

Phytochemical Profiling and Antimicrobial Activity of Aerial Extracts of *Buchholzia Coriacea* with Minimum Inhibitory Concentration Evaluation

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ABSTRACT

This research investigated the phytochemical composition and antimicrobial efficacy of aerial extracts derived from *Buchholzia coriacea* (wonderful kola), with a particular focus on the assessment of minimum inhibitory concentration (MIC). The plant materials were collected, subjected, dried, extracted utilizing ethanol, acetone, and water. Preliminary phytochemical analysis indicated the presence of bioactive compounds, inclusive of alkaloids, flavonoids, tannins, saponins, terpenoids, phenols, and glycosides, with the ethanol extract demonstrating the greatest diversity and concentration of these compounds. Quantitative assessments revealed that flavonoids (24.37 mg/100 g) and alkaloids (21.33 mg/100 g) represented the most prevalent constituents, thereby suggesting substantial pharmacological potential. The evaluation of antimicrobial activity was conducted against selected bacterial (*Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp.) and fungal (*Candida* spp., *Aspergillus* spp.) isolates employing disc diffusion and agar well diffusion methodologies. The findings exhibited pronounced inhibitory effects, particularly with respect to ethanol and acetone extracts, which displayed zones of inhibition akin to those of standard antibiotics. Notably, the ethanol extract exhibited the highest level of antimicrobial potency, particularly against *E. coli* (27.00 mm) and *Candida* spp. (25.00 mm). The MIC evaluation further substantiated the efficacy of the extracts, with the ethanol extract revealing robust activity (0.10– 0.50 mg/mL) against the majority of the test organisms. In contrast, the aqueous extracts exhibited relatively diminished antimicrobial activity. These findings underscore the significant impact of solvent polarity on both extraction efficiency and antimicrobial efficacy.

Keywords: Antimicrobial activity, *Buchholzia coriacea*, medicinal plants, minimum inhibitory concentration (MIC), phytochemical profiling.

INTRODUCTION

The escalating global challenge posed by infectious diseases, exacerbated by the swift emergence of antimicrobial resistance (AMR), has underscored the urgent need for an intensified exploration of innovative therapeutic agents derived from natural sources. Antimicrobial resistance constitutes a significant menace to public health, diminishing the effectiveness of traditional antibiotics and resulting in extended illnesses, escalated healthcare expenditures, and heightened mortality rates (World Health Organization [WHO], 2023). In light of this predicament, medicinal plants have garnered renewed scholarly interest as reservoirs of bioactive compounds endowed with potential antimicrobial efficacy. Historically, these plants have formed the cornerstone of numerous contemporary pharmaceuticals and persist in playing a vital role in primary healthcare frameworks, particularly in developing nations (Newman & Cragg, 2020).

Phytochemicals, colloquially designated as secondary metabolites, are naturally occurring compounds that plants synthesize as integral components of their defense mechanisms against pathogens, herbivores, and environmental stressors. The principal categories of phytochemicals encompass alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, and phenolic compounds. These constituents are recognized for their multifaceted biological activities, which include antimicrobial, anti-inflammatory, antioxidant, and anticancer properties (Kumar *et al.*, 2021). The antimicrobial properties exhibited by phytochemicals are frequently attributed to their capacity to disrupt microbial cell membranes, inhibit enzymatic activity, interfere with nucleic acid synthesis, or modify metabolic pathways within pathogenic organisms (Cowan, 1999; Silva *et al.*, 2022).

One plant that holds considerable ethnomedicinal significance is *Buchholzia coriacea* Engler, often referred to as the wonderful kola. It is part of the Capparaeaceae family and is commonly found in West and Central Africa. This plant is frequently utilized in traditional medicine to address a variety of health issues, such as microbial infections, diabetes, hypertension, and inflammatory disorders (Ajaiyeoba *et al.*, 2001; Olajide *et al.*, 2020). Various components of the plant, especially the seeds, have undergone research to examine their pharmacological effects; however, there has been comparatively less research on the aerial parts (leaves and stems), which might also contain important bioactive compounds.

The aerial parts of plants are especially significant in phytochemical research, as they play a crucial role in photosynthesis and metabolic functions, resulting in the production and accumulation of a wide range of secondary metabolites. The concentration and composition of these compounds may differ from those in other parts of the plant, thus affecting their biological activities. Earlier research has indicated that *Buchholzia coriacea* contains flavonoids, tannins, saponins, and alkaloids, linked to antimicrobial and antioxidant properties (Ezeigbo *et al.*, 2016; Ibrahim & Fagbohun, 2012). Regardless of these results, there is still a necessity for thorough phytochemical profiling of the plant's aerial extracts through contemporary analytical methods to gain deeper insights into their therapeutic capabilities.

Assessing antimicrobial activity is an essential process in establishing the therapeutic potential of plant extracts. Different techniques like agar well diffusion, disc diffusion, and broth dilution are frequently used to evaluate the inhibitory effects of extracts on pathogenic microorganisms. Among these, determining the Minimum Inhibitory Concentration (MIC) is especially important, as it offers quantitative information on the smallest concentration of an antimicrobial agent necessary to prevent visible microbial growth. MIC values are a crucial metric for assessing the efficacy of various extracts and informing dosage recommendations in the development of drugs (Balouiri *et al.*, 2016).

In recent years, there has been a growing interest in exploring plant-derived antimicrobials as alternatives or complements to conventional antibiotics. This is driven not only by the rise in antibiotic resistance but also by the increasing consumer preference for natural and less toxic therapeutic agents. Studies have demonstrated that plant extracts often exhibit broad-spectrum antimicrobial activity and may act synergistically with existing antibiotics, thereby enhancing their efficacy (Gupta *et al.*, 2022). Furthermore, the complex mixture of phytochemicals in plant extracts reduces the likelihood of resistance development compared to single-compound drugs.

Despite the promising potential of *Buchholzia coriacea*, gaps remain in the literature regarding the systematic evaluation of its aerial extracts, particularly in relation to their phytochemical composition and antimicrobial potency. Most existing studies have focused on crude extracts without correlating phytochemical profiles with antimicrobial activity or determining MIC values against clinically relevant pathogens. Such information is essential for validating traditional uses of the plant and for advancing its application in modern pharmacology.

Therefore, this study aims to conduct a comprehensive phytochemical profiling and evaluate the antimicrobial activity of aerial extracts of *Buchholzia coriacea*. Additionally, the study seeks to determine the Minimum Inhibitory Concentration (MIC) of the extracts against selected microbial strains. By integrating phytochemical analysis with antimicrobial assays, this research intends to provide scientific evidence supporting the therapeutic potential of the plant and contribute to the ongoing search for novel antimicrobial agents from natural sources.

MATERIALS AND METHODS

2.1 Sample collection and preparation

The plants were obtained from Awka, Anambra State, Nigeria and identified at Botany dept of Nnamdi Azikiwe University Awka. The samples were sliced into very small sizes and shade dried for 7 days to constant weight. This was then ground into fine powder and stored in an air tight plastic container for extraction.

2.2 Extraction

100g of the sample was weighed into a bottle and acetone was transferred to it to make it 500mL and so it was macerated. It was allowed to stand for 48h and then filtered. The filtrate was evaporated under reduced pressure and was dried using a rotary evaporator at 55°C. This was repeated with water and ethanol to obtain the water extract and ethanol extracts respectively.

2.3 Preliminary Phytochemical Screening

The preliminary phytochemical Screening was conducted on the following: Alkaloids, Phenols, Terpenes, carbohydrates, Cardiac Glycosides, Flavonoids, Saponins, Steroids, Tannins, Proteins and Anthraquinones. The method adopted was that of Harborne (1998).

2.4 Quantitative phytochemical screening

2.4.1 Steroids

One gram (1 g) of the plant extract is macerated with 20 mL of ethanol and filtered to obtain a clear extract. Two milliliters (2 mL) of the filtrate is transferred into a test tube, and 2 mL of chromogen reagent is added. The mixture is allowed to stand for 30 minutes at room temperature for color development. The absorbance of the resulting solution is measured at 550 nm using a UV-Visible spectrophotometer. A standard calibration curve is prepared using varying concentrations of a standard steroid (e.g., cholesterol), treated under the same conditions. The concentration of steroids in the extract is then determined by extrapolation from the standard curve (Harborne J. B., 1998; Trease G. E. & Evans W. C., 2009).

2.4.2 Saponins

One gram (1 g) of the plant extract was weighed into a conical flask and 20 mL of 20% aqueous ethanol was added. The mixture was heated over a water bath at about 55 °C for 4 hours with continuous stirring. The extract was filtered and the residue was re-extracted with another 20 mL of 20% ethanol. The combined extracts were concentrated to about 10 mL over a water bath at 90 °C. The concentrate was transferred into a separatory funnel and 10 mL of diethyl ether was added and shaken vigorously to remove non-polar impurities; the ether layer was discarded. The aqueous layer was then treated with 10 mL of n-butanol and the combined n-butanol extracts were washed twice with 5 mL of 5% sodium chloride solution. The remaining solution was evaporated to dryness in a water bath and dried to a constant weight in an oven.

The saponin content was calculated as a percentage of the initial sample weight using:

$$\% \text{Saponins} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

This method is based on standard gravimetric procedures for saponin determination (Harborne J. B., 1998; Trease G. E. & Evans W. C., 2009).

2.4.3 Alkaloids

Alkaloid content of the plant extract was determined using the alkaline precipitation gravimetric method as described by Harborne (1998) and modified by Obadoni and Ochuko (2001).

One (1.0 g) of the powdered plant extract was weighed into a 250 mL beaker and 100 mL of 10% acetic acid in ethanol was added. The mixture was covered and allowed to stand for 4 hours with intermittent shaking to ensure complete extraction. The solution was then filtered using Whatman No. 1 filter paper. The filtrate was concentrated to one-quarter of its original volume by evaporation on a water bath at about 60°C. Concentrated ammonium hydroxide was then added dropwise to the extract until complete precipitation of alkaloids occurred. The solution was allowed to settle, and the precipitate was collected by filtration. The precipitate was washed with dilute ammonium hydroxide and then filtered again to remove impurities. The residue was dried in an oven at 60°C to a constant weight and recorded.

The percentage alkaloid content was calculated using the formula:

$$\% \text{Alkaloids} = (\text{Weight of residue} \div \text{Weight of sample}) \times 100.$$

2.4.4 Terpenoids

Terpenoid content of the plant extract was determined using the gravimetric method as described by Ferguson (1956) and later modified by Obadoni and Ochuko (2001). Approximately 2.0 g of the powdered plant sample was weighed and extracted with 50 mL of ethanol in a conical flask. The mixture was agitated continuously for 24 hours at room temperature to ensure complete extraction of terpenoids. The extract was then filtered using Whatman No. 1 filter paper. The filtrate was transferred into a separating funnel and mixed with 20 mL of petroleum ether. The mixture was shaken vigorously and allowed to stand for phase separation. The ether layer (containing terpenoids) was carefully collected, while the aqueous layer was discarded. The ether extract was evaporated to dryness in a pre-weighed evaporating dish on a water bath. The residue obtained represents the terpenoid fraction and was dried to a constant weight.

The percentage terpenoid content was calculated using the formula:

$$\% \text{Terpenoids} = (\text{Weight of residue} \div \text{Weight of sample}) \times 100.$$

2.4.5 Tannin

Tannin content of the plant extract was determined using the FolinDenis spectrophotometric method as described by Pearson (1976) and modified by AOAC (2005). About 0.5 g of the powdered plant sample was weighed into a 100 mL conical flask and 50 mL of distilled water was added. The mixture was heated on a water bath for 30 minutes with continuous shaking and then cooled and filtered using Whatman No. 1 filter paper. An aliquot (1 mL) of the filtrate was transferred into a test tube, followed by the addition of 5 mL of FolinDenis reagent and 10 mL of saturated sodium carbonate solution. The mixture was diluted to 50 mL with distilled water, shaken thoroughly, and allowed to stand for 30 minutes at room temperature for color development. The absorbance was measured at 760 nm using a UVVisible spectrophotometer against a reagent blank. Tannin concentration was determined from a standard calibration curve prepared using tannic acid.

The percentage tannin content was calculated as:

$$\text{Tannin (\%)} = (C \times V \times D) / (W \times 1000) \text{ (mg/mL)}$$

Where: C = concentration from calibration curve V = volume of extract (mL) D = dilution factor W = weight of sample (g)

2.4.6 Flavonoids

Flavonoid content of the plant extract was determined using the aluminium chloride colorimetric method as described by Chang et al. (2002) and modified by AOAC (2005). Approximately 0.5 g of the powdered plant sample was extracted with 50 mL of methanol for 24 hours at room temperature with intermittent shaking. The extract was filtered using Whatman No. 1 filter paper. An aliquot (1 mL) of the filtrate was mixed with 4 mL of distilled water in a test tube. Then, 0.3 mL of 5% sodium nitrite (NaNO₂) solution was added. After 5

minutes, 0.3 mL of 10% aluminium chloride (AlCl_3) solution was added. At the 6th minute, 2 mL of 1 M sodium hydroxide (NaOH) was added, and the total volume was made up to 10 mL with distilled water. The mixture was thoroughly mixed, and the absorbance was measured at 510 nm using a UV–Visible spectrophotometer against a blank. Flavonoid concentration was determined from a standard calibration curve prepared using quercetin.

Calculation:

$$\text{Flavonoid (\%)} = (C \times V \times D) / (W \times 1000)$$

Where: C = concentration obtained from calibration curve (mg/mL)

V = volume of extract (mL)

D = dilution factor

W = weight of sample (g)

2.4.8 Phenols

Total phenolic content of the plant extract was determined using the Folin–Ciocalteu spectrophotometric method as described by Singleton and Rossi (1965) and modified by AOAC (2005). Approximately 0.5 g of the powdered plant sample was extracted with 50 mL of methanol for 24 hours at room temperature with intermittent shaking. The extract was filtered using Whatman No. 1 filter paper. An aliquot (1 mL) of the filtrate was mixed with 5 mL of Folin–Ciocalteu reagent (previously diluted 1:10 with distilled water). After 5 minutes, 4 mL of 7.5% sodium carbonate (Na_2CO_3) solution was added to the mixture. The reaction mixture was incubated at room temperature for 30 minutes for color development. The absorbance was then measured at 765 nm using a UV–Visible spectrophotometer against a reagent blank. The total phenolic content was determined from a standard calibration curve prepared using gallic acid and expressed as gallic acid equivalent (GAE). Calculation:

$$\text{Total Phenols (mg GAE/g)} = (C \times V \times D) / W$$

Where: C = concentration from calibration curve (mg/mL) V = volume of extract (mL) D = dilution factor W = weight of sample (g)

2.4.9 Anthraquinones

The anthraquinone level in the plant extract was assessed utilizing a spectrophotometric technique as outlined by Trease and Evans (2002) and adapted by Sofowora (2008).

About 1.0 g of the powdered plant sample was placed in a conical flask and extracted with 50 mL of hot distilled water for 30 minutes. The solution was filtered and cooled with Whatman No. 1 filter paper.

The filtrate was moved to a separating funnel and extracted using 25 mL of chloroform. The chloroform layer was isolated and treated with 10 mL of a 10% ammonia solution. This caused a pink-colored solution to form in the ammoniacal layer, signaling the presence of anthraquinones.

The absorbance of the ammoniacal layer was recorded at 450 nm with a UV–Visible spectrophotometer in comparison to a blank. The concentration of anthraquinone was assessed by utilizing a standard calibration curve created with a known anthraquinone compound (alizarin)

The percentage anthraquinone content was calculated using the formula:

$$\text{Anthraquinones (\%)} = (C \times V \times D) / (W \times 1000)$$

Where: C = concentration from calibration curve (mg/mL) V = volume of extract (mL) D = dilution factor W = weight of sample (g)

2.5 Antimicrobial Screening

Pure clinical isolates of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp.*, *Candida albicans*, and *Aspergillus niger* were obtained from the Microbial Culture Collection Unit, Alpha Research Laboratory, Awka. All cultures used were single isolate cultures to ensure experimental consistency.

the following were the incubation conditions:

Bacteria: 37°C for 18– 24 h

Fungi (*Candida*): 30°C for 24– 48 h

Fungi (*Aspergillus*): 25– 28°C for 48– 72 h.

Fluconazole was used as the standard antifungal agent, while chloramphenicol was used as the standard antibacterial agent.

2.5.1 Standardization of inocula

The cultures were standardized following the approach outlined by Dalitha (2008). Sterile nutrient broth was inoculated with pure isolates, and the cultures were adjusted to match a 0.5 McFarland turbidity standard by comparing their turbidity levels to the standard

2.5.2 Preparation of Disc

Disc of diameter 6mm were perforated from the whatman filter paper using a perforator. The discs were sterilized using a bijoux bottle at 1600C for 2hrs in a hot air oven. It was brought out and allowed to cool before further use according to Robert *et al.*, (2011).

2.5.3 Disc Preparation for Ethanolic/acetone and water Extract

1 gram of each extract were separately mixed with 2mls of Ethanolic/acetone and water. The mixture was properly done in a test tube and 1ml of the mixture was poured into a glass petri dish containing 10 paper discs. The disc was put in the oven to dry so as to allow the plant extract stick to the paper disc for further use (Robert *et al.*, 2011).

2.5.4 Preparation of Antibiotic Disc (standard)

250mg and 200mg each of chloramphenicol and clotrimazole were separately dissolved in 2mls of water each to give 500mg/ml and 400mg/ml each. 1 ml of the various stock solutions was diluted in 1ml of water and 1 ml of this stock was added to 10 paper discs in glass petri dish and allowed to dry in the oven 3hrs at 40 0 C, so that the drug would stick to the discs (Robert *et al.*, 2011).

2.5.5 Media Preparation

All the materials used were sterilized after being washed with detergent and rinsed severally with distilled water. The media used for culturing the organism were Muller -hinton agar for and Sabouraud Dextrose Agar (SDA). Muller-Hinton agar was prepared by dissolving 6.72g of agar powder in 240mls of distilled water and autoclaved at 121 0C for 15 mins and allowed to cool to body temperature. The media was dispensed into petri dishes, it was flamed to remove air bubbles. Sabouraud Dextrose agar media was prepared by dissolving 7.8g of agar powder in 120mls of distilled. It was autoclaved for 15mins at 121 0C and allowed to cool to body. temperature before dispensing into petri dishes. It was flamed was flamed to remove air bubbles according to Cheesbrough (2002).

2.5.6 Screening for Antimicrobial Activity assay

The Antimicrobial activities were carried out based on the method of the Clinical and Laboratory Standards Institute (2021).

2.5.7 Antibacterial Activity (Disc diffusion method)

Mueller-Hinton agar was prepared according to manufacturer's instructions, sterilized, and poured into sterile Petri dishes. The plates were allowed to solidify. Bacterial cultures were adjusted to match 0.5 McFarland standard (approximately 1×10^8 CFU/mL). A sterile cotton swab was dipped into the standardized bacterial suspension and evenly spread across the surface of the agar plate to obtain a uniform lawn culture. Sterile filter paper discs were impregnated with known concentrations of the extract and placed on the inoculated agar surface. The positive control was the standard antibiotic fluconazole, while the negative control the Solvent used for extraction. Plates were incubated at 37°C for 18– 24 hours. After incubation, the diameter of the zone of inhibition around each disc was measured in millimeters.

2.5.8 Antifungal Activity (Agar - well diffusion method).

Mueller-Hinton agar was prepared according to manufacturer's instructions, sterilized, and poured into sterile Petri dishes. The plates were allowed to solidify. Bacterial cultures were adjusted to match 0.5 McFarland standard (approximately 1×10^8 CFU/mL). A sterile cotton swab was dipped into the standardized bacterial suspension and evenly spread across the surface of the agar plate to obtain a uniform lawn culture. Wells of about 6 mm diameter were bored into the agar using a sterile cork borer. A measured volume (e.g., 50– 100 µL) of the extract was introduced into each well. Plates were incubated at 37°C for 18– 24 hours. After incubation, the diameter of the zone of inhibition around each disc or well was measured in millimeters.

2.5.9 Determination of minimum inhibitory concentration (MIC) of plant extract against selected microorganisms (Broth Dilution Method).

A known quantity of the plant extract was dissolved in a suitable solvent to obtain a high concentration stock solution (e.g., 100 mg/mL). Two-fold serial dilutions of the extract were prepared in broth to obtain a range of concentrations. Microbial cultures were standardized to 0.5 McFarland standard ($\sim 1 \times 10^8$ CFU/mL), then diluted to $\sim 10^6$ CFU/mL for the bacteria while $\sim 1 \times 10^6$ spores/mL for the fungi. Equal volumes of standardized inoculum were added to each dilution well containing the extract. The positive control was broth + microorganism (no extract), the negative control was broth only (no microorganism) and the standard drug control was fluconazole. The bacteria was incubated at 37°C for 18– 24 hours while the fungi was Incubated at 25– 28°C for 48– 72 hours. After incubation, wells were examined for turbidity (growth). The MIC was recorded as the lowest concentration of the extract showing no visible growth compared to the control.

2.6 Statistical analysis

Percentages and means of fungal colonies were calculated. All extractions and antimicrobial assays were performed in triplicate (n = 3 biological replicates), and results were expressed as mean ± standard deviation. Statistical analysis was performed using one-way Analysis of Variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) to determine significant differences between means at $p < 0.05$.

RESULTS AND DISCUSIONS

3.1 Extraction

Table1: Percentage Recovery of the Sovents

Acetone	Ethanol	Water
10.00%	14.00%	8.80%

The yield from the aerial parts of *Buchholzia coriacea* indicated that ethanol (14.00%) resulted in the highest yield, followed by acetone (10.00%) and water (8.80%), demonstrating the significant effect of solvent polarity on extraction efficiency. Ethanol is frequently noted as a potent solvent for extracting various phytochemicals because of its moderate polarity and capacity to dissolve both polar and somewhat non-polar compounds (Azwanida, 2015; Do *et al.*, 2014). The moderate yield seen with acetone reinforces its function as a semi-polar solvent that can extract various bioactive components, albeit less effectively than ethanol (Cowan, 1999). Conversely, the reduced yield observed for water might be due to its inability to extract non-polar and certain moderately polar compounds, even though it effectively works for highly polar substances (Ngo *et al.*, 2017). These results align with earlier research highlighting that the choice of solvent is a crucial element in optimizing phytochemical extraction from plant sources (Azwanida, 2015).

3.2 Phytochemical Screening

Table 2: Qualitative phytochemical data

PHYTOCHEMICALS	ACETONE	ETHANOL	WATER
SAPONIN	++	+++	+++
FLAVONOID	-	+++	++
ALKALOID	+	+++	-
TANNIN	++	++	+
STEROIDS	+	-	-
TERPENIODES	+	+++	+++
GLYCOSIDES	++	++	+
CARBOHYDRATES	++	++	+
PROTEIN	+	+	+
ANTHRAQUINONES	-	++	-
PHENOL	++	+++	+
OIL AND RESIN	-	-	-

Key:

+++ = Present in high concentration

++ = Present in moderate concentration

+ = Slightly or sparingly present

- = Absent.

The qualitative phytochemical screening of the acetone, ethanol, and aqueous leaf extracts of *Buchholzia coriacea* revealed the presence of several secondary metabolites with known biological activities. The phytochemicals detected include saponins, flavonoids, alkaloids, tannins, steroids, terpenoids, glycosides, carbohydrates, proteins, anthraquinones, and phenolic compounds, while oil and resin were absent in all extracts (Table 2).

The results indicate that solvent type significantly influenced the extraction efficiency of phytochemicals. The ethanol extract exhibited the highest diversity and concentration of phytochemicals, showing strong presence (+++) of saponins, flavonoids, alkaloids, terpenoids, and phenolic compounds. In contrast, the aqueous extract showed strong presence (+++) of saponins and terpenoids but lacked detectable levels of alkaloids and

anthraquinones. The acetone extract contained moderate to low levels of most phytochemicals. These observations suggest that ethanol was the most effective solvent for extracting phytochemicals *Buchholzia coriacea*.

3.3 Phytochemical quantification

TABLE 3: Quantitative phytochemical composition

Phytochemicals	Quantities (mg/100g)
Alkaloids	21.33 ± 3.20 mg/100g
Quinones	10.01 ± 0.40 mg/100g
Saponins	11.6 ± 0.10 mg/100g
Tannins	14.34 ± 0.40 mg/100g
Flavonoids	24.37 ± 4.10 mg/100g
Steroids	1.01 ± 0.05 mg/100g
Terpenoids	6.74 ± 0.20 mg/100g

Phytochemicals such as tannins, flavonoids, and alkaloids likely contributed to the observed antimicrobial activity. Tannins exert antimicrobial effects by binding to bacterial cell wall proteins, leading to cell membrane disruption and enzyme inhibition. Flavonoids are known to inhibit nucleic acid synthesis and disrupt microbial metabolism, while alkaloids interfere with DNA replication and protein synthesis.

Among the phytochemicals detected, flavonoids recorded the highest concentration (24.37 ± 41 mg/100 g). Flavonoids are an important class of polyphenolic compounds known for their potent antioxidant, antimicrobial, anti-inflammatory, and anticancer properties. Alkaloids were the second most abundant phytochemicals detected (21.33 ± 13.2 mg/100 g). Alkaloids are nitrogen-containing secondary metabolites that are widely distributed in medicinal plants and are well known for their broad spectrum of pharmacological activities. These include antimicrobial, antimalarial, analgesic, and antihypertensive properties. The relatively high alkaloid content observed in this study may therefore partly explain the antimicrobial activity frequently reported for extracts of *Buchholzia coriacea* (Owolabi et al., 2021). Tannins were also present in appreciable amounts (14.34 ± 0.4 mg/100 g). Tannins are polyphenolic compounds capable of forming complexes with proteins and other macromolecules. Their biological activities include antimicrobial, antioxidant, and anti-inflammatory effects. Tannins are known to inhibit microbial growth by binding to bacterial cell walls and enzymes, thereby interfering with metabolic processes essential for microbial survival (Sofowora, Ogunbodede, & Onayade, 2019). Saponins and quinones were averagely present at concentrations of 11.6 ± 0.1 mg/100 g and 10.01 ± 0.4 mg/100 g respectively. Terpenoids were much less (6.74 ± 0.0 mg/100 g) and steroids were the least in quantity at a concentration of 1.01 ± 0.0 mg/100 g.

The predominance of flavonoids and alkaloids, coupled with moderate levels of tannins and saponins, suggests that the leaves may possess strong antioxidant and antimicrobial properties. These findings support the traditional use of *Buchholzia coriacea* in herbal medicine and highlight its potential as a source of bioactive compounds for drug development.

3.4 Antimicrobial Analysis

TABLE 4: Antimicrobial activities of the extracts

ISOLATE	EXTRACT	ZONE OF INHIBITION (mm)	RESULT
<i>Aspergillus spp</i>	Ethanol extract	12.33± 0.28	S
	Acetone extract	12.00± 0.00	S
		10.16± 0.28	1

	Water extract	12.00± 0.00	S
	Fluconazole		
<i>Candida spp</i>	Ethanol extract	25.00±0 .00	S
		15.00± 0.00	S
	Acetone extract	12.00± 0.00	S
	Water extract	32.27± 1.36	S
	Fluconazole		
<i>S. aureus</i>		17.33± 0.28	
	Ethanol extract	20.00± 0.00	S
		7.16± 0.28	S
	Acetone extract	18.00± 0.00	R
	Water extract		S
	Chloramphenicol		
<i>E. coli</i>		27.00±1 .00	S
	Ethanol extract	30.00± 0.20	S
		12.00± 0.02	S
	Acetone extract	32.27± 1.36	S
	Water extract		
	Chloramphenicol		
<i>Salmonella spp</i>		12.00±0 .00	S
	Ethanol extract	17.00± 0.00	S
		20.00± 0.00	S
	Acetone extract	32.27± 1.36	S
	Water extract		
	Chloramphenicol		

*Values are mean scores ± Standard deviation of three (3) replicates

N/B: R = Resistant, I = Intermediate, S = Susceptible

3.5 Antifungal Activity

The antifungal activity of the extracts revealed moderate inhibition against *Aspergillus spp.* with ethanol extract showing the highest zone of inhibition (12.33 ± 0.28 mm), followed closely by acetone extract (12.00 ±

0.00 mm), while the aqueous extract exhibited the lowest activity (10.16 ± 0.28 mm). These inhibition zones were comparable to the standard antifungal drug fluconazole (12.00 ± 0.00 mm). The comparable activity between the organic solvent extracts and the standard drug suggests that ethanol and acetone are effective solvents for extracting antifungal bioactive compounds from the leaves.

The ethanol extract also exhibited strong antifungal activity against *Candida* spp. with a zone of inhibition of 25.00 ± 0.00 mm. However, the activity was still lower than that of the standard antifungal drug fluconazole (32.27 ± 1.36 mm). This observation agrees with earlier reports indicating that extracts of *B. coriacea* possess significant antifungal activity due to the presence of bioactive phytochemicals (Ajaiyeoba *et al.*, 2019).

3.6 Antibacterial Activity

The antibacterial screening of *B. coriacea* leaf extracts demonstrated variable susceptibility among the tested bacterial isolates. For *Staphylococcus aureus*, the acetone extract showed the highest antibacterial activity with a zone of inhibition of 20.00 ± 0.00 mm, followed by ethanol extract (17.33 ± 0.28 mm). The aqueous extract exhibited very low activity (7.16 ± 0.28 mm), indicating resistance according to the interpretation criteria used in the study. The activity of the acetone extract was slightly higher than that of the standard antibiotic chloramphenicol (18.00 ± 0.00 mm).

The high susceptibility of *S. aureus* may be attributed to the structural characteristics of Gram-positive bacteria which lack the outer membrane barrier found in Gram-negative bacteria, thereby allowing easier penetration of antimicrobial compounds (Cushnie & Lamb, 2016).

For *Escherichia coli*, the extracts exhibited strong antibacterial activity. The acetone extract showed the highest inhibition zone (30.00 ± 0.20 mm), followed by ethanol extract (27.00 ± 1.00 mm) and aqueous extract (12.00 ± 0.02 mm). Although the activity of acetone extract was slightly lower than that of chloramphenicol (32.27 ± 1.36 mm), the large inhibition zones indicate significant antibacterial potential. Similar findings have been reported in studies evaluating the antimicrobial properties of *B. coriacea* leaves against enteric bacteria (Nwachukwu *et al.*, 2020).

For *Salmonella* spp., the ethanol extract produced moderate antibacterial activity with a zone of inhibition of 12.00 ± 0.00 mm, while the acetone extract produced a larger inhibition zone (17.00 ± 0.00 mm). This suggests that acetone may extract certain antimicrobial compounds more efficiently than ethanol or water.

3.7 Effect of Extraction Solvent

The findings indicate that the polarity of solvents greatly affects the antimicrobial efficacy of plant extracts. Organic solvents like ethanol and acetone generated larger inhibition zones than aqueous extracts for the majority of the tested organisms. Organic solvents are recognized for their ability to extract a broader variety of phytochemicals, such as phenolics, flavonoids, alkaloids, and terpenoids, which play a crucial role in antimicrobial activity (Sasidharan *et al.*, 2017).

Despite containing some phytochemicals, aqueous extracts showed lower antimicrobial activity. This may be due to the inability of water to efficiently extract moderately polar and non-polar bioactive compounds such as flavonoids and terpenoids, which are better extracted using organic solvents like ethanol.

3.8 Minimum Inhibitory Concentration (MIC)

Table 5: Minimum inhibitory concentration (Mg/mL)

ISOLATES	ACETONE	ETHANOL	WATER	CONTROL DRUG
Salmonella spp	1.0	0.10	1.00	0.10
E. coli	1.00	0.10	0.50	0.50
S. aureus	0.50	0.50	1.00	0.10

C. albicans	0.50	0.50	0.50	0.10
A. niger	13.00	1.00	1.00	0.50

Note:

MIC ≤ 0.1 mg/mL – Very strong antimicrobial activity

0.1– 0.5 mg/mL – Strong antimicrobial activity

0.5– 1.0 mg/mL – Moderate antimicrobial activity

>1.0 mg/mL – Weak antimicrobial activity

The findings show that the ethanol extract demonstrated the highest antimicrobial effectiveness, evident from the lowest MIC values for most of the organisms tested. Ethanol extracts yielded MIC values of 0.10 mg/mL for *Salmonella* spp. and *Escherichia coli*, which are similar to or superior to the control drug employed in the research. Lower MIC values typically suggest greater antimicrobial strength, as a smaller amount of extract is needed to prevent microbial growth. The significant activity of the ethanol extract could be due to ethanol’s capability to dissolve a wide range of bioactive phytochemicals (Cowan, 1999; Akinpelu *et al.*, 2015).

The acetone extract demonstrated moderate antibacterial activity against the majority of bacterial isolates. The MIC values observed for *Staphylococcus aureus* and *Candida albicans* were 0.50 mg/mL, indicating a moderate inhibitory capability. The acetone extract exhibited minimal activity against *Aspergillus niger*, with a MIC value of 13.00 mg/mL, suggesting that a significantly greater concentration of the extract was needed to suppress fungal growth. This implies that the antifungal substances found in the leaf might not be effectively extracted by acetone or could exist in minimal amounts.

The water-based extract typically demonstrated lower antimicrobial effectiveness than the organic solvent extracts. The MIC values for *Salmonella* spp. and *Staphylococcus aureus* were 1.00 mg/mL, suggesting reduced inhibitory efficiency. This finding aligns with earlier research indicating that water usually extracts fewer antimicrobial phytochemicals than organic solvents, as numerous bioactive compounds are either moderately polar or non-polar (Sasidharan *et al.*, 2011). The aqueous extract exhibited a degree of activity against *E. coli* and *Candida albicans*, with MIC values of 0.50 mg/mL, indicating that the water-soluble phytochemicals in *B. coriacea* play a role in antimicrobial effects.

The findings of this study are consistent with previous reports on *Buchholzia coriacea*, which have demonstrated significant antimicrobial activity attributed to its rich phytochemical composition (Ajaiyeoba *et al.*, 2001; Ibrahim & Fagbohun, 2012). However, this study extends existing knowledge by focusing specifically on aerial parts and providing MIC evaluation, thereby contributing additional insight into the plant’s therapeutic potential.

CONCLUSION AND RECOMMENDATION

The phytochemical composition of the aerial parts of *Buchholzia coriacea* show a diverse array of secondary metabolites that may have significant pharmacological potential.

this study is the use of crude extracts, which do not identify specific active compounds responsible for antimicrobial activity. Future studies should employ advanced analytical techniques such as High-Performance Liquid Chromatography (HPLC) and Gas Chromatography– Mass Spectrometry (GC-MS) to isolate and characterize bioactive compounds. These studies may offer greater understanding of the mechanisms that drive the plant's pharmacological effects and support its possible use in drug and nutraceutical development.

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