African Journal of Biotechnology Vol. 5 (18), pp. 1602-1607, 18 September 2006
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2006 Academic Journals

Full Length Research Paper

Evaluation of antibacterial activity and phytochemical analysis of root extracts of *Boscia angustifolia*

Hassan, S. W.[1]*, Umar, R. A.[1], Lawal, M.[1], Bilbis, L. S.[1], Muhammad, B. Y.[2] and Dabai, Y. U.[3]

[1]Department of Biochemistry, Usmanu Danfodiyo University, P.M.B. 2346, Sokoto, Nigeria.
[2]Department of Pharmacology, Usmanu Danfodiyo University, P.M.B. 2346, Sokoto, Nigeria.
[3]Department of Biological sciences, Microbiology unit, Usmanu Danfodiyo University, P.M.B. 2346, Sokoto, Nigeria.

Accepted 21 July, 2006

The aqueous and organic solvents extracts of *Boscia angustifolia* were screened for antibacterial and phytochemical properties. Alkaloids and saponins were detected in aqueous and chloroform extracts. These extract fractions were significantly ($p<0.05$) active against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Streptococcus pneumoniae* but did not show activity against *Salmonella typhi* at concentrations ranging from 10 to 120 mg/ml. The hexane and petroleum ether extracts did not show activity on the bacterial organisms used. All the activities were compared with a standard drug, tetracycline. The Minimum Inhibitory Concentration (M.I.C.) of the aqueous and chloroform extracts was 20 and 10 mg/ml respectively. The results lend scientific credence to justify the use of this plant against some bacterial diseases.

Key words: *Boscia angustifolia,* antibacterial, *in vitro* screening.

INTRODUCTION

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value (Nostro et al., 2000). The acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led researchers to investigate the antimicrobial activity of medicinal plants (Bisignano et al., 1996; Hammer et al., 1999). *Boscia angustifolia* (family Capparidaceae) is commonly called a Rough-leaved shepherd's tree. It is small tree of Savannah with up to 25 ft high with glabrous branches, greenish flowers and fragrance. It is widely spread in northern Nigeria, Sudan and Saudi Arabia (Keay et al., 1960). The leaves and roots are used for the treatment of diarrhea, pneumonia, boils, chest pain, wound infection, urinary infection and typhoid fever and protect cells of the skin (Keay et al., 1960). In Sokoto and other parts of Nigeria, the root extract of *B. angustifolia* is used tradomedically in the treatment of bacterial diseases.

Plants containing terpenoids, steroids, phenolic compounds and alkaloids have been reported to have antimicrobial activity (Hostettmann and Nakanishi, 1979). The plant has not been thoroughly evaluated for antibacterial activity and phytochemical properties. The bacterial organisms used in this study are known to cause wound infection, diarrhoea, pneumonia and chest pain. This study was aimed at investigating the antibacterial activity of the plant by preliminary *in vitro* bioassay screening, using aqueous, hexane, petroleum ether and chloroform extracts.

MATERIALS AND METHODS

Collection of plant materials

B. angustifolia was obtained from Kara market, Sokoto. The portion (root) collected was used by traditional healers in the treatment of bacterial diseases. The plant was identified and verified at Usmanu Danfodiyo University, Sokoto, Herbarium (Botany unit, Department of Biological sciences). Voucher specimen was deposited in the Herbarium. Traditional herbalists in northern Nigeria use the root rather than other parts of the plant in the treatment of bacterial diseases. The root obtained was open-air-dried under the shade, pulverized into a moderately coarse powder (using a wooden pestle and mortar) and stored until required for use (Onoruvwe and Olorunfemi, 1998).

*Corresponding author. E-mail: hassanwara@yahoo.com.

Organisms

The species of bacterial organisms were *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Streptococcus pneumoniae*. They were clinical isolates obtained from Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto. The cultures of bacteria were maintained on nutrient agar slants at 4°C, re-identified by biochemical tests (Cheesbrough, 1982) and sub-cultured on to nutrient broth for 24 h prior to testing.

Extraction and fractionation procedure

Fractionation of the extract was done by activity-guided fractionation using ethanol-water (1:1) and different (hexane, petroleum ether and chloroform) organic solvents. The powdered extract of the plant (40 g) was extracted with ethanol-water (500ml of 1:1) at room temperature overnight (Moris and Aziz, 1976; Isaac and Chinwe, 2001). The extract was filtered; the filtrate was partitioned in hexane (250 ml) and clarified by further filtration. Evaporation of the hexane to dryness in an oven at 50°C yielded 0.76g of residue. The aqueous filtrate (ethanol-water) was further partitioned with petroleum ether (250 ml) and chloroform (250 ml). Evaporation of the petroleum ether, chloroform and the last remaining aqueous filtrate yielded 0.90, 1.0 and 0.80 g of residues, respectively. A separate portion of the powdered plant (40 g) was extracted with 500 ml distilled water at room temperature overnight and filtered. The filtrate was also evaporated to dryness and yielded 3.60 g of residue (Isaac and Chinwe, 2001). The residues were reconstituted in sterilized distilled water and screened for antibacterial activity. The above procedure enabled us obliterate the possible contributory antibacterial effect of the organic solvents.

Phytochemical screening

This was carried out using standard procedures of Harbone (1973), Trease and Evans (1978), El-Olemyl et al. (1994) and Wall et al. (1954).

Antibacterial activity

The antibacterial activity was done by utilizing the hole-in-plate bioassay procedure as reported by Hugo and Russell (1983); Vlietinck et al. (1995). Pure culture of the organisms were inoculated onto Muller-Hinton nutrient broth (Oxoid, England), incubated for 24 h at 37°C, diluted with sterile nutrient broth to a density of 9×10^8 cfu/ml equivalent to MC-Farland test tube number 3. The suspension was used to streak for confluent growth on the surface of Muller-Hinton agar plates with sterile swab. Using a sterile cork-borer of 6 mm diameter, four holes were made in to the set agar in Petri-dishes containing the bacterial culture. Concentrations of 5-120 mg/ml of the extracts were poured in to the wells. Standard antibiotic (tetracycline 10 mg/ml) was used as reference or positive control. The plates were placed in the incubator at 37°C overnight. Antibacterial activity was recorded if the zone of inhibition was greater than 9 mm. The significance of the difference of the antibacterial activities of the extracts were tested by one way analysis of variance (ANOVA)

Determination of minimum inhibitory concentration (MIC)

The water and chloroform extracts showed significant activity ($P<0.05$) and were chosen to assay for MIC. MIC was determined by the standard method of Wariso and Ebong (1996). Nutrient broth was prepared and sterilized using autoclave. One (1) ml of the prepared broth was dispensed in to the test tubes numbered 2-12 using sterile pipette. A stock solution containing 0.8 g of the plant extracts in 10 ml of de-ionized water was prepared. Then 1 ml of the solution was dispensed into each of the tubes numbered 1 and 2. Subsequently, from tube 2, serial dilution was carried out and 1 ml from tube 2 was transferred up to tube number 10 and 1 ml from the tube 10 was discarded. Tube 11 was control for sterility of the medium and tube 12 for viability of the organisms. An overnight culture (inoculums) of each of the test isolates was prepared in sterile nutrient broth 1:100 (10^2 dilution of the broth). From this dilution, 1 ml of the inoculum was transferred into each tube from tube 2 to tube 12 with exception of tube 11, to which another sterile nutrient broth was added. The final concentration of the plant extract in each of the test tubes numbered 1-10 after dilution were 80,000; 40,000; 20,000; 10,000; 5,000; 2,500; 1,250; 625; 312.50 and 156.25 µg/ml, respectively. Tetracycline was used as control. They were incubated at 37°C for 24-48 h and examined for growth. The last tube in which growth failed to occur was the MIC tube.

RESULTS AND DISCUSSION

The results of the *in vitro* assays of antibacterial activity and phytochemical analysis of the residues obtained are shown in Tables 1 - 4. The *in vitro* antibacterial screening of the extracts showed that the crude water and chloroform extracts of the root of *B. angustifolia* possess significant ($P< 0.05$) inhibitory activities against all the tested bacterial isolates with the exception of *S. typhi* at 30-120 and 10-120 mg/ml, respectively, on some of the tested bacteria (Table 1 and 2); with higher inhibitory activity of chloroform extracts compared with aqueous extracts. There was no significant ($P>0.05$) antibacterial activity of the last remaining water-ethanol extract against the test organisms. The hexane and petroleum ether extracts did not show activity at 10-120 mg/ml on all the bacterial isolates tested. The MIC of water and chloroform extracts ranged from 40-80 and 10-80 mg/ml, respectively (Table 3). The chloroform extract has the lowest MIC compared to all the extracts including the control tetracycline. Phytochemical analysis (Table 4) revealed the presence of alkaloids and saponins in water and chloroform extracts.

The findings of our research clearly validate the relatively simple *in vitro* system employed in this investigation as a fast and reliable system for *in vitro* screening of plants. The *in vitro* activities of crude plant extracts provide evidence to support the use of such plants (Wurochekke and Nok, 2004). The aqueous and chloroform extracts indicated significant ($P<0.05$) antibacterial activities against all the tested bacteria with exception of *S. typhi*. The chloroform extract was the most potent of all other fractions as indicated by its lowest MIC (Table 3). The antibacterial potential of the root extracts of *B. angustifolia* has been elucidated by the result of this study. The antimicrobial properties of this plant probably explain its traditional use for treating bacterial diseases. Freiburghans (1996) indicate that different solvent extracts of some plant may exhibit

Table 1. Antibacterial activity of aqueous extract of *Boscia angustifolia*.

Plant extract	Extract conc. (mg/ml)	Zone of inhibition (mm)				
		Staphylococcus aureus	***Pseudomonas aeruginosa***	***Escherichia coli***	***Streptococcus Pneumoniae***	***Salmonella typhi***
Boscia angustifolia	10	-	-	-	-	-
	30	13.00 ± 1.64	11.60 ± 1.05	14.00 ±0.41*	12.60 ± 0.65	-
	90	28.00 ± 0.82*	29.30 ± 0.75*	29.00 ± 1.23*	29.00 ± 0.41*	-
	120	32.50 ± 0.50*	33.00 ± 1.64*	32.50 ± 0.50*	31.25 ± 0.25*	-
Tetracycline	10	22.30 ± 0.40*	-	28.00 ± 1.23*	20.00 ± 0.82*	21.00 ± 0.82*
Water		-	-	-	-	-

- = No activity; values greater than 9 mm indicated some activity.
* = Significantly different from the control (P< 0.05) by using analysis of variance (n=4).
Values are mean ± standard error of the mean.

Table 2. Antibacterial activity of organic solvent extracts of *Boscia angustifolia*.

		Zone of inhibition (mm)				
Plant extract	**Extract conc./Drug (mg/ml)**	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Escherichia coli***	***Streptococcus Pneumoniae***	***Salmonella typhi***
Boscia angustifolia	10 HX	-	-	-	-	-
	90 HX	-	-	-	-	-
	120 HX	-	-	-	-	-
	10 PE	-	-	-	-	-
	90 PE	-	-	-	-	-
	120 PE	-	-	-	-	-
	10 CHL	24.70 ± 1.20*	25.00± 0.41*	22.80± 0.60*	11.50 ± 0.41*	-
	90 CHL	38.5 ± 1.25*	36.25± 0.25*	24.00± 0.41*	20.50 ± 0.50*	-
	120 CHL	48.00 ± 0.82*	48.00± 0.41*	36.50± 0.50*	25.25 ± 0.25*	-
	10 LR	-	-	-	-	-
	90 LR	-	-	-	-	-
	120 LR	-	-	-	-	-
Tetracycline (positive control)	10	21.60 ± 0.15*	-	28.00 ± 0.82*	19.00 ± 0.41*	21.00 ± 0.41*
Water (negative control)		-	-	-	-	-

The chloroform fraction of *Boscia angustifolia* was the most active against the organisms tested.
- = No activity; values greater than 9 mm indicated some activity.
* = Significantly different from the control (P<0.05) by using analysis of variance.
Values are mean ± standard error of the mean (n=4).
HX, Hexane; PE, petroleum ether; CHL, chloroform; and LR, Last remaining water-ethanol.

Table 3. Minimum inhibitory concentration of aqueous and chloroform extracts of *Boscia angustifolia*.

Extract Conc. (mg/ml)	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Streptococcus pneumoniae*
80 (W)	-	-	-	-
40 (W)	-	-	-	-
20 (W)	+	+	+	+
10 (W)	+	+	+	+
5 (W)	+	+	+	+
2.5 (W)	+	+	+	+
1.25 (W)	+	+	+	+
0.63 (W)	+	+	+	+
0.31 (W)	+	+	+	+
0.16 (W)	+	+	+	+
80 (CHL)	-	-	-	-
40 (CHL)	-	-	-	-
20 (CHL)	-	-	-	-
10 (CHL)	-	-	-	-
5 (CHL)	+	+	+	+
2.5 (CHL)	+	+	+	+
1.25 (CHL)	+	+	+	+
0.63 (CHL)	+	+	+	+
0.31 (CHL)	+	+	+	+
0.16 (CHL)	+	+	+	+
Water (Negative control)	+	+	+	+
80 TC (Positive control)	-	+	-	-
40 (TC)	-	+	-	-
20 (TC)	-	+	-	-
10 (TC)	+	+	+	+
5 (TC)	+	+	+	+
2.5 (TC)	+	+	+	+
1.25 (TC)	+	+	+	+
0.63 (TC)	+	+	+	+
0.31 (TC)	+	+	+	+
0.16 (TC)	+	+	+	+

Water and chloroform fractions were the most active against the organisms tested and were selected for MIC test
- = No Growth of test organism, + = Growth of test organism, TC =Tetracycline and CHL = Chloroform.

Table 4. Phytochemical analysis of organic solvents and aqueous extracts of *Boscia angustifolia*.

Plant extracts	Extract Fractions	VLO	ALK	Tannins				Glycosides						SAP
				CDT	HT	PSEU	ET	CG	FG	ATG	CYG	SAG	DG	
Boscia angustifolia	I	-	+++	-	-	-	-	-	-	-	-	-	-	+++
	II	-	-	-	-	-	-	-	-	-	-	-	-	-
	III	-	-	-	-	-	-	-	-	-	-	-	-	-
	IV	-	+++	-	-	-	-	-	-	-	-	-	-	+++
	V	-	-	-	-	-	-	-	-	-	-	-	-	-

Plant extracts	Extract fractions	Flavonoids	Steroids	Free anthraquinones	Balsams	Resins
Boscia angustifolia	I	-	-	-	-	-
	II	-	-	-	-	-
	III	-	-	-	-	-
	IV	-	-	-	-	-
	V	-	-	-	-	-

- = Absence, + =Trace Amounts, +++ = Presence, I = water, II = Hexane, III = Petroleum ether, IV = Chloroform, V = Last remaining Water –Ethanol extract, VLO = Volatile oil, ALK = Alkaloids, FG =Flavonoid glycosides, ATG = Anthraquinone glycosides, CG = Cardiac glycosides, ET = Ellagitannins, DG = Digitalis glycosides, Pseu = Pseudotannins, CyG = Cyanogenic glycosides. CDT = Condensed Tannins, HT = Hydrolysable Tannins, SAP = Saponins, and SAG = Saponin glycosides.

different pharmacological properties. The result suggests that water is not the most effective solvent for extracting the pharmacologically active compounds from *B. angustifolia*. Though water was reported by traditional healers and herbalists to be the most commonly used solvent to extract biologically active compounds due to its easy availability (Shale et al., 1999). Our findings of greater antibacterial activity by the chloroform extract of *B. angustifolia* contradict this assertion. It is reported that the popularity of an herbal recipe in traditional medical practice may not necessarily be an indication of its effectiveness (Atawodi et al., 2003). The bioassay-guided fractionation could in part be responsible for the inactivity of the last remaining water-ethanol, hexane and petroleum ether extracts observed. The significant ($p<0.05$) antibacterial activity of the water and chloroform extracts of root of *B. angustifolia* in our study underscores the need to study all parts of the plant before generalization is made on the plant's pharmacological and therapeutic potentials. The phytochemical screening results have shown the presence of alkaloids and saponins, these classes of compounds have earlier been reported with antimicrobial activity (Hostettman and Nakanishi, 1979). Therefore, these compounds may be responsible for the antibacterial activity of the root extracts of *B. angustifolia*.

The mechanism of action of the constituents of *B. angustifolia* may be difficult to speculate; however, many antibacterial agents may exhibit their action through inhibition of nucleic acid, protein and membrane phospholipids biosynthesis (Franklin et al., 1987). It is probable that the antibacterial agent(s) in the extract of *B. angustifolia* act via some of the above mechanisms. Further studies on the *in vivo* activity, isolation and structural elucidation of the active component(s) and toxicological studies of the plant extract are recommended.

REFERENCES

Atawodi SE, Blus T, Ibrahim S, Ameh DA, Andrew JN, Mamman M (2003). *In vitro* trypanocidal effect of methanolic extract of some Nigerian Savannah plants. Afr. J. Biotechnol. 9:317-321.

Bisignano G, Germano MP, Nostro A, Sanogo R (1996). Drugs used in Africa as dyes: antimicrobial activities. Phytotherapy Research 9: 346-350.

Cheesbrough M (1982). Medical laboratory Manual for tropical countries. Microbiology. English language book service (ELBS) Vol. 11. pp. 283-378.

EL-Olemyl MM, AL- Muhtadi FJ, Afifi AA (1994). Experimental phytochemisry. A Laboratory manual College of Pharmacy, King Saud university. King Saud university press. pp. 1- 134.

Franklin TJ, Snow GA, Barrett-Bee KJ Nolan RD (1987). Biochemistry of antimicrobial action. Fourth edition. Chapman and Hall London New York. pp. 71-73; 112.

Freiburghans F, Kaminsky R, Nkana MHN, Brun R (1996). Evaluation of African Medicinal Plants for their *in vitro* trypanosidal activity J. of Ethnopharmacol. 55: 1-11.

Hammer KA, Carson CF, Riley TV (1999). Antimicrobial activity of essential oils and other plants extracts. J. Appl. Microbiol. 86: 985–990.

Harbone JB (1973). Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis.3rd Charpman and Hall. London pp 7-13, 60, 89, 131-135, 186- 88, 203, 279.

Hostettman K, Nakanishi K (1979). Moronic acid, a simple triterpenoid keto acid with antimicrobial activity isolated from *Ozoroa Mucroanta* J. Med. Plant Res. 31: 358-366.

Hugo WB, Russel AD (1983). Pharmaceutical Microbiology. 3rd edition Blackwell scientific publication. p. 167.

Isaac OO, Chinwe JA (2001). The phytochemical analysis and antibacterial screening of extracts of *Tetra carpidium conophorum.* J. Chem. Soc. Nigeria 26 (1): 53 – 55.

Keay RWJ, Onochie CFA, Stanfield DP (1960). Federal Forest Research. Ibadan, Lagos, F.G. Printer. p. 72.

Morris KS, Aziz K (1976). Tumour inhibitors 114 Aloe Emodin: Antileukemic principle isolated from *Rhamnus frangular L.* Lloydia 39(4): 223-224.

Nostro A, Germano MP, D'Angelo V, Marino A, Cannatelli MA (2000). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Letters in Appl. Microbiol. 30(5):379.

Onoruvwe O, Olorunfemi PO (1998). Antibacterial screening and pharmacognostical evaluation of *Dischrostachys cinerea* root. W. Africa J. of Biol. Science *7*: 91-99.

Shale TL, Stirk WA, Van Staden J (1999). Screening of Medicinal Plants used in Lesotho for antibacterial and anti – inflammatory activity. J. of ethnopharmacol. 67: 347– 354.

Trease GE, Evans WC (1978). A textbook of Pharmacognosy 11th edition Bailliere Tindall London. p. 530.

Vlietinck AJ, Vanhoof,L, Totte J, Lasure A, Vanden berghe D, Rwangabo PC, Mvukiyumwami J (1995). Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties J. Ethnopharmacol. 46: 31-47.

Wall ME, Krider MM, Krewson CF, Eddy CR, Wilaman JJ, Correll S, Gentry HS (1954). Steroidal Sapogenins XII . Supplementary table of data for steroidal sapogenins vii. Agr. Research service circ. Aic. , 363: 17.

Wariso BA, Ebong O (1996). Antimicrobial activity of *kalanchoe pinnaata* (Ntiele. Lam) pers. W. Afr. J. Pharm. Drug Res. 12: 65-68.

Wurochekke AU, Nok AJ (2004). *In Vitro* antitrypanosomal activity of some medicinal plants used in the treatment of trypanosomiasis in Western Nigeria. Afr. J. Biotech. 3 (9): 481-483.