ANTIBACTERIAL EVALUATION OF *Buchholzia coriacea* SEED EXTRACTS

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Abstract: The antimicrobial resistance of microorganisms makes them untreatable using the convectional antibiotics, therefore a need for alternative routes of treatment. Among several different alternative *Buchholzia coriacea* (wonderful kola) is an effective choice because of its numerous phytochemical components. The wonderful kola sample used in this study was purchase from Kure market in Niger State, Nigeria. The phytochemical screening, antibacterial susceptability test, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were performed using standard methods. The phytochemical analysis revealed the presence of alkaloids, tanins, flavonoids, steriod and the absence of saponins. Antibacterial activity of wonderful kola on some medically important bacteria; *Staphylococcus aureus*, *Streptococcus pneumonia* and *Klebsiella pneumonia* shows wonderful kola seed exhibit antibacterial acitivity producing zone of inhibition against all tested bacteria and has the hgihest zone of inhibition at 250 mg/ml concentration. The results from this study show that wonderful kola seed could be used as an antibacterial agent and also be used for the development of therapeutic agents for the treatment of ailment associated with the test organisms.
Key words: *Buchholzia coriacea*, antibacterial, seed.

INTRODUCTION

Buchholzia coriacea has numerous medicinal values. The seed gave it its common name (wonderful k0la) because of its usage in traditional medicine. The parts of the plant usually eaten are the seeds which can either be eaten raw or cooked (Nwachukwu *et al.,* 20l4). In Africa, wonderful kola has the ability to stop migraine headache when applied on the forehead. The stem bark extract is applied as an enema to treat back pain. Non specified bark preparations are also applied externally against smallpox, pleurisy, conjunctivitis, rheumatism, scabies and other skin illnesses. Sterility in women can be treated using leaf decoctions. The seeds which have a peppery taste are used as a substitute of capsicum pepper. The seed is chewed as a substitute for kola nut (Anowi, *et al.,* 20l2, Nwachukwu, *et al*., 20l4).

Wonderful kola as it is commonly called is known worldwide as memory nut because it enhances the memory. It acts as cleanser of the blood, facilitates learning absolutely and strengthens the nervous system and is also effective in the treatment of menstrual complications. It is a brain diet which promotes memory, it is also useful in the treatment of hypertension and also prevents premature aging; it has also been proved in Africa that wonderful kola has the capacity to stop migraine headache on the forehead for about 10 minutes (Ibrahim and Fagbohum, 2014).

The use of medicinal herbs in the prevention and treatment of diseases is attracting the attention of researchers worldwide (Ameen *et al.,* 2010; Falodun *et al*., 2006). Plants usually have phytochemicals which are active constituents technically referred to as drugs, and over the years these drugs have been exploited as traditional medicine for the treatment of various sicknesses troubling man (Shagal *et al.,* 2012). This study therefore seeek to screen the phytochemical components and antibacterial efficacy of *Buchholzia coriacea* seed on some medically important bacteria.

MATERIALS AND METHODS

Sample Collection and Idenfication

Buchholzia coriacea (wonderful kola) were purchsed from Kure Market Minna, Niger State and were identified at the Department of Biological Sciences, Federal University of Technology, Minna.

The seeds were washed several times with clean water after removing the extraneous materials from the seed. The seeds were sliced in pieces with a knife; air dried for 8 days at room temperature and milled using electric blender to powder form.

Sample Extraction

Aqueous (water) and organic (methanol) solvents were used for extraction of the active components of the plant seed. For aqueous extraction, hot water extraction method as described by Mbata *et al.* (2009) was used. 100g of the ground seeds were dispenses into 100 ml hot distilled water and allowed to stay for 2 days. The extracts were filtered using Whatman filter paper and the filtrates concentrated in vacuum at 60°C. For organic extraction, 50 g of the powdered seed sample was extracted in 500 ml of 95% methanol for 6 hours using the Soxhlet apparatus. The volatile oil obtained was concentrated by evaporation using water bath at 100°C for 3 hours. The concentrated filtrate, now the extracts were then stored in sterile universal bottles in the refrigerator at 4°C prior to use (Silver *et al*., l997).

Test Organisms

The microorganisms used for this study, *Staphylococcus aureus, Streptococcus pyogenes and Klebsiella pneumonia* were collected from the General Hospital Minna, Niger state, Nigeria.

Standardization of Organisms

Pure stock culture of test organisms were inoculated into 5 ml of sterile nutrient broth and incubated for 18 hours. Aliquots of 0.2 ml from the overnight culture were dispensed into 20 ml of sterile nutrient broth and incubated for 3-5 hours. Turbidity produced was adjusted to match 0.5 McFarland's standard (Babayi *et al*., 2004).

Phytochemical Screening of *Buchholzia coriacea* Seed

The phytochemical screening of the seed was carried out according to the method described by Trease and Evans (l999).

Test for Alkaloid: one millilitre (l ml) of l% HCl was added to 3mls of the extract in a test tube. The mixture was then heated for 20 minutes, cooled and filtered. Two drops of Wagner's reagent to l ml of the extract was added. A creamy precipitate indicated the presence of alkaloids.

Test for Tannins: one millilitre (l ml) of freshly prepared 10% KOH was added to l ml of the extract. A dirty precipitate showed the presence of tannin.

Test for Saponnin: Three drops of distilled water was added to two drops of each extract and vigorously shaken with the test tube for some seconds. A positive result was indicated by the presence of frothing or bubbling.

Test for Steroids (Salkowski test): Five drops of concentrated H_2SO_4 were added to l ml of the extract in a test tube. Red co1oration was observed which indicated the presence of steroids.

Test for flavonoids: one milliliter (l ml) of each extract was added with NaOH solution. The appearance of a yel1ow co1oration which then disappeared on addition of HCl indicated the presence of flavonoids.

Test for phenolics: Two drops of 5% $FeCl_3$ were added to l ml of the extract. The presence of greenish precipitate indicated the presence of phenols.

Reconstitution of crude extracts of *Buchholzia coriacea* Seed

Each of the extracts were reconstituted by dilution (methanol crude extract in 50% Dimethylsulphoxide (DMS) and aqueous extracts in sterile distilled water) to various concentrations of 250, 200, l50, 100 and 50 mg/ml (Mbata, *et al*., 2009) and used for antibacterial susceptibility testing.

Antibacterial Susceptibility of *Buchholzia coriacea* Seed

Antibacterial susceptibility of extracts was carried out using the agar-gel diffusion method as described by Osadebe and Ukwueze (2004). Sterile Cork borer (diameter 4mm) was used to bore wells on each plate.

Pasteur pipette was then used to transfer different concentrations of each plant extract on each labelled bored well, ciprofloxacin and sterile distilled water were used as positive and negative control respectively. The bottom of the wells were sealed with one drop of the sterile nutrient agar to prevent diffusion of the extract under the agar. Fixed volumes (0.l ml) of the extracts were transferred into the wells using a sterile Pasteur pipette. The control wells shall be filled with 0.1 ml of distilled water and ciprofloxacin. The plates were allowed on the bench for 40 minutes for pre-diffusion of the extract (Esimone *et al.,* l998) and were incubated at 37°C for 24 hours. Antibacterial activity of the extracts were determined by measurement of the resulting zone diameters of inhibition (mm) against each test bacteria using a ruler. The experiment was carried out in triplicates and the mean values of the results were taken as antibacterial activity (Junaid *et al.,* 2006).

Determination of Minimum Inhibitory Concentration (MIC)

The broth dilution method was employed to determine the MIC of the potent extracts. Standardized suspensions of the test organism was inoculated into a series of sterile tubes of nutrient broth containing dilutions (250, 200, l50, 100 and 50 mg/ml) of seed extracts., 8.0 ml of nutrient broth was dispensed into 8 test tubes each, and these were sterilized at 121^{o}C for l5minutes and al1owed to cool at room temperature. Two millilitre (2.0 ml) from the re-constituted extract was introduced into the test tubes. A loop full of the standardized inoculum was then inoculated into the nutrient broth; the test tubes were incubated for 24 hours at 37^{o}C. Turbidity was observed. The MICs were read as the least concentration that inhibited any visible growth (absence of turbidity) of the test organisms (Babayi *et al.,* 2004).

Determination of Minimum Bactericidal Concentration (MBC)

For MBC determination, a loopful of broth from each of the tubes that did not show any visible growth (no turbidity) during MIC determination were subcultured onto freshly prepared sterile Nutrient agar, and further incubated for 24 h at 37°C. The least concentration, at which no visible growth observed, was taken as the MBC (CLSI, 2007).

RESULTS

Phytochemical constituents of *Buchholzia coriacea*

The phyt0chemical screening 0f methan0l and ethan0l crude extract sh0wed that the crude methan0l and aqueous extract had alkal0ids, tanins and flav0n0ids while steriod is only present in the methanol extract and saponin absence in both extracts (Table 1).

Table 1**:** Phytochemical constituents of *Buchholzia coriacea* seed

Phytochemicals	Methanol	Aqueous
Steroids	**+++**	**-**
Tannins	**+**	**+**
Flavonoids	+	+++
Alkaloids	++	++
Saponins	**-**	**-**

KEY: + = present; - = absent; multiple pluses indicate the degree of abundance

Antimicrobial Activity of *Buchholzia coriacea*

Methan0l extracts of *Buchholzia coriacea* seed sh0wed varying antibacterial activities against the test 0rganisms (Table 2). Table 3 shows the results of antibacterial activity of aqeuous extract against the test organisms.

Methanol extract sh0wed the highest z0ne 0f inhibiti0n 0f l8.00 ± 0.17mm, 18.00 ± 0.57 and 14.06 ± 0.06 against *Staphylococcus aureus Streptococcus pyogenes* and

*Klebsiella pneumonia*e respectively while that of Aqueous extract showed the highest zone of inhibition of 16.00 ± 0.00, 14.00 ± 0.00 and 15.00 ± 0.28 against *Staphylococcus aureus Streptococcus pyogenes* and *Klebsiella pneumonia*e respectively at c0ncentrati0ns 250 mg/ml. The result showed that the extracts against antibacterial activity against the test organisms.

Table 2: Antibacterial activity of Methanol crude extracts of *Buchholzia coriacea* seed (zone of inhibition in mm)

Methanol extract (mg/ml)	*Staphylococcus aureus*	*Streptococcus pyogenes*	*Klebsiella pneumonia*
50	6.00 ± 0.17	7.06 ± 0.06	6.96 ± 0.03
100	9.00 ± 0.17	8.00 ± 0.11	10.00 ± 0.11
l50	13.00 ± 0.00	15.00 ± 0.57	12.00 ± 0.00
200	15.00 ± 0.58	17.00 ± 0.00	13.00 ± 0.28
250	18.00 ± 0.17	18.00 ± 0.57	14.06 ± 0.06
Ciprofloxacin	27.00 ± 0.00	25.16 ± 0.16	24.00 ± 0.00
Water	-	-	-

Key - = no zone of inhibition, Values are means of tripicates ± SEM

Table 3: Antibacterial activity of Aqueous crude extracts of *Buchholzia coriacea* seed (zone of inhibition in mm)

Aqueous extract (mg/ml)	*Staphylococcus aureus*	*Streptococcus pyogenes*	*Klebsiella pneumonia*
50	7.00 ± 0.19	6.00 ± 0.28	7.00 ± 0.06
100	9.00 ± 0.00	8.00 ± 0.75	9.00 ± 0.46
l50	11.00 ± 0.11	9.00 ± 0.17	10.00 ± 0.00
200	12.00 ± 0.10	12.00 ± 0.00	12.00 ± 0.06
250	16.00 ± 0.00	14.00 ± 0.00	15.00 ± 0.28
Ciprofloxacin	28.00 ± 0.00	27.00 ± 0.00	25.00 ± 0.00
Water	-	-	-

Key - = no zone of inhibition, Values are means of tripicates ± SEM

4.4 Minimum Inhibitory concentration (MIC) and Minimum Bactericidal concentration (MBC).

The MIC and MBC values obtained for the entired test organisms are 20 mg/ml and at 50 mg/ml respectively.

Table 4: Minimum Inhibitory concentration (MIC) and Minimum Bactericidal concentration (MBC) of the methanol and aqueous crude extract of *Buchholzia coriacea* seed

Test organisms	MIC of extracts (mg/ml)		MBC of extracts (mg/ml)	
	Methanol	Aqueous	Methanol	Aqueous
Staphylococcus aureus	20	20	50	50
Streptococcus pyogenes	20	20	50	50
Klebsiella pneumoniae	20	20	50	50

DISCUSSION

The phytochemical constituent of *Buchholzia coriacea* seed were considerable high, this is in similar to the result of (Esimone *et al*., 2009; Kareem *et al*., 20l0; Ajayi *et al*., 2011). These clases of compound are known to posses therapeutic properties against several pathogens and could therefore be responsible for its antibacterial activity.

The methanol and aqueous extract of *Buchholzia coriacea* seed showed antibacterial activity against all isolates. All isolates are more sensitive at 250 mg/ml concentration for both extracts having the highest zone of inhibition (Table 2 and 3) while the lowest sensitity was recorded at 50 mg/ml for both extracts. The diameter of each zone of inhibition was used to estimate the isolates sensitivity to a particular extract. The zone of inhibition increases with increasing the concentration of the extract on the isolates. The methanol and aqueous extract at 250 mg/ml showed broad spectrum activity as the isolates sensitive to it, with zone of inhibition comparable to the ciproflaxacin (antibiotic) used as standard.

The MICs for both methanol and aqueous extracts was 20 mg/ml against all test isolates Minimum bactericidal concentration (MBC) of the extracts on the test isolates was recorded to be 50mg/ml for both extracts against the three susceptible test organisms used as shown in table 4. The MBC varied from MIC indicating that a different concentration is needed to inhibit the growth of the isolates and a different concentration to kill them (Atukpawu and ozoh, 2014). The antibacterial activities of crude extracts of wonderful kola seed against pathogenic bacteria in this study are agreeable with previous reports that have also reported the medicinal efficacy of wonderful kola plants (Ezeja *et al.,* 2011; Oluseyi and Onyeoziri, 2009; Mbata *et al*., 2009; Nweze,2011; Ejikeugwu *et al*., 2014).

CONCLUSION

This study has presumptively reported the broad spectrum antibacterial activity of *Buchholzia coriacea* seed of methanol and aqueous extract against the test pathogenic bacteria. The result of this study lend credence to the use of this seed in traditional folklore. This implies that *Buchholzia coriacea* seed can be used as therapeutic agent to treat infections. Therefore, there is need for further investigations in terms of toxicological studies and purification of active components with the view to using the seed in novel drug development.

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