Journal of Medical and Biomedical Sciences (2014) 3(2): 7-13

doi: http://dx.doi.org/10.4314/jmbs.v3i2.2

ORIGINAL ARTICLE

Antimicrobial and Antioxidant Activities of the Stem Bark of *Cussonia bancoensis*

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Cussonia bancoensis **is used in Ghana mainly for the management of pain and treating infectious diseases. This study investigated the antibacterial and antifungal effect of the methanol, ethyl acetate and petroleum ether extracts of the stem bark of *C. bancoensis* by the agar dilution method. The antioxidant activity of the methanol extract was also investigated by DPPH free radical scavenging assay, total antioxidant capacity and total phenol content determined. The methanol and ethyl acetate extracts inhibited growth of all tested microorganisms with the exception of *Streptococcus pyogenes* in the antimicrobial assay. The minimum inhibitory concentrations ranged from 625 µg mL^{-1} to 2500 µg mL^{-1} for susceptible organisms. The petroleum ether extract had no antimicrobial activity at the concentrations used. The methanol extract scavenged DPPH with an IC_{50} value of 2.890 µg mL^{-1} and a total antioxidant capacity of 110.6 ± 41.15 mg g^{-1} (ascorbic acid equivalent). The total phenolic content expressed as tannic acid equivalent was 43.40 ± 14.70 mg g^{-1}. The results of this study justifies to some extent the use of *C. bancoensis* in the treatment of microbial infections and supports the ethnomedical evidence that the plant could be a potential source of natural antimicrobial and antioxidant agents.**

Journal of Medical and Biomedical Sciences (2014) 3(2), 7-13

Keywords: Antimicrobial, antioxidant, phytochemical constituents, *Cussonia bancoensis*

INTRODUCTION

The emergence of multiple drug resistance in human pathogenic organisms has necessitated the search for new antibiotics from natural sources including plants (Iwu *et al.*, 1999). The interest in plants, stems from the fact that plants naturally synthesize *de novo* toxic agents following microbial attack to guard against invasive and pathogenic microbes in their environment (Gibbons, 2004). It is believed that the antimicrobial activities of such plants are mediated by mechanisms entirely different from the presently used antibiotics and may thus have clinical value in the treatment of resistant microbial strains (Eloff, 1998). Many plants are used as treatment for microbial infections in African traditional medicine (Martin and Ernst, 2003) of which *Cussonia bancoensis* Aubrév and Pellegr (Araliaceae) is no exception.

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C. bancoensis is a large deciduous tree normally found in wet evergreen forests of Ghana, Ivory Coast, Nigeria, Liberia and Cameroon (Irvine, 1961). The root or stem bark decoction is used to treat gynaecological and sexually transmitted infections, lumbago and to relieve general body pains (Burkill, 1985; Effah, 1991). In Nigerian folklore medicine, the stem bark decoction is known to cause dizziness (De Villiers, 2012). In previous studies, ursolic and 23-hydroxy ursolic acids isolated from the stem bark showed potent anti-inflammatory, antioxidant, anti-nociceptive, anticancer and nitric oxide inhibition properties (Shin *et al.*, 2004; Takaya *et al.*, 2009; Tapondjou *et al.*, 2003a; Tapondjou *et al.*, 2003b; Tapondjou *et al.*, 2006). The ethanolic stem bark extract showed potent central analgesia and sedative properties in mice (Mensah *et al.*, 2014).

The antimicrobial activity of some species of Cussonia have been established in previous studies (De Villiers *et al.*, 2010), however, there is no evi-

dence of the scientific investigation of the antimicrobial property of *Cussonia bancoensis*. This study evaluated the antimicrobial activity of different solvent fractions of the stem bark of *C. bancoensis* and determined its free radical scavenging ability, total phenol content and total antioxidant capacity.

MATERIALS AND METHODS

Plant material collection and extraction

The stem bark of *C. bancoensis* was collected from Kwahu - Asakraka (Lat (DMS) 6°37'42.96"N Long (DMS) 0°41'21.00"W, Altitude 538 m) a town about 30 kilometers from Nkawkaw in the Eastern region of Ghana in September, 2013. The plant material was authenticated at the Department of Herbal Medicine, KNUST and the voucher specimen KNUST/CB1/2013/S005 was deposited in the herbarium of the department. Two hundred grams (200 g) of air dried and coarsely powdered plant material was successively cold macerated with 800 mL of petroleum ether (40-60°C) for 24 h. After filtration, the marc was dried and further macerated with 800 mL of ethyl acetate for 48 h. Furthermore, the marc was again dried and finally cold macerated with 800 mL of methanol for 48 hours. The filtrates were individually evaporated to dryness on a rotary evaporator (R-114, Buchi, Switzerland) at temperatures not exceeding 40 °C. The percentage yields obtained for methanol (CBM), ethyl acetate (CBE) and petroleum ether (CBP) extracts were 5.81 %w/w, 2.03 %w/w and 0.57 %w/w respectively.

Antimicrobial assay

Microorganisms used

Three (3) Gram positive bacteria: *Staphylococcus aureus (ATCC 25923), Bacillus subtilis (NCTC 10073), Streptococcus pyogenes (*clinical strain) and three (3) Gram negative bacteria *Escherichia coli (NCTC 9002), Pseudomonas aeruginosa (ATCC 27853), Salmonella typhi (NCTC 6017)* along with one (1) fungus, *Candida albicans* (clinical strain) were used in the antimicrobial assay. The typed cultures were obtained from the Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST and clinical strains from Komfo Anokye Teaching Hospital (KATH), Kumasi. The bacterial strains were cultured overnight at 37˚C in nutrient broth and fungus was cultured overnight at 25˚C in Sabouraud dextrose agar. For all experiments, 24-hour broth cultures of test organisms were used.

Antimicrobial Assay

The solid agar dilution method described by Vanden-Berghe and Vlientick, (1991) was used to determine the susceptibility of organisms and minimum inhibitory concentration of the extracts. Solubilized extracts (2 mL) at concentrations between 5000 μg mL^{-1} and 312 μg mL^{-1} were mixed with an equal volume of liquid double strength nutrient agar medium at 45 °C to give concentrations between 2500 μg mL^{-1} and 156 μg mL^{-1}. Wells of a sterile micro titre plate were filled with 250 μL of these solutions at 45°C to prevent solidification while filling. After solidification at room temperature, all wells were inoculated with a 1:100 dilution (5 μL) of overnight standardized suspensions of cultures of test organisms. The inoculated wells were then incubated at 37°C (for bacteria) and 25° C (for fungus) for 24 hours. Inhibition of growth of the test organisms was adjudged by comparing it with the amount of growth in the control wells using a light microscope. Ciprofloxacin and ketoconazole were used as positive controls for the bacteria and fungus respectively while 2% Dimethyl sulfoxide (DMSO) used to reconstitute extracts served as negative control.

Antioxidant Assays

DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging activity

The free radical scavenging effect was determined by a method described by (Blois, 1958). Methanol extracts (1 mL each) at concentrations of 3 to 100 μg/mL, was mixed with 3.0 mL of DPPH in methanol (20 mg L^{-1}). The mixture was incubated for 30 min in the dark, after which the absorbance of residual DPPH was measured at 517 nm on a Cecil CE 7200 spectrophotometer, (Cecil Instrument Limited, Milton Technical Centre, England). In this assay, the positive and negative controls were ascorbic acid and blank methanol respectively. The controls were taken through the same procedures

and conditions as the extract. All tests were carried out in triplicates and the results presented as the mean of three values. The percentage inhibition or percentage DPPH scavenging effect was calculated using the formula:

$$\%\ DPPH\ scavenged = \frac{(A0 - A1)}{(A0)} \times 100$$

Where A_0 = absorbance of negative control, A_1 = absorbance of different concentrations of extract or ascorbic acid.

Total Antioxidant Capacity

The total antioxidant capacity was determined by a method described by Athukorala *et al.*, (2006). The reaction mixture consisted of 3.0 mL of the reagent solution (0.6 M sulphuric acid, 28 mM disodium phosphate, 4 mM ammonium molybdate) and 1.0 mL of the extract (10 – 500 µg mL^{-1}). The mixture was incubated for 90 minutes at 95°C, after which the absorbance for each concentration was measured at 695 nm. Ascorbic acid (0.78-25 µg mL^{-1}) was used as the standard drug. The controls were taken through the same procedures and conditions as the extract. Total antioxidant values were expressed in terms of ascorbic acid equivalent (AE) (mg g^{-1} of dry mass) (Prieto *et al.*, 1999).

Total Phenol content

The total phenol content of *C. bancoensis* stem bark was determined by Folin-Ciocateu reagent method (McDonald *et al.*, 2001). The solution mixture contained 0.5 mL of extract (10 - 300 µg mL^{-1}) and 0.1 mL of Folin-Ciocalteu reagent (0.5 N). The mixture was incubated at room temperature for 15 min after which 2.5 mL of saturated sodium carbonate solution was added and further incubated for 120 minutes at room temperature. Absorbance was then measured at 760 nm (Cecil CE 7200 spectrophotometer, Cecil instrument limited, Milton Technical Centre, England). The calibration curve was prepared using tannic acid (5 - 50 µg mL^{-1}). The total phenol content was expressed in terms of tannic acid equivalent (mg g^{-1} of dry mass).

RESULTS AND DISCUSSION

This study investigated the antimicrobial and antioxidant activities of the stem bark of *C. bancoensis*. The extracts were prepared successively with three solvents of different polarities to determine which fraction of the extract had the highest activity and also facilitate the isolation of active antimicrobial constituents in future. The methanol extract was also investigated for total phenol content, free radical scavenging and total antioxidant capacity.

The antimicrobial activity of test extract was performed using the agar dilution method. This is a quantitative susceptibility testing method by which Minimum Inhibitory Concentrations (MIC) values

Table 1: Minimum inhibitory concentrations (µg mL^{-1}) of CBM, CBE, CBP, Ciprofloxacin and Ketoconazole against selected microorganism

Organisms	CBM	CBE	CBP	Ciprofloxacin	Ketoconazole
S. aureus (ATCC 25923)	2500	1250	NI	0.25	NT
B. subtilis (NCTC 10073)	2500	1250	NI	0.10	NT
S. pyogens (clinical strain)	NI	NI	NI	0.10	NT
E. coli (NCTC 9002)	1250	625	NI	0.10	NT
P. aeruginosa (NCTC 9002)	2500	1250	NI	0.10	NT
S. typhi (NCTC 9002)	2500	1250	NI	0.25	NT
C. albicans (clinical strain)	1250	625	NI	NT	5

CME = *C. bancoensis* methanol extract; CBE= *C. bancoensis* ethyl acetate extract; CBP= *C. bancoensis* petroleum ether extract; NI- No inhibition, NT: not tested

can be obtained. The agar dilution method is versatile and does not present problems of sample diffusion or dissolution in growth media (Silva *et al.*, 2005). In the antimicrobial assay, the MIC against susceptible organisms ranged from 625 μg mL^{-1} to 2500 μg mL^{-1} for all tested extracts. The highest activity was shown by the ethyl acetate extract which inhibited growth of all organisms tested except *S. pyogens*. The most susceptible organisms were *E. coli* and *C. albicans* at an MIC of 625 μg mL^{-1}. The methanol extract showed activity against all organisms but *Streptococcus pyogens* with the lowest MICs ranging between 1250 and 2500 μg mL^{-1}. The petroleum ether extract showed no inhibitory action against organisms at the concentrations used (Table 1). The MIC range given by the extracts imply that the semi-polar fraction of *C. bancoensis* is the most active, although the inhibitory effects against Gram positive and Gram negative bacteria as well as the fungus *C. albicans* was moderately low.

In a previous study, the antimicrobial activity of about thirteen *Cussonia* species was reported (De Villiers *et al.*, 2010). The antimicrobial activities of some of the species were associated with particular phytochemical constituents known to have either bacteriostatic or bactericidal properties against most organisms. The antimicrobial activity of *Cussonia arborea* for example was attributed to the presence of polyacetylenes isolated from the plant (Papajewski *et al.*, 1998). Pentacyclic triterpenoids isolated from *Cussonia holstii* were also reported to have significant activity against *Trichomonas* species (He *et al.*, 2003). Phytochemical studies on the stem bark of *C. bancoensis* in previous investigations have revealed the presence of condensed tannins, saponins, flavonoids, triterpenoids and sterols (Mensah *et al.*, 2014). These plant constituents in many cases, serve as defence agents against predation by microorganisms (Cowan, 1999). Their presence in the stem bark can thus be responsible for the observed antibacterial and anti-fungal activity.

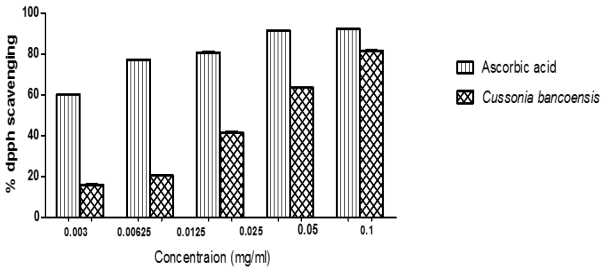

Figure 1: Increasing % DPPH scavenging with increasing concentration of ascorbic acid and *C. bancoensis*

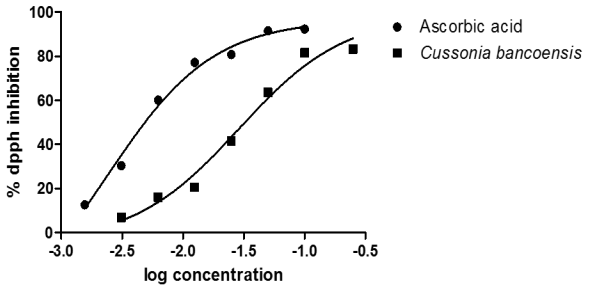

Figure 2: Log concentration versus % DPPH inhibition curve for *C. bancoensis* and Ascorbic acid

The DPPH assay is used to evaluate the ability of an agent to mop up free radicals in a system. DPPH (2, 2-diphenyl-1-picrylhydrazyl) is a stable nitrogen-centered free radical with a deep violet colour and absorption maxima at 517 nm. It is decolorized when it accepts an electron from the antioxidant compound to form a yellow solution, DPPH-H. The amount of residual DPPH after reacting with the antioxidant agent is quantitatively measured from changes in UV absorbance at 517 nm (Blois, 1958). Results from DPPH free radical scavenging assay showed a concentration-dependent scavenging effect of the extract which was observed as a decrease in DPPH absorbance with increasing concentration of extract (Figures 1 & 2).

The IC_{50} values recorded for the extract and ascorbic acid were 2.88 μg mL^{-1} and 2.44 μg mL^{-1} respectively, implying that *C. bancoensis* stem bark has similar radical scavenging effect as the standard antioxidant agent, ascorbic acid. The total phenolic content of the extract was 43.40±14.7 mg g^{-1} dry weight of extract, expressed as tannic acid equiva-

lent and the total antioxidant capacity of the extract was 110.6 ± 41.15 mg g^{-1} dry weight of extract, expressed as ascorbic acid equivalent. The total antioxidant capacity and phenolic content increased with increasing concentration of the extract (Figure 3a and 3b). Plant phenolic constituents including flavonoids and caffeic acid conjugates are known to be responsible for the observed antioxidant properties of many plants (Chanda and Dave, 2009; Cipak *et al.*, 2006).

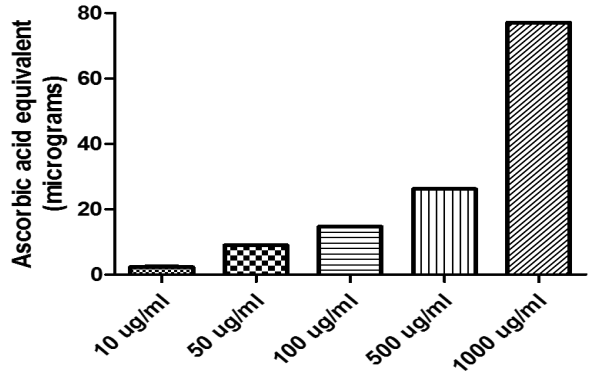

Figure 3a: Total antioxidant capacity of *C. bancoensis* measured as ascorbic acid equivalent at different concentrations of extract

Previous phytochemical investigation showed that the stem bark of *C. bancoensis* posses some amount of antioxidant activity when investigated towards superoxide scavenging and xanthine oxidase inhibition. This activity was attributed to a *p*-caffeoyl phenol derivative trivially named bancoensone (Tapondjou *et al.*, 2006). In the genus Cussonia, *C. arborea* (Biapa *et al.*, 2008) and *C. barteri* (Diallo *et al.*, 2001) have shown potent antioxidant activities, the former's being attributed to the presence of tannins and flavonoids. The antioxidant effect exhibited by the stem bark extract is thus consistent with previous studies and proves *C. bancoensis* as a potential source of natural antioxidants. Free radicals and associated oxidative stress are implicated as the fundamental mechanism underlying most inflammatory conditions hence a therapy using antioxidants has a potential to delay or ameliorate the inflammatory process (Delanty and Dichter, 2000). The antioxidant property of the stem bark may thus contribute to the anti-inflammatory effect observed for the plant in traditional medicine.

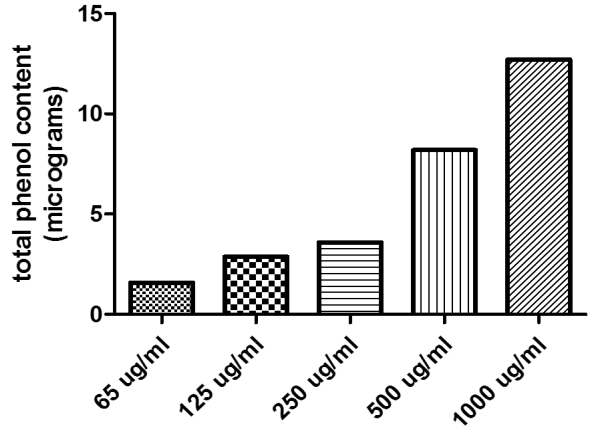

Figure 3b: Total phenolic content of *C. bancoensis* measured as tannic acid equivalent at different concentrations of extract

CONCLUSION

The results of this study justify the ethno-medicinal use of *C. bancoensis* in the treatment of infections and also suggest that the stem bark is a potential source of natural antimicrobial and antioxidant agents.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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ISSN 2026-6294