Nigerian Veterinary Journal
Vol. 32(2): 2011; 143 - 147

ARTICLE

Studies on the Antioxidant and Antimicrobial Activities of the Seed Extracts of *Buchholzia coriacea* (capparaceae)

NWEZE, N. E.

Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.
Correspondence: Nwakaego_ernestina@yahoo.com; +2348054132599

SUMMARY

The seeds of *Buchholzia coriacea* Engler are traditionally used in the treatment of illnesses such as fevers, headaches and gonorrhoea caused by a variety of microbial organisms. Extraction was carried out by cold maceration in hexane, carbon tetrachloride, chloroform, ethyl acetate, acetone, methanol and water. Thin layer and column chromatography techniques were used to study the chemical composition of the extracts. Antioxidant activity was studied using 0.2 % 1, 1-diphenyl-2-picryl-hydrazyl (DPPH). *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Candida albicans, Cryptococcus neoformans var gattii, Aspergillus fumigatus and Trichophyton verrucossum* were used in the antimicrobial assays. Bioautography was done using *S. aureus.* The aqueous extract had the highest yield of 10.7 %. All the extracts had antioxidant activity. Bioautography revealed two antibacterial compounds in the active extracts. The average MIC values of the acetone, chloroform, methanol, hexane and water extracts were 1.6, 2.03, 2.19, >2.5 and >2.5 mg/ml respectively against *E. faecalis, P. aeruginosa* and *S. aureus.* The average MIC values of the chloroform, ethyl acetate, acetone, methanol and hexane extracts were 1.9, 2.2, 2.5, 2.5 and >2.5 mg/ml, respectively against *C. neoformans* and *C. albicans.* The most susceptible bacteria were *E. faecalis* followed by *P. aeruginosa* and *S. aureus.* None of the extracts was active against *E. coli.* The most susceptible fungus was *C. neoformans* followed by *C. albicans.* The extracts showed no activity against *T. verrucossum* and *A. fumigatus.* Extracts of *Buchholzia coriacea* seed possess antioxidant and modest antimicrobial activities.

KEYWORDS: Bioassay; medicinal plant; antibacterial; antifungal

INTRODUCTION

The seeds of *Buchholzia coriacea* Engler are popularly called 'wonderful colas'. They are used traditionally in the treatment of illnesses and conditions caused by a variety of microbial organisms. Such conditions include fevers, headaches and gonorrhoea. For the treatment of headache, the fresh cola is cut and placed on the fore head of the affected individual. For the treatment of systemic diseases, the cola is chopped and soaked overnight in the local gin. The infusion is drunk in order to effect a cure (Nweze *et al*., 2009). Antibiotic resistance is a growing global problem. This is largely due to the indiscriminate use of commercial antibiotic drugs commonly employed in the treatment of infectious diseases (Parekh and Chanda, 2007). In a survey carried out by Chah and Nweze, (2001), it was found out that most of the respondents' rarely or never submitted specimen for laboratory diagnosis/sensitivity testing prior to administering antibiotic therapy in their poultry farms. The spread of resistance to existing antibiotics has led to a diminished effectiveness of these useful agents thereby highlighting the need for novel antibacterial agents.

Plants have been sources of medicines for many generations. More than 80 % of the population in developing countries depend on plants for their medical needs (Farnsworth, 1988). It has been reported that about 2/3 of all plant species are found here in the tropics. Some have been investigated while so many are yet to be studied. In fact, less than 10 % of biodiversity has been tested for biological activity (Nwafor *et al*., 2001). Substances that can either inhibit the growth of pathogens or kill them and have little or no toxicity to host cells are considered good candidates for developing new antimicrobial drugs (Masoko *et al*., 2005). Recent works have revealed the potential of several herbs as sources of drugs. The screening of plant extracts and

products for antimicrobial activity has shown that higher plants are potential sources of novel antibiotic prototypes (Afolayan, 2003). The present plant was studied because of the ethnomedical use of the seeds in treating systemic infections. The aim of the study was to test extracts of *B. coriacea* seeds for antioxidant activity, and to evaluate the antimicrobial activity of the extracts using some pathogenic species of bacteria and fungi.

MATERIALS AND METHODS

Plant material

Buchholzia coriacea seeds were collected in two batches: at first in February, 2008 and then a year later. They were identified by a taxonomist, Mr A. O. Ozioko of Bioresources Development and Conservation Centre, (BDCP) Nsukka, where voucher specimens (Intercedd /708) were deposited. The plants were dried and ground into fine powder.

Extraction

Twenty-five grammes of *B. coriacea* seeds was extracted by cold maceration in hexane, carbon tetrachloride, chloroform, ethyl acetate, acetone, methanol and water. A ten-fold quantity of solvent in relation to plant material was used for the extraction. For each solvent, extraction was done thrice, each for 8 h at room temperature. Extracts were concentrated in a rotary evaporator (BUCHI labortechnik AG, Switzerland) under reduced pressure at 40 ^{0}C and stored at 4 ^{0}C until use.

Phytochemical analysis

Chemical constituents of the seeds were analysed by thin layer chromatography (TLC) using aluminium-backed TLC plates (Merck, silica gel 60 F_{254}). The extracts were reconstituted to a concentration of 10 mg/ml. Ten microlitres of the extracts were spotted onto TLC plates. The TLC plates were developed under saturated conditions with ethyl acetate/methanol/water (40: 5.4: 5): [EMW] (polar/neutral); chloroform/ethyl acetate/formic acid (5: 4: 1): [CEF] (intermediate polarity/acidic); benzene/-ethanol/ammonia hydroxide (90: 10: 1): [BEA] (non-polar/basic). To detect the separated compounds, vanillin-sulphuric acid (0.1 g vanillin (Sigma®): 28 ml methanol: 1 ml sulphuric acid) was sprayed on the chromatograms and heated at 110 ^{0}C to optimal colour development.

Column chromatography

The crude chloroform extract (1.83 g) was mixed with 5 g of silica gel dissolved in chloroform and allowed to dry. A 100: 1 ratio of silica gel to extract was used to pack the column. The extract and silica gel mixture was laid on the packed silica gel. Methanol/ chloroform (1: 100) was used to start the elution after the column had been washed with chloroform. The ratio of methanol to chloroform was steadily increased until 12 % at the end of the column chromatography. The fractions were pooled using TLC to combine similar fractions. Seven fractions were obtained.

Antioxidant activity

Thin layer chromatography (TLC) was used to separate extracts as above. To detect antioxidant activity, chromatograms were sprayed with 0.2 % 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) in methanol as indicator.

Antibacterial assay

A two-fold serial dilution microplate method (Eloff, 1998) was used to determine the minimum inhibitory concentrations (MIC) of crude extracts and fractions against *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212 and *Escherichia coli* ATCC 25922. Iodonitrotetrazolium chloride was used as an indicator of growth.

Bioautography

This was done according to the method of Eloff (2001). Briefly, developed chromatography plates of 10 mg/ml extracts were allowed to dry for 3-4 days and sprayed with a suspension of growing cells of *S. aureus* and incubated at 37^{0}C in a chamber at 100 % relative humidity for 18 h. After spraying with p-iodonitrotetrazolium salt, clear zones on the chromatogram indicated inhibition of growth after incubating for 1 h at 37^{0}C.

Fungal test organisms

Four fungal organisms namely *Candida albicans*, *Cryptococcus neoformans* var *gattii, Aspergillus fumigatus* and *Trichophyton verrucossum* cultured from clinical cases of disease in animals were obtained from the Department of

Veterinary Tropical Diseases, Faculty of Veterinary Science, South Africa. These fungi are yeasts (*Candida albicans*, *Cryptococcus neoformans* var *gattii*) a mould (*Aspergillus fumigatus*) and a dermatophyte (*Trichophyton verrucossum*) which are the most common pathogenic fungi of animals. *Candida albicans* was isolated from a Goldian finch, *Cryptococcus neoformans* from a cheetah and *Aspergillus fumigatus* from a chicken. None of the animals had been treated prior to sampling. All fungal strains were maintained on Sabouraud dextrose agar (Oxoid, Basingstoke, UK).

Antifungal assays

A serial microdilution assay (Masoko *et al.*, 2005) with p-iodonitrotetrazolium violet added as a growth indicator was used to determine the minimum inhibitory concentration (MIC) values for plant extracts and fractions. Residues of the different extracts were dissolved in their respective solvents to a concentration of 10 mg/ml. The extracts (100 μl) were serially diluted up to 50 % with water in 96 well microtitre plates. Fungal cultures were transferred into fresh Sabourand dextrose broth and 100 μl of this was added to each well. Amphotericin B was used as the reference antibiotic and positive control. As an indicator of growth, 40 μl of 0.2 mg/ml of p-iodonitrotetrazolium violet (INT) dissolved in water was added to each of the microtitre wells. The covered microplates were incubated for 2-3 days at 35 ^{0}C and 100 % relative humidity. The MIC was recorded as the lowest concentration of the extract that inhibited fungal growth.

RESULTS

The yields of *Buchholzia coriacea* seed extracts obtained with the various solvents used are shown in Table I. The aqueous extract had the highest yield of 10.7 % while the carbon tetrachloride extract had the least yield of 0.8 %. The results of the antibacterial assays of the extracts are shown in Table II. The acetone extract was most active with an average MIC value of 1.6 mg/ml for all the tested organisms. Chloroform and methanol extracts had average MIC values of 2.03 mg/ml and 2.19 mg/ml, respectively. The hexane and aqueous extracts had MIC values above 2.5 mg/ml. The result of the antibacterial activity of the chloroform fractions is shown in Table III. Fraction F2 had the highest activity with an MIC value of 0.31 mg/ml. This was followed by fractions F6 with an MIC value of 0.62 mg/ml. Fractions F3 and F4 had an MIC value of 1.25 mg/ml. Fractions F1, F5 and F7 had an MIC value of 2.5 mg/ml.

TABLE I: The yields of extracts of *Buchholzia coriacea* seeds

Solvents	% Yield
Acetone	1.4
Methanol	4.6
Chloroform	1.5
Carbon tetrachloride	0.8
Ethyl acetate	1.4
Hexane	1
Water	10.7

TABLE II: Minimum inhibitory concentration values of *Buchholzia coriacea* seed extracts after 24 h incubation

Extracts	Minimum inhibitory concentration values (mg/ml)			
	S. aureus	*E. faecalis*	*E. coli*	*P.aeruginosa*
Acetone	1.25	1.25	>2.5	1.25
Chloroform	2.5	0.625	>2.5	>2.5
Methanol	>2.5	2.5	>2.5	1.25
Hexane	2.5	>2.5	>2.5	>2.5
Ethyl acetate	>2.5	>2.5	>2.5	>2.5
Carbon tetrachloride	>2.5	2.5	>2.5	>2.5
Water	>2.5	>2.5	>2.5	>2.5
Gentamycin	0.019	0.019	0.078	0.078

TABLE III: Minimum inhibitory concentration values of chloroform fractions of *B. coriacea* seeds.

Fractions	Minimum inhibitory concentration (mg/ml)
	S. aureus
F1	2.5
F2	0.31
F3	1.25
F4	1.25
F5	2.5
F6	0.62
F7	2.5
Gentamycin	0.019

TABLE IV: Minimum inhibitory concentration values of *Buchholzia coriacea* seed extracts after 48 h incubation.

Extracts	Minimum inhibitory concentration values (mg/ml)			
	C. neoformans	*C. albicans*	*A. fumigatus*	*T. verrucosum*
Acetone	2.5	>2.5	>2.5	>2.5
Chloroform	0.15	>2.5	>2.5	>2.5
Methanol	>2.5	2.5	>2.5	>2.5
Ethyl acetate	1.25	>2.5	>2.5	>2.5
Hexane	>2.5	>2.5	>2.5	>2.5
Amphotericin B	0.039	0.039	0.039	0.039

The results of the antifungal assay are shown in Table IV. The chloroform extract was the most active with an average MIC value of 1.9 mg/ml. This was followed by the ethyl acetate extract with an average MIC value of 2.2 mg/ml. Acetone and methanol extracts had an average MIC value of 2.5 mg/ml. The hexane extract had an MIC value above 2.5 mg/ml.

DISCUSSION

Extraction with water gave the highest yield of 10.7 %. The high yield could be due to the presence of sugars and other polar substances like tannins in the seeds. Methanol also had a high yield of 4.6 %. The least yield of 0.8 % was obtained on extraction with carbon tetrachloride. The plant yield increased with increasing polarity. Some polar compounds however, may not be so interesting for clinical application (Masoko *et al*., 2005). Virtually all the extracts showed some degree of antioxidant activity. However, activity appeared to be more in the non-polar extracts. The acetone, chloroform, hexane and carbon tetrachloride crude extracts showed some antibacterial activities. No antibacterial activity was observed in the methanol and ethyl acetate extracts. The result of the microplate dilution assay agreed with that obtained by bioautography. From the result of the bioautography, it seemed that the same compound was apparently responsible for the antibacterial activity in all the extracts. The first hexane extract appeared to have a second compound with antibacterial activity.

From the result of the microplate dilution assay, the hexane and aqueous extracts showed no activity against all the tested bacteria. The most susceptible bacteria were *E. faecalis* followed by *P. aeruginosa* and *S. aureus*. None of the extracts was active against *E. coli*. This result disagrees with that reported by Ezekiel and Onyeoziri (2009) who obtained antimicrobial activities against *E. coli* from the fresh seed as well as the hexane and methanolic extracts of *B. coriacea*.

Fractionation of the crude chloroform extract yielded fractions with better antibacterial activities of which fraction F2 was the best with an MIC value of 0.31 mg/ml. Further purification of this fraction could lead to a pure compound with a still better activity. From the results of the antifungal assay in Table IV, the most susceptible fungus was *C. neoformans* followed by *C. albicans*. The extracts showed no activity against *T. verrucossum* and *A. fumigatus*. Similar results have also been obtained by other workers. In a study of antifungal compounds in 24 Combretum species using 5 animal fungi, Masoko and Eloff (2006) found *C. neoformans* the most susceptible and *A. fumigatus* the most resistant. In another study of 96 extracts of *Terminalia* species for antifungal activities, Masoko *et al*. (2005) observed the same trend in susceptibility as above. Ajaiyeoba *et al*. (2003) demonstrated high concentration-dependent antibacterial and antifungal activities against *S. aureus, E. coli, S. typhii, P. aeruginosa, C. albicans* and *A. flavus* from the methanolic extract of *B. coriacea* stem bark.

In conclusion, the extracts of *Buchholzia coriacea* seed possess antioxidant and modest antimicrobial activities. This could be the basis for its traditional use in the treatment of systemic infections.

ACKNOWLEDGEMENTS

The author gratefully acknowledges the Phytomedicine unit, Department of Paraclinical Sciences, Faculty of Veterinary Sciences, University of Pretoria, South Africa for permission to use their facilities in this work.

REFERENCES

AFOLAYAN, A. J. (2003): Extracts from the shoots of *Arctotis artotoides* inhibit the growth of bacteria and fungi. *Pharm. Biol*., **41**: 22-25.

AJAIYEOBA, E. O., ONOCHA, P. A., NWOZO, S. O. and SAMA, N. O, (2003): Antimicrobial and cytotoxicity evaluation of *Buchholzia coriacea* stem bark. *Fitoterapia* **74**(7-8): 706-709.

CHAH, K. F. and NWEZE, N. E. (2001): Antibiotic use in poultry production in Nsukka, South-east Nigeria. *Proceedings of the 26th Annual Conference of the Nigerian Society for Animal Production* **26**: 69-72.

ELOFF, J. N. (1998): A sensitive and quick microplate method to determine the minimum inhibitory concentration of plant extracts for bacteria. *Planta Med*. **64**: 711-713.

ELOFF, J. N. (2001): Antibacterial activity of Marula *(Sclerocarya birrea) (A. rich.) Hochst. subsp. caffra (Sond.) Kokwaro) (Anacardiaceae)* bark and leaves. *J. Ethnopharmacol*., **76:** 305-308.

EZEKIEL, O. O. and ONYEOZIRI, N. F. (2009): Preliminary studies on the antimicrobial properties of *Buchholzia coriacea* (wonderful kola). *Afr. J. Biotechnol*., **8**(3): 472-474.

FARNSWORTH, N. R. (1988): Screening plants for new medicines. In: Wilson, E. O. (ed.). *Biodiversity*, National Academic Press, Washington, DC,. 83-97.

MASOKO, P. and ELOFF, J. N. (2006): Bioautography indicates the multiplicity of antifungal compounds from twenty-four southern African *Combretum* species (Combretaceae). *Afr. J. Biotechnol*., **5**: 1625-1647.

MASOKO, P., PICARD, J. and ELOFF, J. N. (2005): Antifungal activities of six South African *Terminalia* species (Combretaceae). *J. Ethnopharmacol*., **99**: 301-308.

NWAFOR, S. V., AKAH, P. A. and OKOLI, C. O. (2001): Anthelmintic activity of crude aqueous extract of *Nauclea latifolia* stem bark against ovine nematodes. *Fitoterapia* **72**: 12-21.

NWEZE, N. E., FAKAE, L. B. and ASUZU, I. U. (2009): Trypanocidal activity of the ethanolic extract of *Buchholzia coriacea* seed. *Niger. Vet. J*., **29**: (4) 1-6.

PAREKH, J. and CHANDA, S. (2007): Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. *Afr. J. Biomed. Res*., **10**: 175-181.