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Isolation and Identification of Bioactive Antibacterial Component(s) in Root Extracts of *Boscia angustifolia* (Capparidaceae)

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Abstract: The column chromatographic fractions of chloroform (CHL1, CHL2 and CHL3) root extracts of *Boscia angustifolia* were screened for antibacterial activity and phytochemical properties. CHL1 fraction was significantly active ($p<0.05$) at 5 to 60 mg L^{-1} on *Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli* and *Streptococcus pneumoniae* with Minimum Inhibitory Concentration (MIC) of 0.63 to 1.25 mg L^{-1}. The active fraction (CHL1) revealed the presence of only alkaloids with Retention factor (R_f) value of 0.36. The active antibacterial agent in the most potent fraction (CHL1) was isolated and identified by thin layer chromatography (TLC) and phytochemistry. The CHL2 and CHL3 fractions did not show inhibitory activity at 5 to 60 mg L^{-1}. The antibacterial activity of root extract of *Boscia angustifolia* is due to a chloroform-extractable compound. The results support the ethnomedicinal use of root of *Boscia angustifolia* for the treatment of bacterial diseases.

Key words: *Boscia angustifolia*, *in vitro* antibacterial activity, isolation and identification, phytochemical screening, column and thin layer chromatography

INTRODUCTION

The extensive use of natural plants as primary health remedies due to their pharmacological properties is quite common (Conco, 1991). The investigation of the efficacy of plant-based drugs has been paid great attention because of their few side effects, cheap and easy availability (Kumara *et al*., 2001). According to the World Health Organization 80% of the world population still relies mainly on plant drugs (WHO, 1978). Resistance to antibiotics has been the reasons of research for newer drugs to treat microbial infections (Fostel and Lartey, 2000).

B. angustifolia (family Capparidaceae) is a rough-leaved shepherd's tree growing up to 25 ft. high, with glabrous branches, greenish flowers and fragrance. It is distributed in northern Nigeria, Sudan and Saudi Arabia (Keay *et al*., 1960). The leaves and roots are used for the treatment of diarrhoea, pneumonia, boils, chest pain, wound infection, typhoid fever and also protect cells of the skin (Keay *et al*., 1960). In northern Nigeria, the root of *B. angustifolia* is used as a traditional remedy for a wide range of bacterial infections. However, the active substances responsible for its healing properties and antibacterial agent(s) have not previously been isolated. Natural antimicrobial components in plants can inhibit the growth of bacteria by unknown mechanisms other than that of known antibiotics (Eloff, 1998), resistance to almost all the known antibiotics has developed. For these reason, we suggest the continuation of the search for newer antibiotics. In an earlier work we reported

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the chloroform extract fraction of root of *B. angustifolia* having higher activity than the aqueous extract and with no activity on petroleum ether and hexane extracts on some selected bacterial isolates (Hassan *et al*., 2006). In this present study, the *in vitro* antibacterial activity of the column chromatographic fractions of root extracts of *B. angustifolia* was confirmed. Subsequently, the chloroform extract of the root was fractionated using column chromatography and only the fraction (CHL1) which tested positively for antibacterial activity was further isolated and identified. This study would contribute to the development of plant based antibacterial drugs.

MATERIALS AND METHODS

Plant Material

B. angustifolia was obtained from Kara market, Sokoto, Nigeria. The plant samples were authenticated at the Herbarium, Botany unit, Usmanu Danfodiyo University, Sokoto, Nigeria. Voucher specimen was deposited in the Herbarium. The root of the plant is preferred than other parts of the plant for traditional treatment of bacterial diseases. The portion (root) collected was open-air-dried under the shade, pulverized (with a wooden pestle and mortar) in to a coarse powder. It was sieved and stored in a sealed plastic container until required.

Organisms

The bacterial organisms used in this study were *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Streptococcus pneumoniae* and *Salmonella typhi.* All the microorganisms were obtained from Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria. The bacterial cultures were maintained on nutrient agar slants at 4°C, re-identified by biochemical tests (Cheesbrough, 1982) and sub-cultured to nutrient broth for 24 h prior to testing.

Other Materials and Chemicals

Most of the chemicals used were of analytical grade. Silica gel column (60 g, Si 60, 40-63 μm) was from Merck, Germany and commercially prepared TLC plate (GmbH and co. D-3440) was from Eschwege, Germany.

Extraction and Fractionation Procedure

The plant extract was fractionated using activity-guided fractionation with ethanol-water (1:1) and chloroform as described by Moris and Aziz (1976), Springfield and Weitz (2006). The powdered root (120 g) extract was extracted with ethanol-water (1:1, 1500 mL) and separated at room temperature over night. The extract was filtered and partitioned in chloroform separately (750 mL) and clarified by further filtration. Evaporation of chloroform to dryness in an oven at 45°C yielded 7.5% (w/w) residue. The procedure was repeated to obtain more residues. Residues obtained were reconstituted in ethanol-water and chromatographed over silica gel column. The fractions obtained were evaporated and the residues were screened for antibacterial activity.

Column Chromatographic Separation

The chloroform extracts were chromatographed over silica gel column and eluted with ethanol-water (1:1) as described by Brain and Turner (1975). The residues obtained were: CHL1 (0.83 g), CHL2 (0.90 g) and CHL3 (0.81 g). The column fractions were tested for antibacterial activity against five bacterial isolates (Table 1). The fraction(s) that exhibited significant activity was selected for MIC test and the bioactive compound(s) in the most potent fraction (CHL1) was further isolated and identified through TLC and phytochemical screening.

Table 1: Amount of residues obtained from column chromatographic fractions of chloroform extracts of *Boscia angustifolia*

Fractions	Amount recovered (g)
CHL1	0.83
CHL2	0.90
CHL3	0.81

CHL1-3 chloroform fractions

Isolation of Alkaloids

This was done according to standard procedures of Trease and Evans (1978), Nuhu *et al*. (2000) with some modifications. One gram of the sample was extracted with 30 mL of methanol: water (1:1 V: V) mixture. It was filtered through Whatman No. 1 filter paper and the filtrate evaporated. The resultant residue was mixed with 10 mL of 0.0025 M H_2SO_4 and partitioned with ether to remove unwanted materials. The aqueous fraction was basified with strong NH_3 solution and then extracted with excess chloroform to obtain the alkaloidal fraction or separated by filtration. The chloroform extraction was repeated several times and the bulk extract concentrated to dryness. The purity of the substance was determined by means of TLC (Elmarie and Johan, 2001).

TLC of Isolated Alkaloids

Ten microlitres of the bioactive alkaloids were further fractionated by means of commercially prepared thin layer chromatography plates (GmbH and co. D-3440 Eschwege, Germany) using acetone:water: 25% ammonium solution (90:7:3 v:v:v) as a mobile phase. Fifty milligrames of atropine (reference standard) were dissolved in 45 mL of 50% ethanol and 10 μL was applied as a band beside the sample solution. After the plate was developed, the positions of the compounds were detected by spraying first with Dragendorffs reagent and then with 0.1 N H_2SO_4. It was inspected immediately in day light. The R_f values of the bioactive fraction in the sample and the standard were determined (El-Olemyl *et al*., 1994).

Phytochemical Screening

It was done using standard procedures of Wall *et al*. (1954), Persinos and Quimby (1967), Harbone (1973), Trease and Evans (1978) and El-Olemyl *et al*. (1994).

Antibacterial Activity

The antibacterial activity was done using hole-in-plate bioassay procedures as reported by Hugo and Russell (1983), Vlietinck *et al*. (1995). Pure cultures 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^{-1} 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 9 mm diameter, four holes were made in to the set agar in Petri-dishes containing the bacterial culture. Concentrations of 5 to 60 mg mL^{-1} of the extracts were poured in to the wells. Tetracycline (10 mg mL^{-1}), a product of Greenfield Pharmaceutical (Jiang su) Co., Ltd., China, 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 was tested by one-way Analysis Of Variance (ANOVA).

Determination of Minimum Inhibitory Concentration (MIC)

The CHL1 fraction of chloroform extract that showed significant activity ($p<0.05$) was chosen to assay for MIC. This was determined by the standard method of Wariso and Ebong (1996). Nutrient broth was prepared and sterilized using autoclave. One 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 extract 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 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 sample 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^{-1}, respectively. Tetracycline was also used as control. All tubes 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.

Statistical Analysis

The data of the study was subjected to one way Analysis Of Variance (ANOVA), Benferoni compare all columns using Graph Pad Instat Software (San Diego, USA).

RESULTS AND DISCUSSION

Drug resistance in human pathogenic microorganism has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This condition has forced scientists to search for new antimicrobial substances from various sources (Subrammanian *et al*., 2006). The antibacterial properties of root extracts of *B. angustifolia* were shown to be an interesting field for application in pharmaceutical industry. The present study represents a systematic study on isolation and identification of bioactive compounds and antibacterial properties of *B. angustifolia* against some pathogenic bacteria. The results support the view that *B. angustifolia* is a potent antibacterial agent.

The *in vitro* antibacterial activity and phytochemical screening of chloroform column chromatographic fractions of root of *B. angustifolia* against some clinical isolates were presented in Table 2-4. Fractionation of the chloroform extract by column chromatography on silica gel yielded three fractions (CHL1, CHL2 and CHL3). The chloroform extract fraction (CHL1) of the root has shown significant ($p<0.05$) inhibitory activity against the bacterial isolates tested at 5 to 60 mg mL^{-1} with exception of *Salmonella typhi*. The fraction indicated MIC of 0.63 to 1.25 mg mL^{-1} against the isolates used. The CHL2 and CHL3 fractions did not show activity on all the isolates used. As such, different solvent extracts of some plants may have different pharmacological properties. Hassan *et al*. (2006) reported chloroform root extracts of *B. angustifolia* to have higher antibacterial activity than

Table 2: Antibacterial activity of column chromatographic fractions of chloroform root extracts of *Boscia angustifolia*

Fraction conc. (mg mL^{-1})	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Streptococcus pneumoniae*	*Salmonella typhi*
5 CHL1	39.30±1.55*	28.00±0.41*	24.30±1.55*	23.60±1.55*	-
10 CHL1	39.00±0.41*	38.25±0.25*	30.25±0.25*	30.50±0.50*	-
60 CHL1	46.50±1.75*	45.50±0.50*	35.8±0.65*	39.00±1.23*	-
5 CHL2	-	-	-	-	-
10 CHL2	-	-	-	-	-
60 CHL2	-	-	-	-	-
5 CHL3	-	-	-	-	-
10 CHL3	-	-	-	-	-
60 CHL3	-	-	-	-	-
Water (negative control)	-	-	-	-	-
10 TC (positive control)	21.50±0.65*	-	28.50±0.96*	18.00±1.64*	20.00±1.58*

CHL1 was the most active fraction against the isolates and its active component(s) was further identified and isolated, CHL1-3 = Chloroform fractions, TC = Tetracycline, values (mean±standard error of the mean) greater than 9 mm indicated some activity, - = No activity, * significantly different from the control ($p<0.05$), using analysis of variance (n = 4)

Table 3: Minimum inhibitory concentrations of bioactive chloroform column chromatographic fraction of *Boscia angustifolia*

Fraction Conc. (mg mL^{-1})	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Streptococcus pneumoniae*
80 CHL1	-	-	-	-
40 CHL1	-	-	-	-
20 CHL1	-	-	-	-
10 CHL1	-	-	-	-
5 CHL1	-	-	-	-
2.5 CHL1	-	-	-	-
1.25 CHL1	-	-	-	-
0.63 CHL1	-	+	+	+
0.31 CHL1	+	+	+	+
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	+	+	+	+

The CHL1 fraction was active against only these organisms and was selected for MIC test, - = No growth of test organism + = Growth of test organism TC = Tetracycline

Table 4: Phytochemical analysis of chloroform column chromatographic fractions of *Boscia angustifolia*

Extract fractions	Volatile oil	Alkaloids	Tannins	Glycosides	Saponins	Flavonoids	Steroids	Free anthraquinone	Balsams	Resins
CHL1	-	+++	-	-	-	-	-	-	-	-
CHL2	-	-	-	-	+++	-	-	-	-	-
CHL3	-	-	-	-	-	-	-	-	-	-

- = Absence, +++ = Presence

aqueous extract with petroleum ether and hexane extracts lacking activity. Isolation and identification of the active constituents (alkaloids) in CHL1 fraction on TLC indicated R_f value of 0.36 and 0.29 for the reference standard (Atropine).

From the results (Table 2), CHL1 fraction was the only effective fraction and chloroform, probably, the best medium for extracting pharmacologically active compounds from root extract of *B. angustifolia*. However, these findings do not corroborate the practice of traditional herbalist of using water to extract bioactive compounds (Shale *et al*., 1999). The results of the present study showing significant ($p<0.05$) inhibition of the bacterial isolates by the CHL1 fraction only have really contradicted this assertion. It is probable that the bioassay guided fractionation employed in the extraction, is responsible for the inactivity of the CHL2 and CHL3 chloroform fractions. The MIC studies show that *B. angustifolia* chloroform extract (CHL1) fraction was the most potent against the pathogenic bacteria employed at very low doses. Hence, this re-validates its pharmacological and therapeutic potentials. This underscores the ethnobotanical evidence for the selection of *B. angustifolia* in the discovery of new array of bioactive compounds.

Present findings of low MIC values are comparable to those of Subramanian *et al*. (2006) who indicated ethanolic extract of *Aloe vera* leaf gel, although a different family, with antibacterial activity against pathogenic bacteria at very low doses. Alkaloids in the CHL1 fraction may be responsible for the antibacterial actions of *B. angustifolia* (Hostettman and Nakanishi, 1979; Okwute and Mann, 1999). The presence of alkaloid in the active fraction (CHL1) indicates that it is the principal antibacterial agent in the root extract of *B. angustifolia*. The mechanisms of action of the active

components (CHL1) may be due to the pore formation in the cell wall and the leakage of cytoplasmic constituents by the active components (alkaloids) present in the root extract (Shelton, 1991). It could also be by inhibition of nucleic acid, protein and membrane phospholipids biosynthesis (Franklin *et al*., 1987). It can be concluded that root extracts of *B. angustifolia* have compounds with antibacterial potentials that could be harnessed for drug development. Further studies in our laboratory are in progress to elucidate the structure of the active compound.

REFERENCES

Brain, R. and T.D. Tunner, 1975. The Practical Evaluation of Phyto-Pharmaceuticals. Wright-science Technical, Bristol, pp: 90-110, 103-108, 114-117.

Cheesbrough, M., 1982. Medical laboratory manual for tropical countries. Microbiology. English Language Book Service (ELBS), 11: 283-378.

Conco, W.Z., 1991. Zulu traditional medicine: Its role in modern society. Community Health, 5: 8-13.

Elmarie Vander, W. and C.P. Johan, 2001. Purification and identification of active antibacterial components in *Carpobrotus edulis* L. J. Ethnopharmacol., 76: 87-91.

Eloff, J.N., 1998. Which extractant should be used for the screening and isolation of antimicrobial components from plants? J. Ethnopharmacol., 60: 1-8.

EL-Olemyl, M.M., F.J. AL-Muhtadi and A.A. Afifi, 1994. Experimental Phytochemisry. A Laboratory Manual College of Pharmacy, King Saud University, King Saud University Press, pp: 1-134.

Fostel, J.M. and P.A. Lartey, 2000. Emerging novel antifungal agents. Drugs Discov. Today, 5: 25-32.

Franklin, T.J., G.A. Snow, K.J. Barrett-Bee and R.D. Nolan, 1987. Biochemistry of Antimicrobial Action. 4th Edn., Chapman and Hall London New York, pp: 71-73 and 112.

Harbone, J.B., 1973. Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis. 3rd Edn., Chapman and Hall. London, pp: 7-13, 60, 89, 131-135, 186-188, 203, 279.

Hassan, S.W., R.A. Umar, M. Lawal, L.S. Bilbis, B.Y. Muhammad and Y.U. Dabai, 2006. Evaluation of antibacterial activity and phytochemical analysis of root extracts of *Boscia angustifolia*. Afr. J. Biotechnol., 5: 1602-1607.

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

Hugo, W.B. and A.D. Russel, 1983. Pharmaceutical Microbiology. 3rd Edn., Blackwell Scientific Publication, pp: 167.

Keay, R.W.J., C.F.A. Onochie and D.P. Stanfield, 1960. Federal Forest Research. Ibadan, Lagos, F.G. Printer, pp: 72.

Kumara, P.D., G.L. Jayawardane and A.P. Aluwihare, 2001. Complete colonic duplication in an infant. Ceylon Med. J., 46: 69-70.

Morris, K.S. and K. Aziz, 1976. Tumour inhibitors 114 Aloe emodin: Antileukemic-principle isolated from *Rhamnus frangular* L. Lloydia, 39: 223-224.

Nuhu, A.M., M.S. Mshelia and Y. Yakubu, 2000. Antimicrobial screening of the bark extract of *Pterocarpus erinaceus* tree. J. Chem. Soc. Nig., 25: 85-88.

Okwute, S.K. and A. Mann, 1999. Phytochemical and biological screening of *Commiphora kerstingii* Engl. (Burseraceae). Zuma Jpas., 2: 69-71.

Persinos, G.J. and M.W. Quimby, 1967. Nigerian plants. Phytochemical screening for alkaloids, saponins and tannins. J. Pharmaceut. Sci., 56: 1512.

Shale, T.L., W.A. Stirk and J. Van Staden, 1999. Screening of medicinal plants used in lesotho for antibacterial and anti-inflammatory activity. J. Ethnopharmacol., 67: 347-354.

Shelton, R.M., 1991. *Aloe vera*. Its chemical and therapeutic properties. Int. J. Dermatol., 30: 679-683.

Springfield, E.P. and F. Weitz, 2006. The scientific merit of *Carpobrotus mellei* L. base on antimicrobial activity and chemical profiling. Afr. J. Biotechnol., 5: 1289-1293.

Subramanian, S., D. Sathish Kumar, P. Arulselvan and G.P. Senthikumar, 2006. *In vitro* antibacterial and antifungal activities of ethanolic extract of *Aloe vera* leaf gel. J. Plant Sci., 1: 348-355.

Trease, G.E. and W.C. Evans, 1978. A textbook of Pharmacognosy 11th Edn., Bailliere Tindall London, pp: 530.

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

Wall, M.E., M.M. Krider, C.F. Krewson, C.R. Eddy, J.J. Wilaman, S. Correll and H.S. Gentry, 1954. Steroidal sapogenins XII. Supplementary-of data for steroidal sapogenins vii. Agr. Research service circ. Aic., 363: 17.

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

World Health Organization, 1978. The promotion and development of traditional medicine. Tech. Report Series 622.WHO, Geneva.