicated triterpenoids/steroids. *Terpenoids (Salkowski test):* 2 mL of the extract was mixed with 2 mL of chloroform, and 2 mL of concentrated sulphuric acid was carefully layered beneath; a reddish-brown colour at the interface indicated terpenoids.

2.4.7 Alkaloids. About 0.5 g of the extract was dissolved in 10 mL of 1% sulphuric acid, and 1 mL portions of the solution were tested as follows. *Mayer's test:* a few drops of Mayer's reagent; formation of a precipitate indicated alkaloids. *Hager's test:* 2 drops of Hager's reagent; a yellow precipitate indicated alkaloids. *Wagner's test:* 2 drops of Wagner's reagent; a reddish-brown to brown precipitate indicated alkaloids. *Dragendorff's test:* 2 drops of Dragendorff's reagent; an orange to orange-red (reddish-brown) precipitate indicated alkaloids.

2.5 DPPH Free Radical Scavenging Assay

The free radical scavenging activity of the extract was assessed by the method of [32] with minor modifications. A stock solution of the extract and Ascorbic acid (standard drug) were prepared and serially diluted with ethanol to give 500, 250, 125, 62.5, 31.25, 15.63, 7.81, and 3.91 µg/mL. The negative control contained 2 mL of 0.1 mM DPPH in ethanol and 1,000 µL of ethanol, without extract or standard. For the assay, 1,000 µL of each extract or ascorbic acid solution was mixed with 2,000 µL of freshly prepared DPPH solution (0.04 mg/mL; 2 mg in 50 mL of ethanol). The mixtures were incubated at room temperature in the dark for 30 min, and the colour change from deep violet to light yellow was measured at 517 nm with a UV spectrophotometer. The scavenging effect was calculated as:

$$\text{DPPH scavenging effect (\% inhibition)} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

where $A_{control}$ is the absorbance of the negative control, and A_{sample} is the absorbance of the extract or standard. Each concentration was tested in triplicate. The IC_{50} was defined as the concentration of extract or standard causing 50% inhibition of the DPPH radical; a lower IC_{50} indicates higher antioxidant activity [33].

2.6 Antimicrobial Assays

2.6.1 Test organisms. The bacterial isolates were *Staphylococcus aureus* (Gram-positive), *Escherichia coli* and *Klebsiella pneumoniae* (Gram-negative), and the fungal isolates were *Candida albicans* and *Aspergillus niger*; all were clinical isolates obtained from the Pharmaceutical Microbiology Laboratory, Madonna University, Nigeria.

2.6.2 Antibacterial and Antifungal agar well diffusion assays. For antibacterial, Mueller–Hinton agar (120 mL) was prepared according to the manufacturer's specifications, aseptically dispensed into six sterile Petri dishes (about 20 mL each), and allowed to gel. The test isolates, adjusted to the 0.5 McFarland standard, were

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swabbed over the agar surface with sterile swab sticks. For antifungal, Sabouraud dextrose agar (SDA; 80 mL) was prepared according to the manufacturer's specifications and aseptically dispensed into four sterile Petri dishes. The fungal isolates, adjusted to the 0.5 McFarland standard, were swabbed over the agar surface. Each plate was marked into sectors labelled with the extract concentrations (200, 100, 50, 25 and 12.5 mg/mL), both for bacteria and fungi. The ofloxacin (5 µg/mL) and fluconazole (50 µg/mL) were used as positive controls for bacteria and fungi, respectively, while 2% dimethyl sulfoxide (DMSO) was used as a negative control. Wells were bored with a sterile 8 mm cork borer, and 20 µL (0.02 mL, delivered with a calibrated sterile Pasteur pipette) of each extract concentration or control was introduced into the appropriately labelled well. The plates were kept at room temperature in a laminar flow hood for 1 h to allow diffusion into the agar and were then incubated at 37 °C for 24 h for bacteria and 28 °C for 48–72 h for fungi [34]. The mean diameter of the zones of inhibition was recorded after subtracting the 8 mm borer diameter.

2.6.3 Minimum inhibitory concentration (MIC). MICs were determined by agar dilution. For the antibacterial and antifungal assays, 150 mg of the extract was dissolved in 6 mL of 2 % DMSO each, to give the stock solutions of 25,000 µg/mL each. 3 mL of the stock solution was retained as the highest concentration (25,000 µg/mL), and the remaining 3 mL was serially diluted two-fold to give 12,500, 6,250, 3,125, 1,562, 781, 391, 195, 98, 49, and 24 µg/mL. 1 mL of each dilution was placed in a sterile Petri dish and mixed with 9 mL of molten Mueller–Hinton agar (bacteria) or Sabouraud dextrose agar (fungi), giving final plate concentrations of 2,500, 1,250, 625, 312, 156, 78, 39, 19, 9.8, 4.9, and 2.4 µg/mL (exact two-fold values are given in Table 6). Each plate was divided into sectors according to the test isolates (three for the bacteria, two for the fungi), and the organisms were streaked on their respective sectors. Bacterial plates were incubated at 37 °C for 24 h and fungal plates at 28 °C for 48–72 h, after which the plates were observed for growth. The MIC was taken as the lowest concentration that completely inhibited the growth of the isolate [35].

2.6.4 Minimum bactericidal and fungicidal concentrations (MBC/MFC). Samples from the MIC plates were inoculated onto fresh agar plates with a sterile wire loop, using Mueller–Hinton agar for the bacterial isolates and Sabouraud dextrose agar for the fungal isolates. Bacterial plates were incubated at 37 °C for 24 h and fungal plates at 28 °C for 72 h, and the plates were then examined for growth. The MBC or MFC was the lowest concentration that produced no visible colony growth. The MBC/MIC (or MFC/MIC) ratio was calculated for each organism; a ratio of ≤4 was taken to indicate cidal activity and a ratio of >4 indicated static activity.

2.7 Data Analysis and Presentation

Data were analyzed using one-way analysis of variance (ANOVA) employing GraphPad Prism version 5.02 for Windows (GraphPad software, San Diego, California, USA, www.graphpad.com) at $P < 0.05$. Dunnett multiple comparison test at a 95% confidence interval for the difference was considered significant. Results are presented as mean ± standard error of the mean (SEM) of three replicates (n = 3).

3. RESULTS

3.1 Physicochemical Parameters and Extract Yield

The physicochemical parameters of the powdered stem bark are shown in Table 1. The moisture content was 8.48 ± 0.12%, and the total ash was 10.72 ± 0.94%, of which 5.72 ± 0.14% was water-soluble and 1.19 ± 0.03% acid-insoluble. The alcohol extractive value (7.27 ± 0.74%) exceeded the water extractive value (5.89 ± 0.01%). Cold maceration of 463.4 g of powder in 1,600 mL of absolute ethanol yielded 35.3 g of dry extract, a yield of 7.62% (Table 2).

Table 1. Physicochemical parameters of powdered *B. grandiflora* stem bark (mean ± SEM, n = 3)

Parameter	Value (%)
Moisture content	8.48 ± 0.12
Total ash	10.72 ± 0.94
Acid-insoluble ash	1.19 ± 0.03

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Water-soluble ash	5.72 ± 0.14
Alcohol extractive value	7.27 ± 0.74
Water extractive value	5.89 ± 0.01

Table 2. Yield of the ethanol extract

Extract	Weight of powder (g)	Weight of extract (g)	Yield (%)
Ethanol	463.4	35.3	7.62

3.2 Phytochemical Constituents

The phytochemical screening results are summarised in Table 3. Saponins, phenols, tannins, flavonoids, glycosides, steroids, terpenoids and alkaloids were detected, with the alkaloid tests giving a markedly strong reaction. Anthraquinone glycosides and cyanogenic glycosides were not detected.

Table 3. Phytochemical constituents of the ethanol extract of *B. grandiflora* stem bark

Constituent	Test(s) applied	Result
Saponins	Frothing test	+
Phenols	Ferric chloride test	+
Tannins	Lead acetate and ferric chloride tests	+
Flavonoids	Alkaline reagent test	+
Glycosides	Fehling's and Benedict's tests	+
Anthraquinone glycosides	Bornträger's test	−
Cyanogenic glycosides	Sodium picrate test	−
Steroids	Liebermann–Burchard test	+
Terpenoids	Salkowski test	+
Alkaloids	Mayer's, Wagner's and Dragendorff's tests	+

Key: + = present; − = absent.

3.3 DPPH Radical Scavenging Activity

Both the extract and ascorbic acid scavenged DPPH radicals in a concentration-dependent manner (Table 4 and Fig. 1). At 500 µg/mL, the extract produced 84.89 ± 0.37% inhibition compared with 95.21 ± 0.49% for ascorbic acid, and at the lowest concentration tested (3.91 µg/mL), the values were 40.96 ± 0.65% and 54.03 ± 0.06%, respectively. The IC_{50} of the extract was 10.48 µg/mL, compared with 2.71 µg/mL for ascorbic acid; ascorbic acid was therefore about 3.9 times more potent than the crude extract on an IC_{50} basis.

Table 4. DPPH radical scavenging activity (% inhibition, mean ± SEM, n = 3) of ascorbic acid and the ethanol extract.

Concentration (µg/mL)	Ascorbic acid	Ethanol extract
500	95.21 ± 0.49^{C}	84.89 ± 0.37
250	88.64 ± 0.38^{C}	78.42 ± 0.15
125	83.73 ± 0.20^{C}	73.85 ± 0.35
62.5	78.46 ± 0.54^{C}	67.14 ± 0.36
31.25	71.47 ± 0.66^{C}	60.04 ± 0.34
15.63	64.13 ± 0.21^{C}	53.19 ± 0.78

7.81	58.12 ± 0.17^{C}	47.57 ± 0.43
3.91	54.03 ± 0.06^{C}	40.96 ± 0.65
IC_{50} (µg/mL)	**2.71**^{C}	**10.48**

c= P ≤ 0.001 (Ascorbic acid was significantly different from extract)

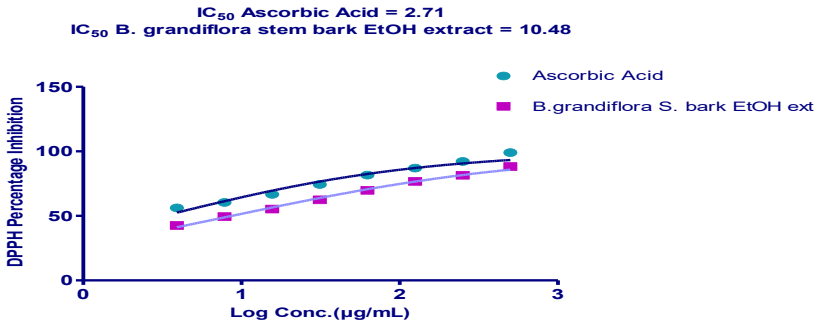


Fig. 1. DPPH Radical Scavenging activity of ethanol extract of *Berlinia grandiflora* stem bark

4. Antimicrobial Activity

The extract inhibited all five test organisms in a dose-dependent manner, whereas the 2% DMSO negative control produced no zone of inhibition (Fig. 2). At 200 mg/mL, the zones of inhibition were 7.3 ± 0.3 mm (*E. coli;* EC), 11.7 ± 0.3 mm (*S. aureus;* SA) and 9.0 ± 0.6 mm (*K. pneumoniae;* KP) for the bacteria, compared with 21.3 ± 0.3, 15.7 ± 0.7 and 20.0 ± 0.6 mm, respectively, for ofloxacin. The fungi were more susceptible: the zones were 17.0 ± 0.0 mm for *A. niger* (fluconazole, 15.3 ± 0.3 mm) and 28.0 ± 0.6 mm for *C. albicans* (fluconazole, 29.7 ± 0.9 mm). Even at 12.5 mg/mL, the extract produced measurable zones against all organisms (2.3–5.7 mm for the bacteria and *A. niger;* AN, and 17.3 mm for *C. albicans;* CA). In the agar dilution assay, the MICs were 156.2 µg/mL for *E. coli*, 78.13 µg/mL for *S. aureus*, 156.2 µg/mL for *K. pneumoniae*, 39.06 µg/mL for *A. niger,* and 19.53 µg/mL for *C. albicans* (Table 6). The MBC/MFC values were 312.5, 156.2, 156.2, 312.5 and 156.2 µg/mL, respectively (Table 7), giving MBC/MIC ratios of 2, 2 and 1 for *E. coli*, *S. aureus* and *K. pneumoniae* and MFC/MIC ratios of 8 for both *A. niger* and *C. albicans* (Table 8). The extract was therefore bactericidal against the three bacteria and fungistatic against the two fungi. − = no growth; + = growth.

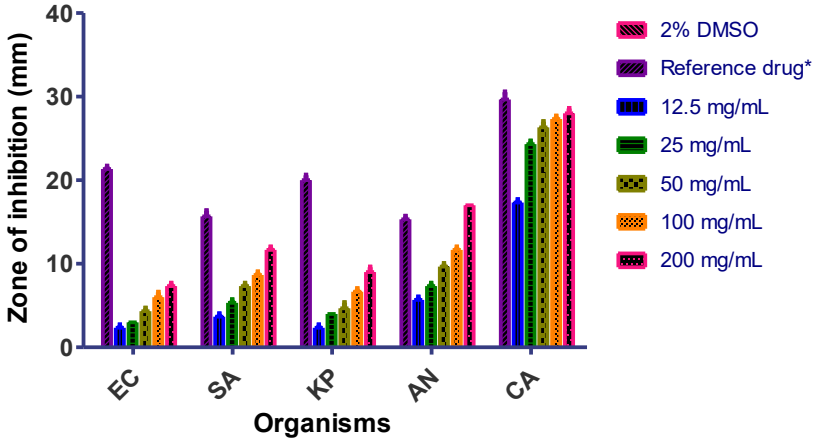


Fig. 2. Zones of inhibition (mm, net of the 8 mm well; mean ± SEM, n = 3) of the ethanol extract of *B. grandiflora* stem bark and reference drugs. Reference drugs*: ofloxacin (bacteria) and fluconazole (fungi).

Table 6. Growth of the test organisms on agar containing the extract (agar dilution; concentrations in µg/mL)

Organism	**2500**	**1250**	**625**	**312.5**	**156.2**	**78.13**	**39.06**	**19.53**	**9.77**	**4.88**	**2.44**
E. coli	−	−	−	−	−	+	+	+	+	+	+

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S. aureus	−	−	−	−	−	−	+	+	+	+	+
K. pneumoniae	−	−	−	−	−	+	+	+	+	+	+
A. niger	−	−	−	−	−	−	−	+	+	+	+
C. albicans	−	−	−	−	−	−	−	−	+	+	+

Key: − = no growth; + = growth. The lowest concentration with no growth is the MIC.

Table 7. Growth after subculture from the MIC plates (MBC/MFC determination; concentrations in µg/mL)

Organism	**2500**	**1250**	**625**	**312.5**	**156.2**	**78.13**	**39.06**	**19.53**	**9.77**	**4.88**	**2.44**
E. coli	−	−	−	−	+	+	+	+	+	+	+
S. aureus	−	−	−	−	−	+	+	+	+	+	+
K. pneumoniae	−	−	−	−	−	+	+	+	+	+	+
A. niger	−	−	−	−	+	+	+	+	+	+	+
C. albicans	−	−	−	−	−	+	+	+	+	+	+

Key: − = no growth; + = growth. The lowest concentration with no growth is the MBC (bacteria) or MFC (fungi).

Table 8. Summary of MIC, MBC/MFC, and MBC/MIC (MFC/MIC) ratios (µg/mL)

Organism	**MIC**	**MBC/MFC**	**Ratio**	**Interpretation**
Escherichia coli	156.2	312.5	2	Bactericidal
Staphylococcus aureus	78.13	156.2	2	Bactericidal
Klebsiella pneumoniae	156.2	156.2	1	Bactericidal
Aspergillus niger	39.06	312.5	8	Fungistatic
Candida albicans	19.53	156.2	8	Fungistatic

4. DISCUSSION

4.1 Physicochemical Parameters and Extract Yield

Physicochemical parameters are used to establish the identity, purity, and quality of crude drugs [36]. The moisture content of the stem bark (8.48%) is reasonably low, which limits microbial proliferation and enzymatic degradation during storage, and the values obtained are in line with those previously reported for *B. grandiflora* [19]. The total ash (10.72%) is relatively high, which may reflect inorganic salts and calcium oxalate crystals in the bark. The acid-insoluble ash was low (1.19%), indicating little contamination with soil or siliceous matter, and the water-soluble ash (5.72%) accounted for about half of the total ash, indicating a substantial water-soluble mineral fraction. The alcohol extractive value (7.27%) was higher than the water extractive value (5.89%), showing that the constituents of the bark are somewhat more soluble in ethanol than in water, which supports the choice of ethanol as the extraction solvent [25]. The extract yield of 7.62% lies within the range of 4.4–8% reported for stem bark and leaf extracts of the same species, depending on solvent polarity [22].

4.2 Phytochemical Constituents

The detection of saponins, tannins, flavonoids, phenols, glycosides, steroids, terpenoids and alkaloids, and the absence of anthraquinones and cyanogenic glycosides, is in broad agreement with earlier phytochemical reports on the stem bark [23] and with the phenolic-rich composition of the leaf [16]. The absence of cyanogenic glycosides is favourable from a safety standpoint, although toxicity was not evaluated here. Flavonoids, tannins and other phenolics are established radical scavengers [4,37] and have antimicrobial properties, including activity against oral pathogens [38]; terpenoids and alkaloids likewise contribute to the antimicrobial effects of

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plant extracts [9,39]. Because the screening was qualitative, however, the contribution of each class cannot be quantified, and total phenolic and flavonoid contents were not determined.

4.3 Antioxidant Activity

The extract scavenged DPPH radicals in a concentration-dependent manner, reaching 84.89% inhibition at 500 µg/mL compared with 95.21% for ascorbic acid. With an IC_{50} of 10.48 µg/mL, the extract is an effective scavenger for a crude preparation, although ascorbic acid (IC_{50} 2.71 µg/mL) was about 3.9 times more potent. This comparison is between a pure compound and a complex mixture in which the active constituents may represent only a fraction of the mass. The activity is plausibly attributable to the phenolics, flavonoids, and tannins detected, which donate hydrogen atoms or electrons to the DPPH radical [4,33]. The earlier finding that a tannin-rich residue of the stem bark was the most active fraction in an anthelmintic assay [23] also points to tannins as important constituents of the bark, and the antioxidant activity reported for the leaf [16] suggests that radical scavenging is a general feature of the plant. These results indicate that the stem bark could be explored as a source of natural antioxidants. In addition, the IC_{50} of ascorbic acid (2.71 µg/mL) lies below the lowest concentration tested (3.91 µg/mL) and was therefore obtained by extrapolation; a lower concentration range should be included in future work.

4.4 Antimicrobial Activity

Ofloxacin, a fluoroquinolone that inhibits bacterial nucleic acid synthesis [40], and fluconazole, an azole antifungal [41], served as reference drugs. The extract inhibited Gram-positive and Gram-negative bacteria and both fungi in a dose-dependent manner, and the absence of inhibition by 2% DMSO confirms that the activity was due to the extract. Activity against the bacteria was modest relative to ofloxacin (net zones of 7.3–11.7 mm at 200 mg/mL versus 15.7–21.3 mm), but it was marked against the fungi, particularly *C. albicans* (28.0 mm at 200 mg/mL and 17.3 mm even at 12.5 mg/mL) and *A. niger* (17.0 mm). These zone diameters should not be taken to indicate equal potency with the reference drugs: the extract was tested at 12.5–200 mg/mL, whereas ofloxacin and fluconazole were applied at µg/mL concentrations, and zone size also depends on diffusion through the agar. The greater susceptibility of *S. aureus* than of *E. coli* and *K. pneumoniae* is often seen with plant extracts and may reflect the barrier presented by the Gram-negative outer membrane, since Gram-positive cell walls are single-layered with a thick peptidoglycan layer, while Gram-negative cell walls are double-layered with a thin peptidoglycan layer and an outer membrane

In the agar dilution assay, MICs ranged from 78.13 to 156.2 µg/mL for the bacteria and from 19.53 to 39.06 µg/mL for the fungi, so the fungi were again the more susceptible. MBC/MIC ratios of 1–2 indicate bactericidal action, whereas the ratio of 8 for both fungi indicates fungistatic action under the convention that a ratio of ≤ 4 denotes cidal activity. The MICs are much lower than the concentrations needed to produce zones in the well diffusion assay. This probably reflects the poor diffusion of large or polar constituents through agar, whereas in agar dilution the organisms are exposed to the extract throughout the medium.

The activity is consistent with earlier reports that leaf and stem bark extracts of *B. grandiflora* inhibit pathogenic bacteria [22,24] and with the bioactivities reported for stem bark of the congener *B. confuse* [42]. Phenolics, tannins, flavonoids, terpenoids, and alkaloids are recognised antimicrobial classes [9,38,39], and mixtures of constituents may act synergistically [15]. Concurrent antioxidant and antimicrobial activities have also been reported for other African medicinal plants [43]. The lower IC_{50} and MIC recorded in the DPPH and agar dilution assays, respectively, could be linked to the presence of tannins and flavonoids; betulinic acid has previously been isolated from the bark [24]. Future work should therefore include quantification of phenolics and flavonoids, LC–MS profiling, bioassay-guided fractionation and isolation of active compounds, and toxicological evaluation.

5. CONCLUSION

The ethanol extract of *Berlinia grandiflora* stem bark contains a diverse range of phytochemicals and displays concentration-dependent free radical scavenging activity (IC_{50} 10.48 µg/mL) together with antibacterial and antifungal activity. It was bactericidal against *E. coli*, *S. aureus* and *K. pneumoniae* (MIC 78.13–156.2 µg/mL)

and fungistatic against *A. niger* and *C. albicans* (MIC 39.06 and 19.53 µg/mL), with the strongest effects against the fungi. The physicochemical parameters provide quality-control reference values for the stem bark. These findings give preliminary support for the traditional use of the plant and provide a basis for isolating and characterising its active antioxidant and antimicrobial constituents.

ETHICS STATEMENT

The study did not involve human participants or animals; hence, ethical approval is not applicable.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest and that all agreed to submit this manuscript to the International Journal of Research and Innovation in Applied Science.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are already inserted in the manuscript, and no further data are required.

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