

PHYTOCONSTITUENTS AND ANTIBACTERIAL EFFECTS OF CRUDE ETHANOL EXTRACT AND FRACTIONS OF *BUCHHOLZIA CORIACEA* SEEDS

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Abstract

The present study evaluated the phytoconstituents and antibacterial activities of crude ethanol extract and fractions of *Buchholzia coriacea* seeds against pure isolates of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Streptococcus mutans* at various concentrations. The presence of alkaloid, anthocyanin, flavonoid, phenols, saponins, steroids and terpenoids, tannins, carotenoids and other phytochemicals were detected in the pulverized sample and crude extract. Crude methanol extract demonstrated antibacterial effect against *S. mutans* and *S. aureus* at 100 mg/ml concentration. Free flavonoid-rich fraction showed antibacterial effect against *S. aureus* at both 50 and 100 mg/ml and against *E. coli* at 100 mg/ml. Bound flavonoid-rich fraction showed antibacterial effect against *S. aureus* and *E. coli* at 100 mg/ml. Alkaloid-rich fraction exhibited antibacterial effect against *S. mutans* and *S. aureus* at all the concentrations studied except 6.25 mg/ml. Antibacterial effect against *E. coli* at both 50 and 100 mg/ml and *B. cereus* at all the concentrations studied were observed in alkaloid-rich fraction. These findings showed that flavonoids and alkaloids in *B. coriacea* seeds possess antibacterial effects.

Keywords: *Buchholzia coriacea*, antibacterial effects, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Streptococcus mutans*, phytochemicals

Introduction

Medicinal plants are rich in secondary metabolites which are potential sources of drugs and are hence, of therapeutic values [1]. There has been a gradual renewal of the interest in the use of medicinal plants in most developing countries because the use of herbal drugs has been reported to be less toxic when compared with synthetic drugs. Aside nutrients, medicinal plants bear important chemical compounds that are biologically active and effective against diseases [2]. In addition, the alarming development and spread of antibiotics resistance necessitates the search for alternative sources of antimicrobial agents especially from plant origin. Plant-based antimicrobials have enormous therapeutic potentials; they contain chemical compounds that act individually and synergistically, with wider spectrum and fewer side effects when compared with synthetic antimicrobial agents [3].

Buchholzia coriacea was named after Reinhold Wilhelm Buchholz who collected the plants in Cameroon in the late 19th century [5]. It belongs to the family *Capparidaceae*. The family *capparidaceae* is described from Carpe Verde Islands. It comprises of 45 genera and approximately 1000 species, distributed in the tropical and sub-tropical regions, especially east Africa and South America. The plant is an evergreen under-storey tree of lowland rainforest, up to 20 metres high occurring in Cameroon, Congo, Central African Republic, Gabon, Angola, Nigeria, Ghana among others [5]. It is a shrub or medium-sized tree, evergreen, with a dense crown, large, glossy, leathery leaves arranged spirally and clustered at the ends of the branches and conspicuous cream-white flowers in racemes at the end of the branches [6]. The bark of the plant is smooth, blackish-brown/dark-green. Slashes are deep red turning dark brown. The leaflets are large, obviate, oblanceolate to elliptic, shortly acute at apex, cuneate at base, 15x30x5-11 cm, thinly coriaceous, glabrous, midrib very prominent below, stalk 10-15 cm long, swollen for about 1 cm at the both ends, pale green [7]. The fruits are large, long-stalked, ellipsoid, resembling, avocado peas, 12x15-8 cm, endocarp up to 1.3 cm thick and woody, yellowish when ripe, flash yellow, edible, containing

a few large blackish seeds about 2.5 cm long [8]. The plant is taxonomically classified as follows:

Family: Capparaceae juss
Super Order: Rosanae takhl
Order: Brassicales bromahead
Genus: Buchholzia engl
Class: Eqissetopsida c. agardh
Sub-class: Magnoliidae nov' ak ex takht
Specie: *Buchholzia coriacea*.
Common name: Wonderful kola

The seed of the plant is used in folk medicine for the treatment of diabetes, ulcer, malaria, helminthic disorders, hypercholesterolemia and other diseases, hence the origin of its common name "wonderful kola". The plant part commonly eaten is the seeds which are either cooked or eaten raw [9]. Preliminary study has shown that the seed extract possesses antimicrobial activity [10-12]. Olaiya and Omolekan [13] demonstrated the antihypercholesterolemic effect of ethanol seed extract of the plant. Eze et al. [6] showed that seed extract of the plant has immunomodulatory effects. Ezike et al. [15] showed that the plant leaf extract possess anti-inflammatory effect. Ibrahim and Fagbohun [15] showed that *B. coriacea* seeds are rich in many phytochemicals, some of which possess great antimicrobial effects. Phenolics such as alkaloids, flavonoids, glucosinolates and sterols have been implicated in this plant family which possess antimicrobial properties [16-17]. Ajaiyeoba et al. [16] and Onwuka et al. [18] showed that extract of the *B. coriacea* exhibited antihelminthic effect. The two main compounds present in the most active fraction were isolated and identified as lupeol and β -sitosterol [16]. The hypoglycaemic effect of the seed extract has been evaluated [19-21]. We have earlier shown from our laboratory that methanol extract of the plant's seed possess anti-inflammatory [22], hypolipidaemic [23] and antiulcer and gastric anti-secretory effects [24]. Nwaichi and Oluwa [25] demonstrated that the plant extract possesses antioxidant potential. Recently, Okere et al. [26] showed that the seed extract has anti-inflammatory effect on carrageenan-induced inflammation. Also, Lapshak et al. [27] showed that aqueous extract of the plant seed is rich in phytochemicals and possess anti-diabetic effect. The

present study was therefore aimed to evaluate the phytoconstituents and antibacterial activities of crude ethanol extract and fractions of *B. coriacea* seeds

Methods

Collection and authentication of plant material

Fresh Fruits of *Buchholzia coriacea* were purchased from Orba Main market, Enugu State of Nigeria and were authenticated at the herbarium of Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. The seeds were removed, washed with clean water to remove dirt and sand, chopped into pieces and air-dried for 21 days. The dried seeds were pulverized into fine powder.

Preparation of crude plant extract

Powdered seed sample of *B. coriacea* (1,000 g) was macerated in 3.5 litres of absolute ethanol for 24 hours. The extract was filtered using Whatman No.1 filter paper and the filtrate was concentrated to a semi-solid residue using an incubator at 27°C.

Phytochemical analysis of the pulverized dried seeds and crude ethanol extract

The phytochemical analyses were done using the methods of Harborne [28], Trease and Evans [29] and AOAC [30].

Preparation of flavonoid-rich extract

One hundred grams (100 g) of the finely powdered sample was soxhlet-extracted with absolute ethanol. The filtrate was re-extracted successively with petroleum ether (Fraction 1), ethyl ether (Fraction 2) and ethyl acetate (Fraction 3) using separating funnel. Ethyl acetate fractions were analysed for free and bound flavonoid respectively. The ethyl acetate fraction of each of the samples was hydrolysed by refluxing with 7% concentrated sulphuric acid for 2 hours (removal of bounded sugars) and the filtrate was extracted with ethyl acetate in separating funnel. Ethyl acetate extract obtained was washed with distilled water to

neutrality. Ethyl ether (free flavonoid) was dried in open air followed by heat concentration at 27°C. Ethyl acetate fractions (bound flavonoid) was dried in the oven at 40°C and stored at 4°C in an air-tight sample bottle.

Preparation of alkaloid-rich extract

The method previously described by Yubin et al. [31] was used. Pulverized *B. coriacea* seed (100 g) was extracted with 10% acetic acid in ethanol for 4 hours. Extracts were concentrated and were made alkaline by NH₄OH. The precipitate obtained was collected by centrifugation, washed with 1% NH₄OH in distilled water, filtered and dried in a vacuum. Alkaloid-rich extract obtained were stored at 4°C.

Study organisms

The test organisms used in this study are associated with various forms of human infections from a clinical point of view. Pure isolates of four bacteria species used to test for the antibacterial effect of the extract and fractions of *B. coriacea* include: gram negative bacteria- *Escherichia coli* and gram positive bacteria- *Staphylococcus aureus*, *Bacillus cereus* and *Streptococcus mutans*.

Preparation of the stock culture, sub-culture and working culture

The method described by Holt et al. [32] was adopted. Stock culture is a culture of microorganisms maintained solely for the purpose of keeping the microorganism in viable condition. To prepare this, nutrient agar (28 g) was dissolved in 100 ml of distilled water in a bijou bottles. The suspension was homogenized and sterilized in an autoclave at 15 psi at 121°C for 15 mins. The mixture was then allowed to cool and poured into bijou bottles, one for each bacterial isolate and was left to solidify in a slanting position. Aseptic procedures were maintained throughout the experiment. After the slants had solidified, the agar was incubated for 24 hours at 37°C to check for signs of growth. The isolates were cultured in representative bottles using a wire loop and allowed to grow for 24 hours at 37°C after which the isolates were preserved at 4°C. Prior to study proper, a sub-culture was prepared from the stock culture as follows: Fresh

nutrient agar was prepared as described above and poured in sterile petri dishes and allowed to solidify. They were incubated for 24 hours at 37°C and observed for growth/sterility. Microorganisms were inoculated on them and allowed to grow for 24 hours at 37°C. For working culture, nutrient broth (1.3 g) was dissolved in 100 ml of distilled water, autoclaved and was poured in the sterilize test tubes, one test tube for each microorganism. Using a wire loop and flame, the respective isolates were obtained and cultured into test tubes and left to grow in a slanting position for 24 hours at 37°C.

Standardization of the microorganism

Standardization of the bacterial species to 1×10^6 cells/ml was done using 0.5 Macfalan standard. This was achieved by adjusting the turbidity of the microorganism-containing broth to that of Macfalan standard using normal saline.

Preparation of various concentrations of the extracts, standard and negative controls

Crude extract and fractions (0.6 g each) were dissolved in 1 ml of the extracting solution and the volume was made up to 5 ml with distilled water to give a concentration of 100 mg/ml. Using double fold serial dilution, concentrations of 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml were achieved. The standard drug, used in this study was chloramphenicol. Chloramphenicol (1 g) was dissolved in 10 ml normal saline which gave 100 mg/ml and was serially diluted to 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml. As a negative control, each of the dissolving solvents (Ethyl ether, ethyl acetate, 10% acetic acid in ethanol, 80% ethanol in distilled water, distilled water and normal saline) were tested for antibacterial activities against all four microorganisms.

Determination of antibacterial activity of the extracts

On freshly prepared plates, 0.1 ml of the standardized microorganism was inoculated, one isolate per plate, and was smeared all-round the plate using a sterile swab stick. By method of agar-well diffusion, using a hole-borer of 4 mm, five holes

were made on the plate. One in the middle for the control and the other four holes for the four extracts for a particular concentration and test sample. For an isolate, five plates would be used for the five different concentrations. This was done for *B. cereus*, *S. mutans*, *E. coli* and *S. aureus*, which gave a total of 20 plates for all the isolates. The experiments were done in triplicates, hence, a total of 60 plates was used. The plates were then kept at 4°C to enhance diffusion, and then the bacteria were incubated at 37°C for 24 hours for growth, after which the plates were observed for zones of inhibition. The minimum inhibitory concentration (MIC) was obtained by noting the lowest concentration of extract and standard drug which had activity against the test microorganisms, i.e. the lowest concentration of an antimicrobial agent that will inhibit visible growth of a microorganism after overnight incubation [5].

Statistical analysis

Raw data were entered in IBM Statistical Product and Service Solution (SPSS), version 18 and analyzed using one-way analysis of variance (ANOVA). The results were presented as mean \pm standard deviation (SD) in Tables. Mean differences were considered significant at $p < 0.05$.

Results

Phytochemical constituents of the pulverized seed samples and crude ethanol extract of *Buchholzia coriacea*

The results of the phytochemical analysis of pulverized seed samples and crude ethanol extract of *B. coriacea* are shown in Table 1. The presence of alkaloid, anthocyanin, flavonoid, phenols, saponins, steroids and terpenoids, tannins, carotenoids and other phytochemicals were detected in the pulverized sample and crude extract. The result of the quantitative phytochemical analysis showed that except for saponins, polyphenols, anthocyanin, resins, flavonoids and carotenoids, the pulverized seed sample had significantly ($p < 0.05$) higher phytoconstituents compared with the crude ethanol extract.

Minimum inhibition concentrations of crude extract and fractions of *Buchholzia coriacea* on *Streptococcus mutans*

The mean inhibition zone diameters for different concentration of crude ethanol extract, free flavonoid-rich fraction, bound flavonoid-rich fraction, alkaloid-rich fraction and the standard drug (chloramphenicol) on *S. mutans* is shown in Table 2. Chloramphenicol exhibited good antibacterial effect which is concentration-dependent. Similarly, alkaloid-rich fraction of the plant exhibited an appreciable antibacterial effect which is concentration-dependent except at concentration of 6.25 mg/ml where no activity was observed. Notably, the antibacterial activity of chloramphenicol was significantly ($p < 0.05$) higher compared with the extract and fractions in all the concentrations considered. Meanwhile, crude extract exhibited antibacterial effect on *S. mutans* only at concentration of 100 mg/ml. However, flavonoid-rich fractions did not show any antibacterial effect on the test organism considered at all the concentrations studied.

Minimum inhibition concentrations of crude extract and fractions of *Buchholzia coriacea* on *Staphylococcus aureus*

The inhibition zone diameters for different concentrations of crude ethanol extract, free flavonoid-rich fraction, bound flavonoid-rich fraction, alkaloid-rich fraction and the standard drug (chloramphenicol) on *S. aureus* is shown in Table 3. In all the concentrations studied, chloramphenicol exhibited the highest antibacterial effect. Crude methanol extract and free flavonoid-rich fraction of the plant seed possess antibacterial effect against *S. aureus* at concentrations of 100 and 50 mg/ml while bound flavonoid-rich fraction of the plant seed possess antibacterial effect against *S. aureus* only at concentration of 100 mg/ml. However, alkaloid-rich fraction of the plant seed showed antibacterial effect against *S. aureus* in all the concentrations studied except at 6.25 mg/ml. The findings showed that the antibacterial activity of chloramphenicol was significantly ($p < 0.05$) higher compared with

the extract and fractions at all the concentrations considered.

Minimum inhibition concentrations of crude extract and fractions of *Buchholzia coriacea* on *Escherichia coli*

Table 4 shows inhibition zone diameters for different concentration of different seed fractions, crude ethanol extract, free flavonoid-rich fraction, bound flavonoid-rich fraction, alkaloid-rich fraction and the standard drug (chloramphenicol) on *E. coli*. Flavonoid-rich fraction (both free and bound) of *B. coriacea* exhibited antibacterial effect against *E. coli* only at concentration of 100 mg/ml. Similarly, alkaloid-rich fraction of the plant possesses antibacterial effect at 50 and 100 mg/ml while crude ethanol extract showed no antibacterial effect against *E. coli*. Meanwhile, chloramphenicol exhibited a concentration-dependent antibacterial effect against *E. coli* at all the concentrations studied.

Minimum inhibition concentrations of crude extract and fractions of *Buchholzia coriacea* on *Bacillus cereus*

Table 5 shows inhibition zone diameters for different concentration of different seed fractions, crude ethanol extract, free flavonoid-rich fraction, bound flavonoid-rich fraction, alkaloid-rich fraction and the standard drug (chloramphenicol) on *B. cereus*. Crude ethanol extract and flavonoid-rich fraction (both free and bound) showed no antibacterial effect against *B. cereus*. Meanwhile alkaloid-rich fraction and chloramphenicol demonstrated antibacterial effects against *B. cereus* in a concentration-dependent manner. However, the antibacterial activity of t chloramphenicol was significantly ($p < 0.05$) higher compared with the extract and fractions at all the concentrations considered.

Discussion

As part of the general response to the public health problems associated with increasing drug resistance among bacteria, more attention is focused on herbal medicine. Hence, the present study evaluated the

phytoconstituents of pulverized seeds and crude ethanol extract of *B. coriacea* seeds. The presence of various phytochemicals such as alkaloid, flavonoid, anthocyanin, phenols, saponins, steroids and terpenoids were detected, both in the pulverized seed powder and crude ethanol extract. Phytochemicals are important chemicals found at different concentrations in virtually all plants. Various factors such as part of plant studied, location of the plants, solvent of extraction and extraction methods affect the phytoconstituents of plants. Ezekiel and Onyeoziri [10], Nwaichi and Olua [25], and Ijarotimi et al. [33] in their separate studies detected the presence of some of these phytochemicals such as saponins, alkaloids and flavonoids in the raw seeds of *B. coriacea*. These secondary metabolites have been previously shown to have antibacterial activities [34-35]. The result of the present study and those from other researchers observed that alkaloids and flavonoids are among the most abundant phytochemicals in seeds of *B. coriacea*. Nwachukwu et al. [36] showed that flavonoids and alkaloids are present in high concentration in *B. coriacea*. Alkaloids can occur in all parts of the plants but frequently depending on the plant species, they accumulate only in particular organs such as the bark, root, fruits and seed. Several alkaloids such as berberine and pyrrolizidine alkaloids have been shown to possess antibacterial effect [37], and flavonoids have been shown to have antimicrobial effects [38]. Hence, we prepared alkaloid-rich, flavonoid-rich (both bound and free) fractions and crude ethanol extract of the seed and subjected them to antibacterial effects against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Streptococcus mutans* at various concentrations.

Crude ethanol extract and alkaloid-rich fraction of *B. coriacea* seeds displayed appreciable antibacterial effects against *S. mutans* at concentrations of 100 mg/ml for crude extract and 12.5-100 mg/ml for the alkaloid-rich fraction. Similarly, chloramphenicol, the standard drug used in this study demonstrated concentration-dependent antibacterial activity against *S. mutans*. However, the antibacterial effect extrapolated from the mean diameter of inhibition zone of chloramphenicol is significantly ($p < 0.05$)

higher compared with the crude ethanol extract and alkaloid-rich fraction (Table 2). *Streptococcus mutans* is a facultative anaerobic gram positive coccus-shaped bacterium found in the human oral cavity and a significant contributor to tooth decay. Similarly, crude ethanol extract and free flavonoid-rich fraction of the plant seed possess antibacterial effect against *S. aureus* at concentrations of 50 and 100 mg/ml while bound flavonoid-rich fraction of the plant seed possess antibacterial effect against *S. aureus* only at concentration of 100 mg/ml. However, alkaloid-rich fraction of the plant seed showed antibacterial effect against *S. aureus* at all the concentrations studied except at 6.25 mg/ml. Chloramphenicol exhibited antibacterial activity against *S. aureus* at all the concentrations studied. The findings showed that the antibacterial activity of chloramphenicol against *S. aureus* was significantly ($p < 0.05$) higher compared with the extract and fractions at all the concentrations considered (Table 3). *S. aureus* is the most important member of the staphylococcus genus and a gram positive coecal bacterium that is frequently found in the respiratory tract and on the skin. It is associated with pimple, boil, impetigo, food poisoning and many other diseases [39].

Flavonoid-rich fraction (both free and bound) of *B. coriacea* exhibited antibacterial effect against *E. coli* only at a concentration of 100 mg/ml. Alkaloid-rich fraction possesses antibacterial effect at 50 and 100 mg/ml but crude ethanol extract showed no antibacterial effect against *E. coli*. Meanwhile, chloramphenicol exhibited a concentration-dependent antibacterial effect against *E. coli* (Table 4). *Escherichia coli* is a rod-shaped gram negative bacteria found commonly in the lower intestine of warm blooded organisms (endotherms). It has been implicated in septicaemias and can infect the gall bladder, surgical wounds, skin lesions and the lungs especially in immune-deficient patients [40]. Our results agree with the findings of Ezekiel and Onyeoziri [10] that showed that hexane and methanol extract of *B. coriacea* seed possesses antibacterial effects against *S. aureus* and *E. coli*.

It was observed that crude ethanol extract and flavonoid-rich fraction (both free and bound) showed no antibacterial effect against *B. cereus*. Meanwhile, alkaloid-rich fraction and chloramphenicol

demonstrated antibacterial effects against *B. cereus* in a concentration-dependent manner. However, the antibacterial activity of chloramphenicol was significantly ($p < 0.05$) higher when compared with the extract and fractions at all the concentrations considered. Our results and that of other researchers [10-11], have shown that the plant seeds are rich reservoirs of antimicrobials which usually interfere with growth and metabolism of microorganisms in a negative manner [41]. *Bacillus cereus*, an endemic, soil-dwelling, gram-positive, rod-shaped bacterium commonly found in the food that has been sitting at room temperature and in turn causes food-borne illness. The varying degree of sensitivity of the bacterial isolates may be due to the intrinsic tolerance of the microbes and the nature and combination of phytochemicals present in the extract and the fractions. Hence, the present investigation clearly revealed the antibacterial effects of different fractions and crude ethanol extract of the seeds against *E. coli*, *S. aureus*, *B. cereus* and *S. mutans* and suggest that the alkaloid contents of the plant seed be isolated, characterized and subjected to clinical trials for management of bacterial infection.

It can be concluded from the findings of this study that crude ethanol extract and fractions of *Buchholzia coriacea* seeds have appreciable antibacterial potentials and thus can be used alone or in combination with synthetic drugs/other antibacterial medicinal plants for the treatment of diseases caused by the tested isolates. The antibacterial activity of the seed is therefore attributed to the flavonoid and majorly, the alkaloids contents of the plant.

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Table 1: Phytochemical analysis of the dried seeds and the ethanol extract of *Buchholzia coriacea*

Phytochemicals	Amount in powdered seed sample (mg/100g)	Amount in crude ethanol extract (mg/100g)
Alkaloid	971.67± 46.07 ^a	607.67± 36.10 ^b
Flavonoid	231.67± 30.44 ^b	470.95± 73.07 ^a
Reducing sugar	1241.33± 70.19 ^a	760.67± 73.82 ^b
Saponin	0.96 ± 0.02 ^b	1.19± 0.40 ^a
Steroids	114.91± 21.54 ^a	72.33± 12.85 ^b
Carotenoids	1.93± 0.36 ^b	7.88± 0.59 ^a
Glycosides	7.41± 0.34 ^a	5.50± 0.59 ^b
Tannins	5.12± 0.18 ^a	0.79±0.12 ^b
Cyanogenic glycoside	74.52± 75.24 ^a	33.37± 13.84 ^b
Phenols	17.76± 1.91 ^a	15.23± 1.89 ^b
Polyphenols	19.04±2.05 ^a	24.28±12.22 ^a
Resins	53.40±25.5 ^a	27.69±22.02 ^a
Protein	11.37± 0.30 ^b	124.91±21.74 ^a
Terpenoids	423.07±9.42 ^a	255.93±35.05 ^b
Anthocyanins (citrate)	67.91±11.37 ^a	71.73±10.81 ^a
Anthocyanins (water)	65.27±8.15 ^a	64.50±7.21 ^a

Data are mean ± SD (n = 3). Values with different superscripts in a column are considered significantly different at p < 0.05.

Table 2: Minimum inhibition concentrations of crude extract and fractions of *Buchholzia coriacea* on *Streptococcus mutans*

Extract and fractions/ Concentration	Diameters of inhibition zones (mm)				
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml
Crude ethanol extract	1.00±1.732 ^b	0.00±0.000 ^a	0.00±0.000 ^a	0.00±0.000 ^a	0.00±0.000 ^a
Free flavonoid-rich fraction	0.00±0.000 ^a	0.00±0.000 ^a	0.00±0.000 ^a	0.00±0.000 ^a	0.00±0.000 ^a
Bound flavonoid-rich fraction	0.00±0.000 ^a	0.00±0.000 ^a	0.00±0.000 ^a	0.00±0.000 ^a	0.00±0.000 ^a
Alkaloid-rich fraction	7.00±3.000 ^c	5.33±3.055 ^b	3.33±3.055 ^b	0.67±1.155 ^b	0.00±0.000 ^a
Chloramphenicol	16.00±2.646 ^d	13.67±3.512 ^c	11.67±3.512 ^c	9.67±3.512 ^c	7.00±2.646 ^b

Values are mean diameters of inhibition zones (mm) ± SD (n = 3). Values with different superscripts in a column are considered significantly different at p < 0.05.

Table 3: Minimum inhibition concentrations of crude extract and fractions of *Buchholzia coriacea* on *Staphylococcus aureus*

Extract and fractions/ Concentration	Diameters of inhibition zones (mm)				
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml
Crude ethanol extract	6.33±1.16 ^c	4.33±1.16 ^c	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.000 ^a
Free flavonoid-rich fraction	3.67±1.16 ^b	1.00±1.73 ^b	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.000 ^a
Bound flavonoid-rich fraction	0.67±1.16 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.000 ^a
Alkaloid-rich fraction	8.00±1.00 ^d	5.67±0.58 ^c	3.00±1.00 ^b	0.67±1.16 ^b	0.00±0.000 ^a
Chloramphenicol	15.67±2.82 ^e	13.33±3.06 ^d	12.67±4.16 ^c	8.67±2.31 ^c	6.00±1.000 ^b

Values are mean diameters of inhibition zones (mm) ± SD (n = 3). Values with different superscripts in a column are considered significantly different at p < 0.05.

Table 4: Minimum inhibition concentrations of crude extract and fractions of *Buchholzia coriacea* on *Escherichia coli*

Extract and fractions/ Concentration	Diameters of inhibition zones (mm)				
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml
Crude ethanol extract	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
Free flavonoid-rich fraction	2.33±0.14 ^b	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
Bound flavonoid-rich fraction	2.00±0.26 ^b	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
Alkaloid-rich fraction	7.00±1.00 ^c	3.33±3.06 ^b	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
Chloramphenicol	13.33±4.73 ^d	12.67±2.52 ^c	11.33±2.08 ^b	9.00±2.65 ^b	7.33±2.08 ^b

Values are mean diameters of inhibition zones (mm) ± SD (n = 3). Values with different superscripts in a column are considered significantly different at $p < 0.05$.

Table 5: Minimum inhibition concentrations of crude extract and fractions of *Buchholzia coriacea* on *Bacillus cereus*

Extract and fractions/ Concentration	Diameters of inhibition zones (mm)				
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml
Crude ethanol extract	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
Free flavonoid-rich fraction	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
Bound flavonoid-rich fraction	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
Alkaloid-rich fraction	7.33±1.16 ^b	4.00±0.00 ^b	1.67±0.58 ^b	0.67±0.28 ^b	0.43±0.16 ^b
Chloramphenicol	13.00±5.29 ^c	9.67±4.73 ^c	8.00±3.61 ^c	6.67±3.06 ^c	4.67±2.08 ^c

Values are mean diameters of inhibition zones (mm) ± SD (n = 3). Values with different superscripts in a column are considered significantly different at $p < 0.05$.