

Full Length Research Paper

Antibacterial activity of crude seed extracts of *Buchholzia coriacea* E. on some pathogenic bacteria

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The antibacterial efficacy of hot water and methanol extracts of dried seeds of *Buchholzia coriacea* against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Bacillus cereus*, and *Vibrio cholerae* were determined using the Agar-gel diffusion method. The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and phytochemistry of the extracts were also evaluated. Results obtained showed that the methanol extracts of the dried seed was potent, inhibiting the isolates with diameter zone of inhibition ranging from 7.0 - 35.0 mm. The extracts inhibited the growth of the bacterial isolates in a concentration dependant manner with MICs ranging between 9.3 - 50 mg/ml, and MBCs of 4.1 - 17.4 mg/ml. Phytochemical analysis of dried seed extracts revealed the presence of alkaloids, anthraquinones, carbohydrates, cardiac glycosides, flavonoids, glycosides, resins, saponin, steroidal rings, steroidal terpenes and tannins. The findings from this study could be of interest and suggests the need for further investigations in terms of toxicological studies and purification of active components with the view to using the plant in novel drug development.

Key words: Antibacterial activity, phytochemical analysis, *Buchholzia coriacea*, bacterial isolates.

INTRODUCTION

Traditional medicine is widespread throughout the world and it can be described as the total combination of knowledge and practices, whether explicable or not, used in diagnosing, preventing or eliminating a physical, mental or social disease and which may rely exclusively on past experience and observation handed down from generation, verbally or written (Sofowora, 1984). Medicinal plants has been defined by WHO consultative group as any plant which in one or more of its organs contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Andrews, 1982).

For many years medicine depended exclusively on leaves, flowers and barks of plants; only recently have synthetic drugs came into use and in many instances, there are carbon copies of chemicals identified in plants (Conway, 1973). In orthodox medicine, a plant may be subjected to several chemical processes before its active

ingredient is extracted, refined and made ready for consumption while in traditional medicine a plant is simply eaten raw, cooked or infused in water or native wine or even prepared as food (Conway, 1973).

Buchholzia coriacea E. (Capparidaceae) is a forest tree with large, glossy, leathery leaves and conspicuous cream white flowers in racemes at the end of the branches. The plant is easily recognized by the compound pinnate leaves and the long narrow angular fruits containing large, usually aligned seeds. In Nigeria the plant has various common names including; 'Ovu' (Bini), and 'Aponmu' (Akure). *B. coriacea* is found widely distributed in other African countries such as Ivory Coast and Gabon (Keay et al., 1964; Koudogbo et al., 1972).

The plant's fruit is about 5 inches long and 2 - 3 inches in diameter and resembles avocado pear, yellowish when ripe with a yellow flesh containing a few large, blackish seeds about 1 inch long. They are edible and taste peppery.

It has been used for years to meet a variety of illnesses; since it has been used continually over many generations it is likely that the kola (seed) actually has an effect against illnesses.

The leaves and stem bark of *Buchholzia* in various for-

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mulations, decoctions and concoction exhibit antihelmintic, antimicrobial and cytotoxicity effects on microorganisms (Ajaiyeoba et al., 2001; Ajaiyeoba et al., 2003; Nweze and Asuzu, 2006; Ezekiel and Onyeoziri, 2009). In Ghana fresh bark of the plants were used for earache (Irvine, 1961).

Despite the various reports, information on the antibacterial properties of seeds of the plants on gastrointestinal pathogens is scarce. The study was therefore undertaken in order to evaluate the antibacterial activities and phytochemical profile of the crude extracts of seeds of *B. coriacea* on some gastrointestinal bacterial pathogens.

MATERIALS AND METHODS

Plants collection

The seeds of *B. coriacea* were collected from Awka, Nigeria and were authenticated by a Taxonomist at the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria. The seeds were air-dried for 5 days to constant weight, cut into pieces and grinded into powder using a sterile electric blender. The powder was then used for extraction of bioactive components.

Extraction of plant material

Aqueous (water) and organic (methanol) solvents were used for extraction of the active components of the plant part. For aqueous extraction, hot water extraction method as described by Asuzu (1986) was used. 20 g of each of the grounded seeds were extracted by successive soaking for 2 days using 40 ml of hot distilled water in a 250 ml sterile conical flask. The extracts were filtered using Whatman filter paper and the filtrates concentrated in vacuum at 60°C. The concentrated filtrate, now the extracts were then stored in universal bottles in the refrigerator at 4°C prior to use. For organic extraction, 25 g of the powdered plant part was extracted in 250 ml of 95% methanol for 6 h using the soxhlet apparatus as described by Harbone (1993). The volatile oil obtained was concentrated by evaporation using water bath at 100°C for 1 h.

Preparation of crude extract

Each of the extracts were reconstituted by dilution (methanol crude extract in 50% Dimethylsulphoxide (DMSO) and aqueous extracts in sterile distilled water) to various concentrations of 250, 200, 150, 100 and 50 mg/ml as described by Akujobi et al. (2004) and used for antibacterial susceptibility testing.

Photochemical screening

This was carried out according to the methods described by Trease and Evans (1989).

Test bacteria

Clinical isolates of *Bacillus cereus*, *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Vibrio cholerae* used for this work were collected from the Bacteriology Laboratory Center, University of Nigeria, Teaching Hospital, Enugu. The bacterial isolates were further purified by subculturing each isolate onto fresh

plates of Nutrient Agar (NA). The pure isolates were identified using standard biochemical methods (Holt et al., 1994) and then maintained as described by Cruickshank et al. (1980).

Determination of antibacterial susceptibility of extracts

This was carried out using the agar-gel diffusion method as described by Osadebe and Ukwueze (2004). In this method, broth culture of the test isolates (0.1 ml) containing 1×10^5 cells/ml of organism was aseptically inoculated by spreading evenly onto the surface of NA plates using a bent sterile glass rod. Six wells (5.0 mm diameter) were then made in the plates using a sterile cork borer. The fifth and sixth wells served as negative and positive control. The sterile distilled water served as the negative control, ciprofloxacin used as the positive control. The bottom of the wells 1 - 4 was sealed with one drop of the sterile nutrient agar to prevent diffusion of the extract under the agar. Fixed volumes (0.1 ml) of the extracts were transferred into the wells 1 - 4 using a sterile Pasteur pipette. The control wells were filled with 0.1 ml of distilled water and ciprofloxacin. The plates were allowed on the bench for 40 min for pre-diffusion of the extract (Esimone et al., 1998) and then incubated at 37°C for 24 h. 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 (Abayomi, 1982; Junaid et al., 2006).

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC and MBC of the potent extracts was determined according to the macro broth dilution technique (Boron and Fingold, 1990). Standardized suspensions of the test organism was inoculated into a series of sterile tubes of nutrient broth containing dilutions (250, 200, 150, 100 and 50 mg/ml) of leaf extracts and incubated at 37°C for 24 h. The MICs were read as the least concentration that inhibited any visible growth (absence of turbidity) of the test organisms. For MBC determination, a loopful of broth from each of the tubes that did not show any visible growth (no turbidity) during MIC determination was subcultured onto extract fresh free NA plates, and further incubated for 24 h at 37°C. The least concentration, at which no visible growth was observed, was noted as the MBC.

RESULTS AND DISCUSSION

Results of preliminary phytochemical screening of the seed extracts of *B. coriacea* are shown in Table 1. Results showed the presence of alkaloids, anthraquinones, carbohydrates, cardiac glycosides, flavonoids, glycosides, resins, saponin, steroidal rings, steroidal terpenes and tannin. The presence of phytochemicals in the seed extracts (Table 1) showed that the extracts possess antibacterial properties. These results are in agreement with similar study by Ajaiyeoba et al. (2003).

Table 2 shows the results of antibacterial effects of seed extracts of the plant against the test bacteria. Results showed that the activity of the extracts against the test bacteria decreased with decrease in the concentration with the methanol extracts demonstrating higher activity (35 mm, 250 mg/ml,) than the hot water extracts (3 mm, 50 mg/ml). This could be because the

Table 1. Phytochemical analysis of dried seed extracts of *Buchholzia coriacea*.

Phytochemicals	Hot water seed extract	Methanol seed extract
Alkaloids	+	++
Anthraquinone	+	+
Carbohydrates	++	++
Cardiac glycosides	+	+
Flavonoids	+	++
Glycosides	++	++
Resins	++	++
Saponin	+	++
Steroidal ring	+	+
Steroidal terpenes	++	++
Tannin	+	++

+ = Present.

Table 2. Antibacterial activity of dried seed extracts of *Buchholzia coriacea* on test Isolates.

Isolates	Mean zone diameter of inhibition (mm)						Extracts	
	250	200	150	100	50	+ve control	-ve control	
<i>Staph. aureus</i>	30	25	18	16	12	17	0	METH
	22	18	18	10	8	15	0	HH ₂ OD
<i>S. typhimurium</i>	25	20	16	12	10	20	0	METH
	18	17	10	6	5	18	0	HH ₂ OD
<i>B. cereus</i>	35	25	20	17	12	16	0	METH
	24	18	16	11	10	15	0	HH ₂ OD
<i>V. cholerae</i>	24	19	15	11	7	17	0	METH
	8	13	9	7	3	15	0	HH ₂ OD
<i>E. coli</i>	18	16	12	10	7	21	0	METH
	13	11	8	6	3	18	0	HH ₂ OD
Conc. of extracts (mg/ml)	250	200	150	100	50	+ve control	-ve control	

HH₂OD = Hot water (Dried seed), METD = Methanol (Dried seed).

active component must be a highly polar compound. It has been observed that the more polar the solvent the higher the yield of extraction (Chang et al., 1977), although the inhibitory effects of aqueous extract of medicinal plants has been reported (Tignokpa et al., 1986; Olayinka et al., 1992; Omer et al., 1998).

Figure 1 and Table 3 showed the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts on the test isolates respectively. Results showed that the values obtained are quite higher for the aqueous (hot) extracts than that of methanol extracts suggesting that extraction with methanol could produce better active antimicrobial phytochemicals which are contained in the seed. The presence of phytochemicals in the seed extracts (Table 1) showed

that the extracts possess antibacterial properties. These results are in agreement with similar study by Ajaiyeoba et al. (2001). The observed antibacterial effects of the seeds on the bacterial isolates though *in-vitro* is an indication that the seed extracts could be effective in the management of infections cause by these organisms (Tables 1 and 2).

Conclusion

Results of the study showed that seed extracts of *B. coriacea* possessed phytochemical substances that can be used as components of new antimicrobial agents. Therefore there is need for further investigations in terms

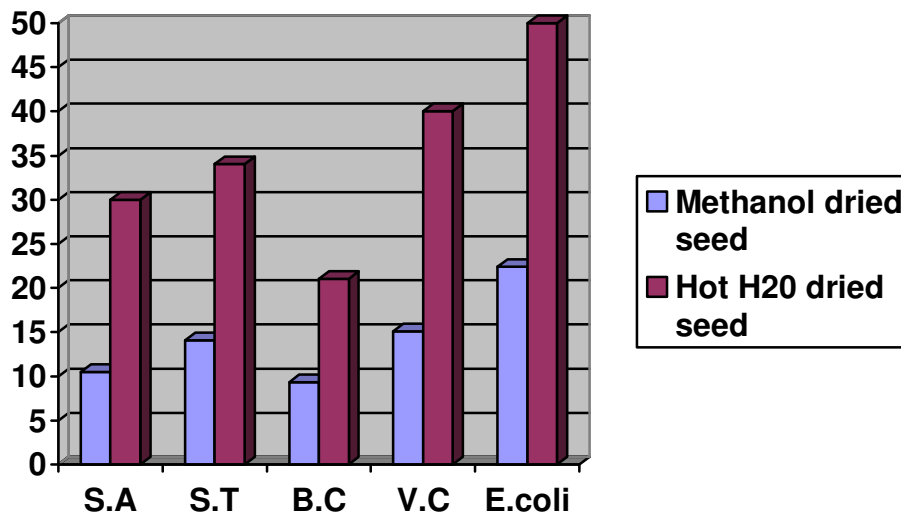


Figure 1. Minimum Inhibitory Concentration of seed extracts of *Buchholzia coriacea*. HH₂OD= Hot water (Dried seed), METH= Methanol (Dried seed), S.A = *Staphylococcus aureus*, S.T.= *Salmonella typhimurium*, B.C = *Bacillus cereus*, V.C = *Vibrio cholerae*, E. coli = *Escherichia coli*

Table 3. Minimum bactericidal concentration (MBC) of seed extracts of *Buchholzia coriacea*.

Isolates	Extracts	MBC (mg/ml)
<i>S. aureus</i>	METH	4.1
	HH ₂ OD	9.3
<i>S. typhimurium</i>	METH	5.2
	HH ₂ OD	11.2
<i>B. cereus</i>	METH	3.9
	HH ₂ OD	8.1
<i>V. cholerae</i>	METH	6.1
	HH ₂ OD	12.4
<i>E. coli</i>	METH	10.5
	HH ₂ OD	17.4

HH₂OD= Hot water (Dried seed), METH= methanol (Dried seed).

of toxicological studies and purification of active components with the view to using the plant in novel drug development. The study has also justified the traditional usage of this plant as health remedy.

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