Journal of Medicinal Plants Research Vol. 5(20), pp. 5116-5122, 30 September, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

In-vitro evaluation of the antimicrobial activity of extracts of *Bridelia micrantha* on selected bacterial pathogens

Adefuye A. O.[1], Samie A.[2] and Ndip R. N.[1,3]*

[1]Department of Biochemistry and Microbiology, Faculty of Science and Agriculture, University of Fort Hare, P/Bag X1314, Alice 5700, South Africa.
[2]Department of Microbiology, University of Venda, Thohoyandou, South Africa.
[3]Department of Biochemistry and Microbiology, Faculty of Science, University of Buea, Box 63, Buea, Cameroon.

Accepted 4 July, 2011

Medicinal plants have been age long remedies for human diseases because they contain components of therapeutic value. In this study, six solvent extracts [dichloromethane, ethyl acetate, acetone, ethanol, methanol (100%), and 40% methanol hydroxide] of the stem bark of *Bridelia micrantha* and ciprofloxacin were investigated for antimicrobial activity by the agar-well diffusion method against strains of *Staphylococcus aureus* NCTC 6571*, Shigella sonnei* ATCC 29930, *Salmonella typhimurium* ATCC 13311, *Helicobacter pylori* ATCC 43526, and *Helicobacter pylori* 252c. The most active extracts were assayed for MIC_{50} using the 96-well microdilution technique and one way ANOVA test was used to determine if there was any statistically significant difference in the MIC_{50} of the most active extracts and the control antibiotic (ciprofloxacin). Results obtained indicated that methanol was quantitatively the best solvent for extraction, while ethyl acetate was the least. Zone diameters of inhibition ranged from 0 to 28 mm for the six extracts and 29 to 38 mm for ciprofloxacin. Ethyl acetate and acetone extracts were the most active of all the extracts exhibiting a broad spectrum activity. However, Gram-positive bacteria were more sensitive compared to Gram-negative bacteria. The MIC_{50} value ranged from 0.078 to 1.25 mg/ml and 0.078 to 0.625 mg/ml for the acetone and ethyl acetate extracts respectively, with no statistically significant difference in potency (p value = 0.187) when compared to ciprofloxacin. Our findings demonstrate the *in-vitro* antibacterial activity of the crude extracts of *B. micrantha*, and therefore provide preliminary scientific evidence to justify the use of the plant in traditional medicine.

Key words: Medicinal plant, solvent extract, *Bridelia micrantha*, MIC_{50}, bacteria.

INTRODUCTION

Throughout life, humans carry a greater number of cells of our indigenous bacteria than of our own human cells, with the skin, respiratory, and gastrointestinal tracts being portals of entry for the introduction of exogenous organisms into the body. It is well established that bacterial pathogens can survive in air, soil, and water for long periods and their persistence in the environment leads to increased risk of infection in human and animal hosts (Lemunier et al., 2005). Although the vast majority of bacteria are harmless or beneficial, quite a few bacteria are pathogenic, thus causing medically important diseases globally. *Staphylococcus aureus, Shigella sonnei, Salmonella enterica serova Tyhimurium*, and *Helicobacter pylori* are medically important pathogens causing life threatening infections such as pneumonia and meningitis, nosocomial infections (Lowy, 1998; National Nosocomial Infection Surveillance, 2001), various gastric ailments; shigellosis (Kotloff et al., 1999), non-typhoidal salmonellosis (Madigan et al., 2000), chronic gastritis, peptic ulcer, duodenitis (Ahmed et al., 2007), and an important risk factor for the development of gastric adenocarcinoma and mucosal associated lymphoid tissue (MALT) lymphoma (Mbulaiteye et al., 2009). These infections have become more difficult to treat due to the emergence of multidrug resistant strains

*Corresponding author. E-mail: rndip@ufh.ac.za or ndip3@yahoo.com. Tel: +27 782696191. Fax: +27 866224759.

(MRSA, VRSA, and *S. typhimurium* DT104) (Akoachere et al., 2009; Nkwelang et al., 2009) and resistance to conventional antibiotics such as ampicillin, amoxicillin, chloramphenicol, and trimethoprim - sulfamethoxazole (Chiu et al., 2006; Ndip et al., 2008; Tanih et al., 2010); as well as to the quinolones and derivatives of fluoroquinolones (nalidixic acid and ciprofloxacin) have emerged (Hakanen et al., 2006).

According to the World Health Organization (WHO), as many as 80% of the world's population depend on traditional medicine for their primary healthcare needs (Muthu et al., 2006). In sub-Saharan Africa medicinal plants have shown great promise in the treatment of infectious diseases (Ndip et al., 2007).

Bridelia micrantha (Euphorbiaceae) is a semi-deciduous to deciduous tree up to 20 m tall with a dense rounded crown and tall, bare stem. It does well in a wide variety of climates and it is naturally distributed from the Sudan in the north to the Eastern Cape in South Africa where it is known locally as: bruinstinkhout, mitserie (Afrikaans), umHlahla-makwaba (Xhosa), isiHlalamangewibi, umHlahle, umHlalamagwababa, umShonge (Zulu) (Orwa et al., 2009). Virtually all parts of the plant are used traditionally to treat different human ailments including gastritis, salmonellosis, gastro-enteritis, diarrhea, tapeworms and as an emetic for poisons (causes vomiting) (Orwa et al., 2009), joint aches, cough, conjunctivitis, skin problems such as ulcers, boils and rashes, as an antimalarial, for toothache and gum diseases, psychological problems such as neurosis and psychosis (www.blackherbals.com). Extracts of the plant have been reported to be active against *E. coli, K. pneumoniae, S. flexneri,* and *P. aeruginosa* (Samie et al., 2005). It has also been shown to be a possible principle inhibitor to HIV-1 reverse transcriptase (Bessong et al., 2005). However, we are not aware of studies which have investigated *B. micrantha* for its antimicrobial activity against the selected pathogens which constitute potential health problems in our environment, necessitating the current study.

MATERIALS AND METHODS

Bacterial species

Bacterial species used in this study include the following reference strains; *S. aureus* NCTC 6571, *S. tyhimurium* ATCC 13311, *S. sonnei* ATCC 29930, *H. pylori* ATCC 43526, and *H. pylori* 252c (a local drug resistant strain) (Tanih et al., 2010). These strains were selected based on the frequency of infections and antimicrobial resistance they exhibit in the developing world (Gangoue-peiboji et al., 2009; Akoachere et al., 2009; Nkwelang et al., 2009).

Bacterial resuscitation

H. pylori was resuscitated in freshly prepared Brain Heart Infusion (BHI) broth (Oxoid, England) supplemented with Skirrow's supplement (Oxoid, England), and later plated on BHI agar (Oxoid, England) supplemented with 7% horse blood (Oxoid, England), Skirrow's supplement and incubated microaerobically at 37°C for 5 days. Confirmed isolates were suspended in 20% glycerol and stored at -80°C (Ndip et al., 2008), as working stock. The other organisms were resuscitated in BHI broth and later sub-cultured on fresh nutrient agar plates 24 h before use (Adeleye et al., 2008). Pure isolates were then inoculated on fresh nutrient agar slant and kept at 4°C as working stock.

Preparation of plant extracts

The stem bark of *B. micrantha* was selected based on ethnobotanical information and identified in collaboration with botanists at the University of Venda, Limpopo Province, South Africa where voucher specimens (BPO3) have been deposited. The method described by Ndip et al. (2007) was used with modifications. The harvested plant was air dried for 2 weeks and ground to fine powder using a blender (ATO MSE mix, 702732, England). Analytical grade ethyl acetate, acetone, ethanol, methanol (100%), and (40%) methanol hydroxide (Merck) were used for extraction. Briefly, dried plant material (2.5 kg) was macerated in five fold excess of the solvent in extraction bottles with the level of the solvent above that of the plant material. The mixture was placed in a shaker (Edison, N.J., USA) at room temperature (RT) for 48 h and then centrifuged (Model TJ-6 Beckman, USA) at 3000 rpm for 5 min, and later filtered using filter paper of pore size 60^{Å}. This process was repeated twice for a total of three extractions (exhaustive extraction) for each solvent. The combined filtrate was concentrated in a rotavapor (BUCHI R461, Switzerland) and the plant extract obtained transferred to labelled vials and allowed to stand at RT for 24 h to permit evaporation of the residual solvent. Stock solutions were prepared by dissolving 3 g of the extract in Dimethyl Sulphoxide (DMSO) and the remainder kept in the extract bank.

Determination of solvent extraction strength

To determine the extraction efficiency of different solvents, 100 g of fresh plant material was macerated in 300 ml of each extracting solvent, and extraction was done as described previously. The mass of each solvent extract was measured and presented in a bar chart, as mass of extract against solvent (Masoko et al., 2008).

Screening of crude extracts for antimicrobial activity

The agar-well diffusion method was used as previously described (Boyanova et al., 2005; Ndip et al., 2007). In performing this experiment, a total of six solvent extracts were used; the five stated earlier and a dichloromethane extract that already existed in our laboratory. A three day old culture of *H. pylori* was suspended in sterile normal saline and the density adjusted to equal 0.5 McFarland standard.

This was carefully swabbed on fresh BHI agar plates (Oxoid, England) supplemented with 7% laked horse blood (Oxoid, England), and Skirrow's supplement (Oxoid, England). The plates were allowed to dry for 3 to 5 min before use. Thereafter, the crude extracts (dichloromethane, ethyl acetate, acetone, ethanol, methanol and 40% methanol hydroxide) were reconstituted in 10% DMSO to 50, 100, and 200 mg/ml concentrations respectively. Wells were punched with sterile stainless 6 mm cork borer on the plates and 20 µl of the reconstituted extracts applied into each well. The plates were then incubated microaerophically at 37°C for 3 days (Anaerocutt, Baringstoke, England). A similar procedure was used for *S. typhimurium*, *S. aureus,* and *S. sonnei*, but the organisms were plated on Meuller-Hington agar and incubated at 37°C for 24 h. Ten percent DMSO was used as a negative control, because it was established not to be inhibitory to any of the

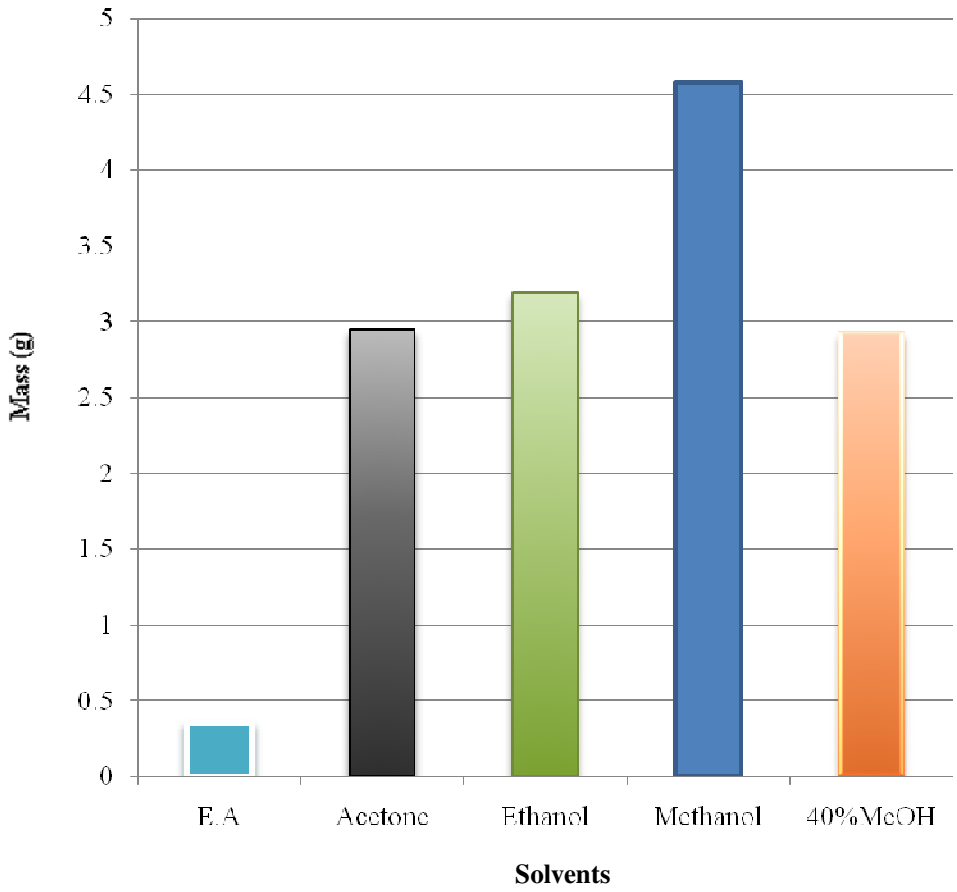

Figure 1. Solvent yield (gram of extract per 100 g of dried plant material).

organisms while ciprofloxacin (2.5 µg/ml) was used as the positive control. The zones of inhibition was measured in millimeters; each experiment was repeated twice and the mean taken.

Determination of MIC_{50}

The MIC_{50} was determined using the 96-well microdilution method as described by Banfi et al. (2003) with modification. Extracts that gave a zone diameter of inhibition ≥ 11 mm (break point for ciprofloxacin) (CLSI, 2008) in all the organisms were used. Two-fold dilutions of the active extracts (Ethyl acetate and acetone) were prepared in the test wells in complete BHI broth, the final extract concentrations being 0.0048 to 10 mg/ml. Each bacterial strain was subcultured in 5 ml of BHI broth for 3 days (*H. pylori*), and 24 h for the other organisms. Twenty microlitres of each bacterial suspension was added to 180 µl of extract-containing culture medium. Control wells were prepared with culture medium and bacterial suspension only.

The plates were sealed and incubated under microaerophilic condition for 3 to 5 days (*H. pylori*), and aerobically for 24 h (*S. sonnei*, *S. aureus*, *S. typhimurium*) at 37°C. After incubation, 20 µl of resazurin solution was added per well, colouring them blue. Plates were then incubated at 37°C for an additional 1 h and observed for colour change from blue to pink in live microorganism-containing wells and then read with a microtitre plate reader (Model 680 Bio-Rad, Japan) adjusted to 620 nm. MIC_{50} was then determined as the lowest extract concentration that inhibited bacterial growth by 50%. Ciprofloxacin was included as a positive control.

Statistical analyses

Statistical analysis was performed using the SPSS statistical package version 17.0 (Illinios USA, 2009). The one way ANOVA test was used to determine if there was any statistically significant difference in the diameter of zones of inhibition of the plant extracts and ciprofloxacin, the MIC of the most active extracts and the control antibiotic (ciprofloxacin). P-values <0.05 were considered significant.

RESULTS

Extract efficiency

The total mass of plant material extracted by the different solvents (ethyl acetate, acetone, ethanol, methanol, and 40% methanol hydroxide) from 100 g of plant material are presented in Figure 1. Methanol was the best solvent in terms of mass extracted (4.58 g) compared to ethanol (3.19 g), acetone (2.95 g), 40% methanol hydroxide (2.93 g), and ethyl acetate (E.A) (0.36 g).

Table 1. Antimicrobial activity (zones of inhibition in mm) of stem-bark extracts of *B. micrantha* and ciprofloxacin.

Crude extract	Zones diameters of inhibition (mm)				
	S. aureus	***S. sonnei***	***S. typhimurium***	***H. pylori 252c***	***H. pylori 43526***
(50 mg/ml)					
Dichloromethane	20	12	0	0	0
Ethyl acetate	20	14	11	12	13
Acetone	19	11	8	0	0
Ethanol	21	0	0	0	0
Methanol	19	0	0	0	0
40% Methanol hydroxide	22	11	0	0	0
(100 mg/ml)					
Dichloromethane	21	12	0	0	0
Ethyl acetate	25	17	12	14	15
Acetone	21	14	8	11	13
Ethanol	23	0	0	0	0
Methanol	21	0	0	0	0
40% Methanol hydroxide	22	13	0	0	0
(200 mg/ml)					
Dichloromethane	22	15	0	0	0
Ethyl acetate	28	21	17	17	18
Acetone	25	16	11	13	15
Ethanol	24	0	0	0	0
Methanol	23	0	0	0	0
40% Methanol hydroxide	23	14	0	0	0
(2.5 µg/ml)					
Ciprofloxacin	38	35	35	29	31

Antibacterial activity

Results of the antimicrobial activity of the crude extracts against the five test strains are summarized in Table 1. An inhibition zone of ≥ 11 mm by the test bacteria was considered susceptible to the tested crude extracts and antibiotic. Generally, all the extracts exhibited antimicrobial activity depending on the concentration of extracts and microorganisms. At 50 mg/ml, the best activity (22 mm) was exhibited by 40% methanol hydroxide against *S. aureus*; while at 100 mg/ml ethanol was very active (23 mm) against *S. aureus*. The ethyl acetate extract demonstrated the best activity of all the extracts (28 mm) at 200 mg/ml against *S. aureus*.

It is important to note that the ethyl acetate extract exhibited a broad spectrum activity, that is, being active against the Gram-positive and all Gram-negative bacteria used in this study at the lowest concentration tested and the pattern of activity observed was such that the zone diameter of inhibition for each organism was directly proportional to the concentration of the crude extract used (that is, concentration dependent inhibition). Although the acetone extract exhibited same spectrum of activity, this could only be achieved at the highest concentration used in the study. The Gram-positive bacteria (*S. aureus*) was the most sensitive while the Gram-negative bacteria (especially *S. typhimurium* and *H. pylori*) were more resistant.

MIC_{50} determination

In determining MIC_{50}, the crude extracts that gave activity (zone of ≥ 11 mm) in all the test organisms at the highest concentration used (200 mg/ml) were considered; hence ethyl acetate and acetone extracts were used. Ciprofloxacin was also included in the experiment as a positive control.

MIC_{50} for the ethyl acetate extract ranged from 0.078 to 0.625 mg/ml, while that of the acetone extract ranged from 0.078 to 1.25 mg/ml (Table 2). It was observed that both extracts had their lowest MIC_{50} value against *S. aureus.* MIC_{50} for ciprofloxacin ranged from 0.0000122 to 0.0078 mg/ml, with *S. typhimurium* being the most

Table 2. Minimum inhibitory concentration (MIC_{50}) of extracts of *B. micrantha* and ciprofloxacin on test organisms.

Test organisms	*B. micrantha* Ethyl acetate (mg/ml)	*B. micrantha* Acetone (mg/ml)	Ciprofloxacin (mg/ml)
S. aureus	0.078	0.078	0.0000977
S. sonnei	0.156	0.312	0.0000977
S. typhimurium	0.625	1.25	0.0000122
H. pylori 252c	0.625	0.625	0.0078
H. pylori 43536	0.312	0.312	0.0048

sensitive and *H. pylori* 252c the least sensitive to the antibiotic. There was no statistically significant difference between the MIC_{50} value of ethyl acetate extract and ciprofloxacin ($P = 0.189$), but there was for acetone and ciprofloxacin ($P = 0.048$).

DISCUSSION

The increase in bacterial resistance to conventional antibiotics has necessitated the search for new and cost effective ways for the control of infectious diseases. Many studies have shown that medicinal plants constitute a great source for the isolation of active antimicrobials (Samie et al., 2005; Bessong et al., 2006). Quinine used in the treatment of malaria, an endemic disease in Africa was isolated from a plant (Cowan, 1999), as well as emetine used in the treatment of amebiasis. The use of medicinal plants in the treatment of diseases is an ancient tradition that has co-existed with human habitation (Bizimenyera et al., 2007).

B. micrantha was selected for this study based on ethnobotanical information, the plant has been widely used ethnomedically (Steenkamp et al., 2009). In South Africa, the stem bark of the plant is used in traditional medicine for gastrointestinal ailments, paralysis, and painful joints (Lin et al., 2002). Steenkamp (2003) reported the use of the bark as an abortifacient. In a study carried out by Bessong et al. (2006), crude methanol extract of the plant was shown to be active against the RNA-dependent DNA polymerization (RDDP) function of HIV-1 reverse transcriptase enzyme.

In this study, 6 solvent extracts of *B. micrantha* were tested for their antibacterial activity against four species of bacteria; *S. aureus* (Gram-positive), and *S. sonnei*, *S. typhimurium* and *H. pylori* (all Gram-negative); after which MIC was done to establish the potency of the most active extract(s).

The majority of traditional healers use water to extract active compounds from this plant, because water is not harmful to humans and is generally cheap and easily acquired. However, successful isolation of compounds from plant material is largely dependent on the type of solvent used in the extraction process (Masoko et al., 2008). Use of water alone leads to difficulties in isolating non-polar active compounds. In this study, methanol was quantitatively the best solvent, extracting a greater quantity (4.58 g) of plant material than any of the other solvents used. The non-polar solvent, ethyl acetate was a poor solvent for extraction for *B. micrantha* as the extract yield was below 1 g as previously reported (Masoko et al., 2008).

Similarly, in many reports on plant extraction, methanol or ethanol are used for alkaloid, sterol, and tannins extraction; ethyl acetate for esters and acetone for flavonoids extraction (Njume et al., 2009). However, phytochemical analysis of *B. micrantha* had revealed that the alcohol (ethanol) extract of the plant is devoid of alkaloid (Adeleye et al., 2008), which seems to be in line with our study. For example, from an interpretation of Figure 1 it could be deduced that the stem bark of *B. micrantha* posses little store of esters but high deposit of tannins, sterol and flavonoids.

From the results of the antimicrobial susceptibility testing, the activity of extracts was more potent on the Gram-positive coccus (*S. aureus*) when compared to the Gram-negative bacilli (*S. sonnei, S. typhimurium, and H. pylori*). This finding had equally been reported (Gangoue-peiboji et al., 2009) and could be explained by the difference in cell wall structures of these bacteria. Gram-negative bacteria possess an outer phospholipidic membrane with structural lipopolysaccharide components which is not found in Gram-positive bacteria. This composition makes the cell wall impermeable to lipophilic solutes, and porins in the cell wall do not allow the penetration of high molecular weight hydrophilic solutes, with an exclusion limit of about 600 Da (Gangoue-peiboji et al., 2009).

The ethyl acetate extract was the most active of all the extracts, showing activity against all the microorganisms tested at the lowest concentration (50 mg/ml) used in the study with a MIC_{50} value that ranged from 0.078 to 0.625 mg/ml. The acetone extract also demonstrated activity but a higher concentration of the extract was required; the MIC_{50} value ranged from 0.078 to 1.25 mg/ml. These results agree with previous studies (Lin et al., 2002) where the methanol extract of *B. micrantha* demonstrated inhibitory and potent anti-diarrhoeic activities against different bacterial species including; *S. typhi, S. enteritidis, S. flexneri,* and *E. coli* (Adeleye et al., 2008).

In this study, the ethyl acetate extract was mostly active against *S. aureus* with inhibition zone diameter of 20 mm (50 mg/ml) and the least MIC_{50} value of 0.078 mg/ml. This compares favourably with the report of Gangoue-pieboji et al. (2009). *S. typhimurium* was the most resistant organism against the acetone extract, followed by *H. pylori* 252c, while both organisms exhibited the same level of resistance (0.625 mg/ml) against the ethyl acetate extract, thus suggesting that it has the same inhibitory effect on both organisms. *S. typhimurium* was the most resistant to the plant extract, this conforming to the findings of Samie et al. (2005) who reported resistance of *Salmonella cholerasuis* a member of the *Salmonella enterica* subspecie to the plant extract. Similarly, the pattern of activity exhibited by the ethyl acetate extract was found to be concentration dependent, which is in concordance with the report of Oboh and Abulu (1997), that antimicrobial activity is a function of the active ingredients reaching an organism.

Conclusion

The finding of this study provides preliminary evidence justifying the traditional use of this plant in folklore medicine. However, a detailed assessment to identify the active principle in the extract is required.

ACKNOWLEDGEMENTS

We are grateful to the National Research Foundation, South Africa (grant reference CSUR2008052900010), and the Govan Mbeki Research and Development Centre, University of Fort Hare, South Africa for funding this study. We are also grateful to Mr. Tshikhawe P for technical assistance.

REFRENCES

Adeleye A, Omonigbehin AE, Stella S, Oluwatosin O, Jumoke S (2008). Antibacterial activity of extracts of *Alchornea cordifolia* (Schum and Thonn) Mull.Arg, *Boerhavia diffusa* (L) and *Bridelia micrantha* (Hoscht) Baill. used in traditional medicine in Nigeria on *Helicobacter pylori* and four diarrhoeagenic bacterial pathogens. Afr. J. Biotechnol., 7(20): 3761-3764.

Ahmed KS, Khan AA, Ahmed I, Tiwari SK, Habeeb A, Ahi JD, Abid Z, Ahmed N, Hahibullah CM (2007). Impact of household hygiene and water sources on the prevalence and transmission of *Helicobacter pylori:* a South Indian perspective. Singapore Med. J., 48(6): 543-549.

Akoachere JF, Tanih NF, Ndip LM, Ndip RN (2009). Phenotypic characterization of *Salmonella Typhimurium* isolates from food animals and abattoir drains in Buea, Cameroon. J. Health Popul. Nutr., 27(5): 612-618.

Banfi E, Scialino G, Monti-Bragadin C (2003). Development of a microdilution method to evaluate *Mycobacterium tuberculosis* drug susceptibility. J. Antimicrob. Chemother., 52: 796-800.

Bessong PO, Rojas LB, Obi LC, Tshisikawe PM, Igunbor EO (2006). Further screening of Venda medicinal plants for activity against HIV type 1 reverse transcriptase and integrase. Afr. J. Biotechnol., 5: 526-528.

Bizimenyera ES, Swan GE, Samdumu FB, Mc Gaw LJ, Eloff JN (2007). Safety profiles of *Peltophorum africanum* Sond (Fabaceae) extracts. South Afr. J. Sci., 7: 66-78.

Boyanova L, Gergova G, Nikolov R, Derejian S, Lazarova E, Katsarov N, Mitov I, Krastev Z (2005). Activity of *Bulgarian propolis* against 94 *Helicobacter pylori* strains *in vitro* by agar-well diffusion, agar dilution and disc diffusion methods. J. Med. Microbiol., 54: 481-483.

Chiu CH, Chuang CH, Chiu S, Su LH, Lin TY (2006). *Salmonella enterica serotype Cholerasius* infection in pediatric patients. Pediatrics, 117(6): 1193-1196.

Clinical Laboratory Standards Institute (2008). Performance standards for antimicrobial sensitivity testing; disc diffusion supplemental tables. M100-S18, Vol. 28. CLSI, Wayne, PA.

Cowan MM (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12(4): 564-82.

Gangoue-pieboji J, Eze N, Djintchui AN, Ngameni B, Tsabang N, Pegnyemb DE, Biyti L, Ngassam P, Koulla-shiro S, Galleni M (2009). The *in-vitro* antimicrobial activity of some traditionally used plants against beta-lactam-resistance bacteria. J. Infect. Dev. Ctries, 3(9): 671-680.

Hakanen AJ, Kotilanen P, Pitkanen S, Hukko S, Siitonen A, Huovinen P (2006). Reduction in fluoroquinolone susceptibility among non typhoidal strains of *Salmonella enterica* isolated from Finish patients. J. Antimicrob. Chemother., 57: 569-572.

Kotloff KL, Winickoff JP, Ivanoff B, Clemens JD, Swerdlow DL, Sansonetti PJ, Adak GK, Levine MM (1999). Global burden of *Shigella* infections: implications for vaccine development and implementation of control strategies. Bull. World Health Organ, 77: 651-666.

Lin J, Puckree T, Mvelase TP (2002). Anti-diarrhoeal evaluation of some medicinal plants used by Zulu traditional healers. J. Ethnopharmacol., 79(1): 123-128.

Lemunier M, Francou C, Rousseaux S, Houot S, Dantigny P, Piveteau P, Guzzo J (2005). Long-term survival of pathogenic and sanitation indicator bacteria in experimental bio waste composts. Appl. Environ Microbiol., 71: 5779-5786.

Lowy FD (1998). *Staphylococcus aureus* infections. N. Engl. J. Med., 339: 520-532.

Madigan MT, Martinko JM, Parker J (2000). Brock's Biology of Microorganisms, (9th edition). e-Book, p. 120.

Masoko P, Mmushi TJ, Mogashoa MM, Mokgotho MP, Mampuru LJ, Howard RL (2008). *In vitro* evaluation of the antifungal activity of *Sclerocarya birrea* extracts against pathogenic yeasts. Afr. J. Biotechnol., 7(20): 3521-3526.

Mbulaiteye SM, Hisada M, El-Omar EM (2009). *Helicobacter pylori* associated global gastric cancer burden. Front Biosci., 14: 1490-504.

National Nosocomial Infections Surveillance (NNIS) (2001). System report, data summary from January 1992–June 2001. Am. J. Infect. Control, 29: 404-421.

Ndip RN, Malange Tarkang AE, Mbullah SM, Luma HN, Malongue A, Ndip LM, Wirmum C, Efange SMN (2007). In *vitro* anti-*Helicobacter pylori* activity of extracts of selected medicinal plants from North West Cameroon. J. Ethnopharmacol. 114(3): 452-457.

Ndip RN, Alertia EMT, Ojongokpoko JEA, Luma HN, Malongue A, Akoachere JFTK, Ndip LM, MacMillan M, Weaver LT (2008). *Helicobacter pylori* isolates recovered from gastric biopsies of patients with gastro-duodenal pathologies in Cameroon: current status of antibiogram. Trop. Med Int. Health, 13 (6): 1-7.

Njume C, Afolayan AJ, Ndip RN (2009). An overview of antimicrobial resistance and future of medicinal plants in the treatment of *Helicobacter pylori* infections. Afr. J. Pharm. Pharmacol., 3(13): 685-699.

Nkwelang G, Jane-Francis T, Akoachere K, Kamga LH, Nfoncham ED, Ndip RN (2009). *Staphylococcus aureus* isolates from clinical and environmental samples in a semi-rural area of Cameroon. Phenotypic characterization of isolates. Afr. J. Microbiol. Res., 3(11): 731-736.

Oboh PA, Abulu EO (1997). The antimicrobial activities of extracts of *Psidium guajava* and *Citrus auratifolia*. Nig. J. Biotechnol., 8: 25-27.

Orwa C, Mutua A, Kindt R, Jamnadass R, Anthony S (2009). Agroforestree Database: a tree reference and selection guide version 4.0 http://www.worldagroforestry.org.

Samie A, Obi CL, Bessong PO, Namrita L (2005). Activity profiles of fourteen selected medicinal plants from rural Venda communities in South Africa against fifteen clinical bacterial species. Afr. J. Biotechnol., 4 (12): 1443-1451.

Steenkamp V (2003). Traditional herbal remedies used by South African women for gynaecological complaints. J. Ethnopharmacol., 86: 97-108.

Steenkamp V, Mokoele TL, Van Rensburg CEJ (2009). Toxicity testing of two medicinal plants, *B. micrantha* and *A. Venosum.* Open Toxicol. J., 3: 35-38.

Tanih NF, Okeleye BI, Clarke AM , Naidoo N , Mkwetshana N, Green E, Ndip LM, Ndip RN (2010). Marked susceptibility of South African *Helicobacter pylori* strains to ciprofloxacin and amoxicillin: clinical implications. South Afr. Med. J., 100: 49-52.