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The Antimicrobial Activities of Root Extract of *Buchholzia coriacea* Engler (Wonderful Kola) against Gram-positive and Gram-negative Pathogens

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Abstract

This study was conducted to investigate antimicrobial activity of aqueous and methanol root extract of *Buchholzia coriacea* against selected Gram positive and Gram-negative clinically significant bacteria as well as determine phytochemical constituent of the root of the plant. The extraction of the plant root parts was carried out using the Soxhlet extraction method and the antimicrobial screening of the root extract was performed using the agar diffusion technique. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration of root extract against test microorganisms such as (*Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*) and fungus (*Aspergillus niger* employing the seeded plate method. Tioconazole and Ciprofloxacin were used as positive controls. Phytochemical screening showed the presence of tannins saponins, terpenoids, and flavonoids while alkaloids and anthraquinones were absent in the dried root extract of *Buchholzia coriacea*. Antibacterial antifungal activities were observed. The IZD for methanol root range from 7 mm at 6.25 mg/ml to 19 mm at 100 mg/ml for the bacterial isolates, while the IZDs for the *A. niger* range from 16 mm at 100 mg/ml and 6.25 mg/ml respectively. Meanwhile IZD of the aqueous extract range between 6 mm to 17 mm at 6.25 mg/ml and 100 mg/ml respectively for the bacterial isolates. The IZD of the *A. niger* range from 7 mm to 15 mm at 6.25 mg/ml and 100 mg/ml respectively. MIC of the methanol root extract of *B. coriacea* range between 25 mg/ml to 50 mg/ml for both bacteria and the fungus. The

MIC of aqueous root extract was between 50 mg/ml to 100 mg/ml for the bacteria, while the MIC for *A. niger* was 50 mg/ml for both methanol and aqueous root extracts. *Buchholzia coriacea* root bark can be formulated into suitable dosage forms and used for treatment of infections by susceptible organisms. The extract had activity against gram-positive and gram-negative organisms and showed some anti-fungal activity. The aqueous extract had higher MIC concentrations than the methanol root extract.

Keywords: Antimicrobial, Phytochemical, *Buchholzia coriacea*, Sensitivity, Root extract

Introduction

In recent years, the focus of research has been on finding novel compounds from plant, animals and microbial origin. Several plants do synthesize substances that could be very helpful to control microbial growth and of course these plants usually serve as the potential source of microbial agents (Adesina *et al.*, 2000). Plants based, traditional medicine systems continue to play an essential role in the health care system, with about 80% of the world's inhabitants relying mainly on the traditional medicines for primary health care (Maiti *et al.*, 2011).

The use of plant, plant extracts or plant derived chemicals to treat disease; topical, subcutaneous and systemic, has stood the test of time (Oladunmoye, 2006). In the recent times, there has been a steady renewal of interest in the use of medicinal plant in the developing nations due to the fact that herbal medicines have been reported



to be safe and without adverse side effects particularly when it is compared to synthetic drugs (Iniaghe *et al.* 2009).

There are plants with medicinal values in which one of its parts are known to contain secondary metabolites that can be used for therapeutic purposes or which its precursors for the synthesis of other useful drugs. An example of such plant is *Buchholzia coriacea* Engler. (Mbata *et al.*, 2009). *Buchholzia coriacea* earned its name after R.W Buchholz who harnessed the plant in the late 19th century in Cameroon (Keay, 1989). Recently, human pathogenic bacteria resistant to antibiotics has been regularly reported worldwide. There is an increasing interest in the use of either plant extracts in pharmaceutical industries and food. Screening of some plant extracts for these properties has become of a rising relevance. The secondary plant metabolites formerly with the unidentified pharmacological actions have been thoroughly investigated as a good source of medicinal agents. Since the time of old, man has utilized a variety of plant parts in the prophylaxis and treatment of a range of disease conditions. Consequently, it is predictable that phytochemicals with sufficient antimicrobial efficacy will be of use in the treatment of infections caused by bacteria (Mazid *et al.*, 2011).

Buchholzia coriacea is known to possess analgesic, antimalarial, antihelminthic, larvicidal, and antimicrobial activity on its seed and leaf extract. *Buchholzia coriacea* Engler is one of the thirty six species of the family *capparidaceae* (Pierre *et al.*, 1973). The problem of bacterial resistance to commonly used antibiotics has necessitated the search for newer and another compounds used in the treatment of drug resistant infections and also the high cost of orthodox drugs, in reserve poor communities of Africa has led to the enlarged use of plants as another source of treatment of infectious diseases (Sibandu *et al.*, 2008). The aim of this study is to determine the antimicrobial properties of the root extract of this wonderful kola on various pathogenic bacteria such as *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and fungal susceptibility.

Materials and Methods

Culture Media and Chemicals

The culture media used include, Nutrient agar, Distilled water, Nutrient broth all from Fluka Biochemika (Germany) and Mueller Hutton Agar, Methanol, Dragendorff Reagent Meyer's Reagent Wagner's reagent, Hager's reagent, Concentrated tetraoxosulphate (VI) acid Concentrated Hydrochloric acid and Chloroform, all the organisms used in this work were collected from Pharmaceutical Microbiology Laboratory, Faculty of Pharmacy. Tioconazole and Ciprofloxacin (Pfizer, Inc. New York, USA) were used as positive control.

Preparation of Plant material: Root parts were collected from Faculty of Pharmacy Botanical Garden Delta State University, Abraka Botanical identification and authentication was done by Odewo, Akinniyi Samuel of Forestry Institute of Nigeria, Jericho, Ibadan.

Experimental

Extraction procedure: The powdered root barks (25 g) each were extracted using 200 ml of methanol in a Soxhlet apparatus and the extracts were dried *in vacuo* by a rotary evaporator. (Trease *et al.*, 1989).

Phytochemical Screening

The Phytochemical tests were conducted employing standard procedures to make out the constituents (Akujobi *et al.*, 2004; Trease *et al.*, 1989).

Preparation of McFarland turbidity equivalent standard

McFarland equivalent standard was prepared by adding 0.6 ml barium chloride solution to 99.4 ml of 1 % v/v sulphuric acid and mixed properly.

Test micro-organisms

Isolates of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* were gotten from Delta State University Health Centre Abraka.

Antibiotics used.

The standard antibiotic used was Ciprofloxacin and Tioconazole.

Sensitivity Test

This test was carried out using the agar diffusion technique. Broth culture of the test organisms (0.1 ml) of standardized broth cultures organisms was aseptically seeded into molten Mueller Hutton Agar plates (20ml). Holes were bored with a cork borer. Ciprofloxacin and Tioconazole were used as the control. A volume of (0.1 ml) of extract was aseptically transferred into the wells with the aid of a sterile Pasteur pipette. The ciprofloxacin of (0.1 ml) was poured into the wells as control. The plates were then allowed for pre-diffusion of the extract on the bench for 40 minutes and later incubated at 37°C for 24 h. The diameter of zones of inhibition (IZD) was measured with a meter rule. This experiment was conducted in triplicates and the mean values of the results were taken as antibacterial activity Baker *et al.* (2001).

Antimicrobial activity

The concentrations used included 6.25, 12.5 25, 50 and 100 mg/ml. Individual concentration was examined for antimicrobial activity using seeded nutrient agar and Sabouraud carpeted plates. For every bacterial seeded plate, the positive control

was ciprofloxacin at a concentration of 1 mg/ml while tioconazole was used for the fungal carpeted plates. Triplicate determinations of the above procedure were done.

The MICs of the root extract against the test organisms were carried out using the agar well diffusion method. Serial dilutions of the extract were prepared in peptone water and then challenged with small volume (0.1 ml) of overnight culture of the test organisms. These plates were incubated for 24 hours at the temperature 37^oC. The least concentration of the root extract that inhibits the growth of the test organisms was taken as MIC.

Results

Results of the extraction yield and preliminary phytochemical screening of the dried root extracts of *Buchholzia coriacea* are shown in Table 1 and 2. Phytochemical screening showed presence of tannins saponins, reducing sugar, terpenoids, cardiac glycosides and flavonoids, while alkaloids and anthraquinones were absent in the dried root extract of *Buchholzia coriacea*.

Table 1: Extraction yield based on different extraction method

Extraction Method	Weight of powdered Sample (g)	Weight of Extract Yield	Percentage Yield
Water	50	4.9	9.8
Methanol	50	6.0	12

Table 2. Phytochemical analysis of the dried root extracts of *Buchholzia coriacea*

Phytochemicals	Methanol Root Extract	Aqueous Root Extract
Alkaloids	+	+
Tannins	+	+
Terpenoids	+	+
Flavonoids	+	+
Saponins	+	+
Anthroquinone	+	+

Key: + = Present

The IZD for methanol root range from 7 mm at 6.25 mg/ml to 19 mm at 100 mg/ml for the bacterial isolates, while the IZDs for the *A. niger* range from 16 mm at 100 mg/ml and 6.25 mg/ml respectively. Meanwhile IZD of the aqueous extract range

between 6 mm to 17 mm at 6.25 mg/ml and 100 mg/ml respectively for the bacterial isolates. The IZD of the *A. niger* range from 7 mm to 15 mm at 6.25 mg/ml and 100 mg/ml respectively as shown in Table 3 and Fig 1.

Table 3: Antibacterial activities of methanol and aqueous leaf extracts of *B. coriacea* on bacterial and fungal isolates

Isolates	Mean zone of inhibition (mm) at different concentration (mg/ml) of methanol leaf extract <i>Buchholzia coriacea</i> .						
	100	50	25	12.5	6.25	Ciprofloxacin positive control	Tioconazole Negative control
<i>Staphylococcus Aureus</i>	19	18	17	13	12	35	-
<i>Pseudomonas Aeruginosa</i>	17	16	15	12	110	32	-
<i>Escherichia Coli</i>	18	18	17	8	6	27	-
<i>Bacillus subtilis</i>	15.5	14	12	11	7	30	-
<i>Aspergillus Niger</i>	16	19	17	15	13	-	22
Isolates	Mean zone of inhibition (mm) at different concentration (mg/ml) of aqueous leaf extract of <i>B. coriacea</i> .						
	100	50	25	12.5	6.25	Ciprofloxacin Positive control	Tioconazole Positive control
<i>S. aureus</i>	17	16	14	12	9	30	-
<i>P. aeruginosa</i>	16	13	12	10	7	29	-
<i>E. coli</i>	17	10	10	8.5	8	28	-
<i>B. subtilis</i>	15	12	11	9	9	31	-
<i>A. Niger</i>	15	15	12	9	7		21

Table 4 shows the MIC of methanol leaf extract of *B. coriacea* on bacterial and fungal isolates. The results show that every isolate had growth at 6.25 mg/ml concentration but none of them had growth at 100 mg/ml – 50 mg/ml concentrations. MIC of the methanol root extract of *B. coriacea*

range between 25 mg/ml to 50 mg/ml for both bacteria and the fungus, while the MIC of aqueous root extract is between 50 mg/ml to 100 mg/ml for the bacteria, while the MIC for *A. niger* was 50 mg/ml for both methanol and aqueous root extracts.

Table 4: MIC of methanol and aqueous and leaf extracts of *B. coriacea* on bacterial and fungal isolates

Isolates	MIC (mg/ml)	
	Methanol extract	Water extract
<i>Staphylococcus Aureus</i>	25	50
<i>Pseudomonas Aeruginosa</i>	50	100
<i>Escherichia Coli</i>	25	50
<i>Bacillus subtilis</i>	25	50
<i>Aspergillus Niger</i>	50	50

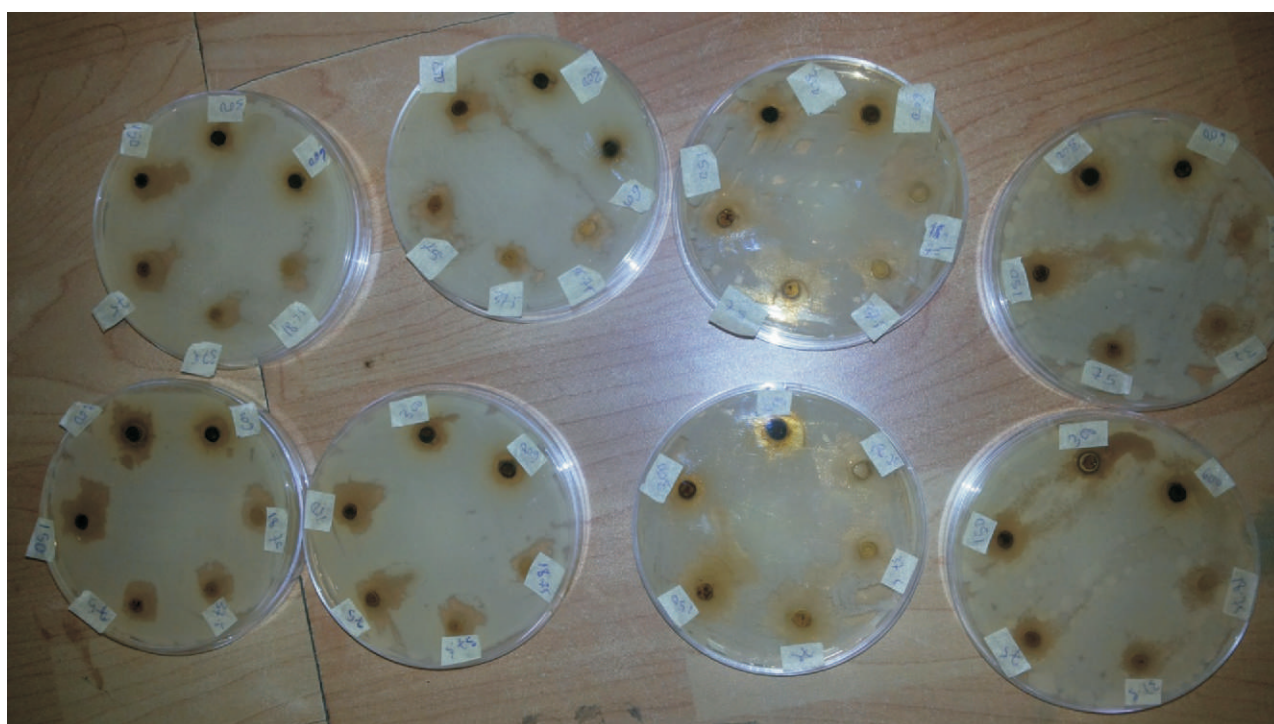


Fig 1. The IZD for methanol root and aqueous extract of *A. Niger*



Discussion

According to Ikpeazu *et al.* (2017), plants are used globally in the treatment of different diseases using varying approach. Several research works have been carried out on the antibacterial activity of *Buchholzia coriacea*. Various researchers have shown that *Buchholzia coriacea* leave extract exerts bacteriostatic and bactericidal activities against various organisms including both gram-positive and gram-negative organisms.

The results of phytochemicals screening of the root extract are shown in Table 1. The analyses revealed that the root extract of *B. coriacea* are rich in phytonutrient such as alkaloids, glycosides, saponins, flavonoids, tannins and phenol, purine, reducing sugar, phlebotomine and steroids. These results are in line with the earlier study by Ajaiyeoba *et al.* (2003). Secondary metabolites which include alkaloids, tannins, steroids, and terpenoids have been observed to possess curative capacity against pathogens such as *Staphylococcus aureus* and *Escherichia coli* and this suggests that of any plant containing those metabolites could be used for the treatment of various illness. The degree of inhibitory activity of extract depends on the type of bioactive ingredient present in the extract. These ingredients include flavonoids, saponins, tannins, alkaloids, terpenoids e.t.c. These bioactive ingredients interfere with microbial enzymes by acting as metabolic analogs thus inactivating the enzymes (Mazid *et al.*, 2011). A similar result of phytochemical constituents of the methanol leaves extract of *Buchholzia coriacea* done by Anowi *et al.* (2012).

The results of this study revealed that the aqueous and methanol root extract possess antibacterial and fungal, which is in agreement with the work of Ezekiel *et al.* (2009). The activity of methanol and aqueous root of *B. coriacea* extract on bacterial and fungal isolates. The inhibition zone diameters (IZD) indicates bacteria and fungi isolates showed the greatest susceptibility to methanol leaf extract (20 mm) at 100 mg/ml concentration than aqueous extract at same concentration. The IZD for methanol root range from 7 mm at 6.25 mg/ml to 19 mm at 100 mg/ml for the bacterial isolates, while the IZDs

for the *A. niger* range from 16 mm at 100 mg/ml and 6.25 mg/ml respectively. Meanwhile IZD of the aqueous extract range between 6 mm to 17 mm at 6.25 mg/ml and 100 mg/ml respectively for the bacterial isolates. The IZD of the *A. niger* range from 7 mm to 15 mm at 6.25 mg/ml and 100 mg/ml respectively this is in line the work of Ezekiel *et al.* (2009) who showed that *B. coriacea* has an antifungal activity.

The MICs of methanol and aqueous root extracts of *Buchholzia coriacea* on bacterial and fungal isolates are presented in Table 4. Table 4 shows the MIC of methanol root extract of *B. coriacea* on bacterial and fungal isolates. The results show that every isolate had growth at 6.25 mg/ml concentration but none of them had at 100 mg/ml – 50 mg/ml concentrations. MIC of the methanol root extract of *B. coriacea* range between 25 mg/ml to 50 mg/ml for both bacteria and the fungus, while the MIC of aqueous root extract is between 50 mg/ml to 100 mg/ml for the bacteria, while the MIC for *A. niger* was 50 mg/ml for both methanol and aqueous root extracts as depicted in Table 4. The aqueous extract had higher MIC concentrations than the methanol leaf extract in agreement with earlier report (Mbata *et al.*, 2009).

Ejikeugwu *et al.* (2012) reported that n-hexane, methanol and chloroform extracts of wonderful kola leave possess modest antibacterial activities against *E. coli*, *Staphylococcus aureus*, *Shigella* species, *Klebsiella pneumoniae* and *Bacillus subtilis*. At 100 mg/ml of the extract, there was increase in the number of susceptible organisms, however, there was no significant increase ($p > 0.05$) in the zones of inhibition when compared to those susceptible at 50 mg/ml of extract. The two-gram negative bacteria (*Pseudomonas aeruginosa* and *E. coli*) were observed to susceptible to root extract of *Buchholzia coriacea*. It therefore means that it can be used to treat gastroenteritis associated with *E. coli* (Enatani *et al.*, 1998). These results showed that, extract of the root of *Buchholzia coriacea* did inhibit the growth of all fungus species at all concentration (Table 3). This finding is in line with the earlier study carried out on the methanolic seed extract of *Buchholzia coriacea* by Nweze *et al.* (2006) which inhibited the

growth of fungi at different concentration. Therefore the plant can be used to treat fungal infections. The result of the antibacterial activity showed that the plant leaves are rich reservoirs of antibacterial agents. The active components usually interfere with growth and metabolism of microorganism in negative manner. The varying degree of sensitivity of the bacteria isolates may be due to intrinsic relevance of the microbes and the nature and combination of phytochemicals present in the extract. Furthermore, the antimicrobial properties have been widely attributed to bioactive components (Doherty et al. 2010).

Conclusion

This study has shown that *Buchholzia coriacea* root barks can be used for treatment of infections by susceptible organisms, because of its sensitivity to the extract's concentration of 50 mg/ml or more of the various extract. The extract had activity against *S. aureus* than any other microorganism used in this study.

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