Full Length Research Paper

In vitro antibacterial activities of crude aqueous and ethanolic extracts of the stem bark of *Acacia mearnsii* De Wild

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The antibacterial activities of both water and ethanol extracts of *Acacia mearnsii* de Wild against Gram-positive and Gram-negative bacteria were investigated. The two extracts were very active against the two groups of bacteria. While the minimum inhibitory concentration (MIC) of the ethanol extract ranged between 78.1 and 625 µg/ml, the MIC for aqueous extract ranged between 156.3 and 625 µg/ml. The minimum bactericidal concentrations (MBC) of ethanol extract ranged between 156.3 and 625 µg/ml while that of aqueous extract was between 312.5 and 5000 µg/ml. Water appeared to be a good extraction medium and its extract could be as potent as alcoholic extracts.The MIC_{index} showed that the two extracts were bactericidal at high concentrations and bacteriostatic at low concentrations. The strong bactericidal activities of the two extracts may be attributed to the presence of some secondary metabolites which are soluble in both ethanol and water. The antibacterial activity of the extracts indicated the great therapeutic potential of this plant and has justified its ethnomedicinal use against infectious diseases.

Key words: *Acacia mearnsii*, extracts, antibacterial, bacteriostatic, bactericidal.

INTRODUCTION

Despite the huge advances in modern medicine, most people in the developing world still rely on traditional and effective knowledge to treat illness and disease. The valued trado-medical practices providing affordable healthcare have been recognized by the World Health Organization (WHO). While scientists anticipated that phytochemicals with adequate antibacterial efficacy will be useful for the treatment of bacterial infections (Balandrin et al., 1985), national and international policymakers are calling for partnerships between modern and traditional medicine to bridge the gap in global public health. Consequently, there are rising interests in the search for natural products from plants for the discovery of new antimicrobial agents in the recent times (Nascimento et al., 2000).

In view of the expensive treatment regimen by synthetic drugs already in practice, their gross side effects due to indiscriminate use (Sharif, 2001; Tomoko et al., 2002) as well as increasing trend in the emergence of resistance to antimicrobial agents resulting from poor quality drugs manufactured, patient non-compliance, and spontaneous mutations within the microbial populations (Nester et al., 2002; Denyer et al., 2004), there is a constant need to search for new and effective antimicrobial agents (Ahmad et al., 1998; Bhavnani and Ballow, 2000). In search of remedies for human ailments from the environment, particularly from natural resources (Habeeb et al., 2007), the plants turn out to be a significant source of therapeutics (Potier et al., 1990) and many potent and powerful drugs (Srivastava et al., 1996) used medicinally in different countries. In many developing countries, about 80% of available drugs come from medicinal plants. While progresses in antimicrobial drugs have introduced many antibiotics most of which are nontoxic but having side effects (Shafiei and Ghanbarpour, 1992), plants have provided a good source of anti-infective agents and many of them remain highly effective in the

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fight against microbial infections. Apart from being cost-effective, they have fewer side effects (Samsam and Moatar, 1991). Hence, ethnopharmacology and traditional knowledge-inspired approaches have become useful in drug discovery and development (Patwardhan, 2005; Cooper, 2008) while traditional medicine-inspired approaches remain important in the management of chronic diseases and discovery of drugs of natural origin (Patwardhan et al., 2004; Patwardhan and Mashelkar, 2009) as a result of their long history of use and better safety profiles than synthetic drugs.

Acacia mearnsii de Wild (Fabaceae) is a fast-growing leguminous tree. The genus *Acacia* is a cosmopolitan taxon containing more than 1350 species (Seigler, 2003). In Australia, there are approximately 960 species, which makes *Acacia* the largest genus of vascular plants in that region (Maslin, 2001). The species of Acacia was introduced to South Africa about 150 years ago primarily for the tanning industry (Sherry, 1971). They are of immense value for the reforestation and reclamation of wastelands (Skolmen, 1986). They are used for fuel wood, timber and shelter (Palmberg, 1981). While the bark of *A. mearnsii* is known to contain about 20 to 40% tannins and 70% proanthocyanidins (Young et al., 1986), ethanolic extract of *A. mearnsii* had the highest total flavonoid contents with aqueous extracts having the least of the phytochemicals while the total phenolic content correlated well with the antioxidant activity of the extracts (Olajuyigbe and Afolayan, 2011 accepted). Although *A. mearnsii* is widespread, relatively little is known about its pharmacological potentials. This may be due to the difficulty associated with the identification of *Acacia* species and the insufficient clarity about their taxonomic relationships (Seigler, 2003). There is lack of scientific studies on the pharmacological importance of this plant especially antimicrobial study. Though ethanol and water are the solvents traditionally used in herbal preparations by soaking or being allowed to simmer, this study is aimed at evaluating the antibacterial activities of both aqueous and ethanol extracts of this plant.

MATERIALS AND METHODS

Plant collection and identification

The bark materials of *A. mearnsii* were collected from the plant growing within the University of Fort Hare campus in Alice, South Africa. The plant was authenticated in the Department of Botany and a voucher specimen was prepared and deposited in the Griffen Herbarium of the University.

Extract preparation

The bark samples were air-dried at room temperature and pulverized with a milling machine. About 100 g of the pulverized sample was extracted with 500 ml of ethanol for 48 h. The extract was filtered with Whatman No. 1 filter paper and evaporated to dryness under reduced pressure at a maximum temperature of

40°C using a rotary evaporator. The water extract was prepared by soaking 100 g of the pulverized sample in 500 ml of sterile de-ionized distilled water. The mixture was brought to boil in a water bath for 30 min and allowed to stand for 24 h on rotary shaker. The mixture was filtered through Whatman No. 1 filter paper. The filtrate of water extract obtained was quickly frozen at -40°C and dried for 48 h using a freeze dryer (Savant Refrigerated vapor Trap, RV T41404, USA).The dried crude aqueous and ethanol extracts were redissolved in their respective solvents to the required concentrations for the bioassay analyses.

The reconstituted extract solutions were sterilized by filtering through 0.45 µm membrane filter. They were tested for sterility after filtration by introducing 2 ml of each extract into 10 ml of sterile nutrient broth and incubated at 37°C for 24 h. A sterile extract was indicated by the absence of turbidity of the broth after the incubation period (Ronald, 1995).

Test organisms

Organisms used in this study included seven Gram positive and eight Gram negative bacteria. The Gram positive strains included three American Type Culture Collection [*Staphylococcus aureus* (ATCC 6538), *Streptococcus faecalis* (ATCC 29212) and *Bacillus cereus* (ATCC 10702)], two environmental isolates [*Bacillus subtilits* (KZN) and *Micrococcus luteus*] as well as two clinical isolates [*S. aureus* (OK1) and *S. aureus* (OK2a)]. The Gram negative strains were five American Type Culture Collection [*Pseudomonas aeruginosa* (ATCC 19582), *Shigigella sonnei* (ATCC 29930), *Salmonella typhi* (ATCC 13311), *Escherichia coli* (ATCC 25922) and *Enterobacter cloacae* (ATCC 13047)] and three environmental isolates [*Klebsiella pneumonia* (KZN), *Proteus vulgaris* (KZN), *Shigella flexneri* (KZN)]. All the strains were obtained from the Department of Biochemistry and Microbiology, University of Fort Hare, Alice, South Africa. The organisms were maintained in nutrient broth and nutrient agar (Biolab) slant incubated at 37°C for 24 h.

Determination of the minimum inhibitory concentration (MIC)

The activities of the extracts and their minimum inhibitory concentrations were determined by the macrobroth dilution methods (Jones et al., 1985; NCCLS, 1993). The inoculum of each test strain was standardized at 5×10^6 cfu/ml using McFarland Nephe-lometer standard. The extracts were serially diluted with nutrient broth to give final concentrations of 19.53, 39.06, 78.13, 156.25, 312.50, 625.00, 1250.00, 2500.00, 5000.00 and 10,000 µg/ml. The tubes were inoculated with 100 µl of each bacterial suspension. Blank nutrient broth was used as negative control while different concentrations of 0.01, 0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 1.28, 2.56 and 5.2 µg/ml ciprofloxacin prepared by serial dilution was used as positive controls. The tubes were incubated aerobically at 37°C for 24 h. The MIC was the lowest concentration of the extract that yielded no visible growth after the incubation (Sung et al., 2006).

Determination of minimum bactericidal concentration (MBC)

The minimum bactericidal concentration (MBC) was determined according to the method of Doughari (2006). Sterile nutrient agar plates were inoculated with a loopful of culture taken from each of the first three test tubes that showed no growth in the MIC tubes. After incubation for 24 h at 37°C, the least concentration of the extracts that showed no colony formation on the agar was taken as the MBC.

Table 1. Antibacterial activities of ethanol and aqueous extracts of *A. mearnsii.*

Listofbacteria used	Ciprofloxacin			Ethanol extract			Water extract		
	MIC (µg/ml)	MBC (µg/ml)	MIC index	MIC (µg/ml)	MBC (µg/ml)	MIC index	MIC (µg/ml)	MBC (µg/ml)	MIC index
Pseudomonasaeruginosa(ATCC 19582)	0.08	0.16	2	156.25	312.50	2	312.50	312.50	1
Shigellasonnei(ATCC 29930)	0.16	0.32	2	156.25	312.50	2	312.50	625.00	2
Salmonellatyphi(ATCC 13311)	0.02	0.08	2	156.25	156.25	1	312.50	312.50	1
Escherichia coli(ATCC 25922)	0.02	0.02	1	312.50	312.50	1	625.00	2500.00	4
Enterobactercloacae(ATCC 13047)	0.08	0.32	4	156.25	156.25	1	625.00	1250.00	2
Klebsiellapneumoniae(KZN)	0.02	0.04	2	156.25	312.50	2	312.50	625.00	2
Proteusvulgaris(KZN)	0.32	0.64	2	312.50	625.00	2	625.00	2500.00	4
Shigellaflexneri(KZN)	0.16	0.32	2	78.13	312.50	4	156.25	1250.00	8
Serratiamercescens(ATCC 9986)	0.04	0.08	2	625.00	625.00	1	625.00	5000.00	8
Klebsiellapneumonia(ATCC 4352)	0.16	0.32	2	156.25	312.50	2	625.00	2500.00	4
Staphylococcus aureus(ATCC 6538)	0.32	0.64	2	312.50	312.50	1	625.00	2500.00	4
Streptococcusfaecalis(ATCC 29212)	0.64	2.56	4	156.25	312.50	2	625.00	2500.00	4
Bacilluscereus(ATCC 10702)	0.08	0.08	1	156.25	312.50	2	312.50	312.50	1
Bacillussubtilits(KZN)	0.08	0.08	1	312.50	312.50	1	625.00	2500.00	4
Staphylococcus aureus(OK2a)	0.64	0.64	1	156.25	312.50	2	312.50	312.50	1
Micrococcusluteus	0.16	0.32	2	78.13	156.25	2	625.00	1250.00	2
Staphylococcus aureus(OK1)	0.04	0.04	1	78.13	156.25	2	312.50	1250.00	1

**Determination of mechanisms of antibiosis (bactericidal or bacteriostatic)**

The mechanism of antibiosis of the extracts was calculated using the ratio of MBC/MIC as described by Shanmughapriya et al. (2008) to elucidate whether the observed antibacterial effects were bactericidal or bacteriostatic. When the ratio of MBC/MIC was less than or equal to 1.0, the extract was considered bactericidal. However, when the ratio of MBC/MIC was equal to or greater than 2.0, the extract was considered bacteriostatic. If the ratio was equal to or greater than 16.0, the extract was considered ineffective.

RESULTS AND DISCUSSION

The two extracts of *A. mearnsii* were active against both Gram-negative and Gram-positive bacteria (Table 1). The antibacterial activities of ethnol extract against the different bacterial strains are presented in Figure 1 while those of water extract are as indicated in Figure 2. From Figure 1, *S. flexneri* (KZN), *M. luteus* and *S. aureus* (OK1) had the least minimum inhibitory concentrations (78.13 µg/ml) and *S. mercescens* (ATCC 9986) had the highest minimum inhibitory concentration (625 µg/ml). With the exception of *S. flexneri* (KZN) for which the minimum bactericial concentration (MBC) was four times higher than the MIC values, the MBC values of other bacteria were either similar or two folds higher than the MIC values. From Figure 2, *S. flexneri* (KZN) was the most susceptible of all the

bacteria to water extract of *A. mearnsii*. The organism had the least MIC value (156.25 µg/ml) while other bacteria had MIC values ranging between two and four folds higher. With the exception of *P. vulgaris* (KZN) and *S. mercescens* (ATCC, 9986), the MBC values of all the bacteria were similar or two to four folds higher than the MIC values. Comparatively, Figure 3 showed that the two extracts were not as effective as ciprofloxacin used as control. The MIC and MBC values of the ethanol extract were lower than those of water extract. While the two extracts were active at very low concentrations and exerted a high degree of inhibitory effect on the organisms; ethanol extract had better antibacterial effects against all the organisms than the water
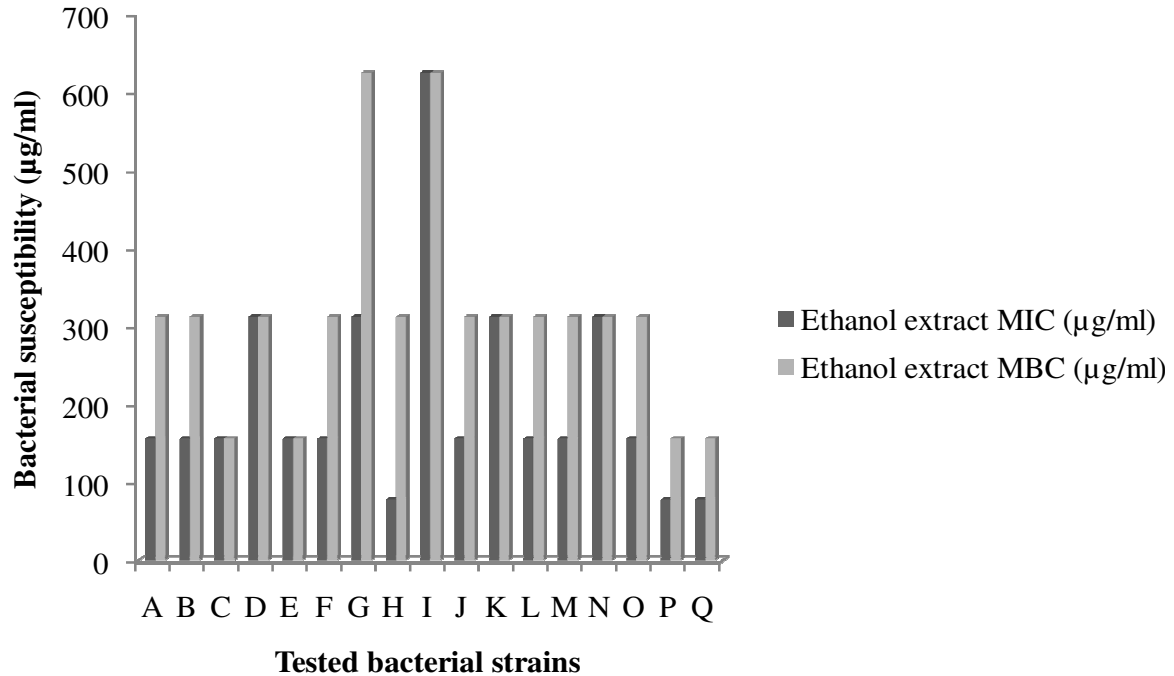
Figure 1. Antibacterial activity of ethanol extract of *A. mearnsii* against tested bacterial strains. A, *P. aeruginosa* (ATCC 19582); B, *S. sonnei* (ATCC 29930); C = *S. typhi* (ATCC 13311); D, *Escherichia coli* (ATCC 25922); E, *Enterobacter cloacae* (ATCC 13047); F, *K. pneumoniae* (KZN); G, *P. vulgaris* (KZN); H, *S. flexneri* (KZN); I, *S. mercescens* (ATCC 9986); J, *K. pneumonia* (ATCC 4352);   K, *S. aureus* (ATCC 6538); L, *S. faecalis* (ATCC 29212); M, *B. cereus* (ATCC 10702); N, *B. subtilits* (KZN); O, *S. aureus* (OK2a); P = *M. luteus;* Q = *S. aureus*(OK1).
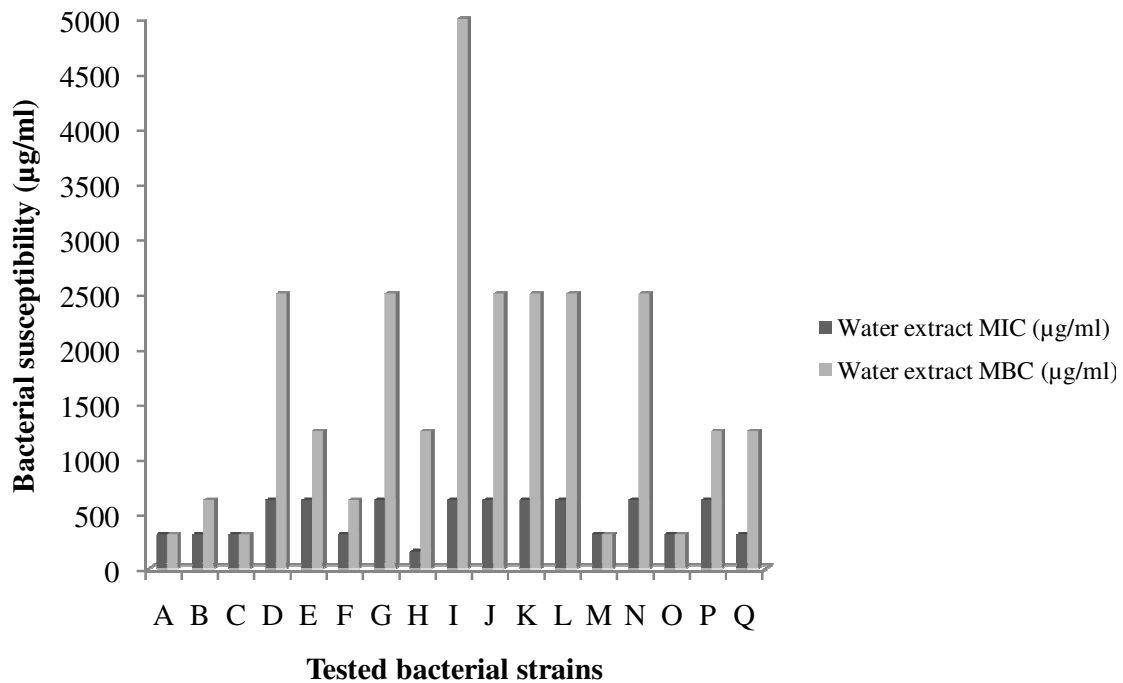
Figure 2. Antibacterial activity of water extract of *A. mearnsii* against tested bacterial strains. A, *P. aeruginosa* (ATCC 19582); B, *S. sonnei* (ATCC 29930); C = *S. typhi* (ATCC 13311); D, *Escherichia coli* (ATCC 25922); E, *Enterobacter cloacae* (ATCC 13047); F, *K. pneumoniae* (KZN); G, *P. vulgaris* (KZN); H, *S. flexneri* (KZN); I, *S. mercescens* (ATCC 9986); J, *K. pneumonia* (ATCC 4352);   K, *S. aureus* (ATCC 6538); L, *S. faecalis* (ATCC 29212); M, *B. cereus* (ATCC 10702); N, *B. subtilits* (KZN); O, *S. aureus* (OK2a); P = *M. luteus;* Q = *S. aureus*(OK1).
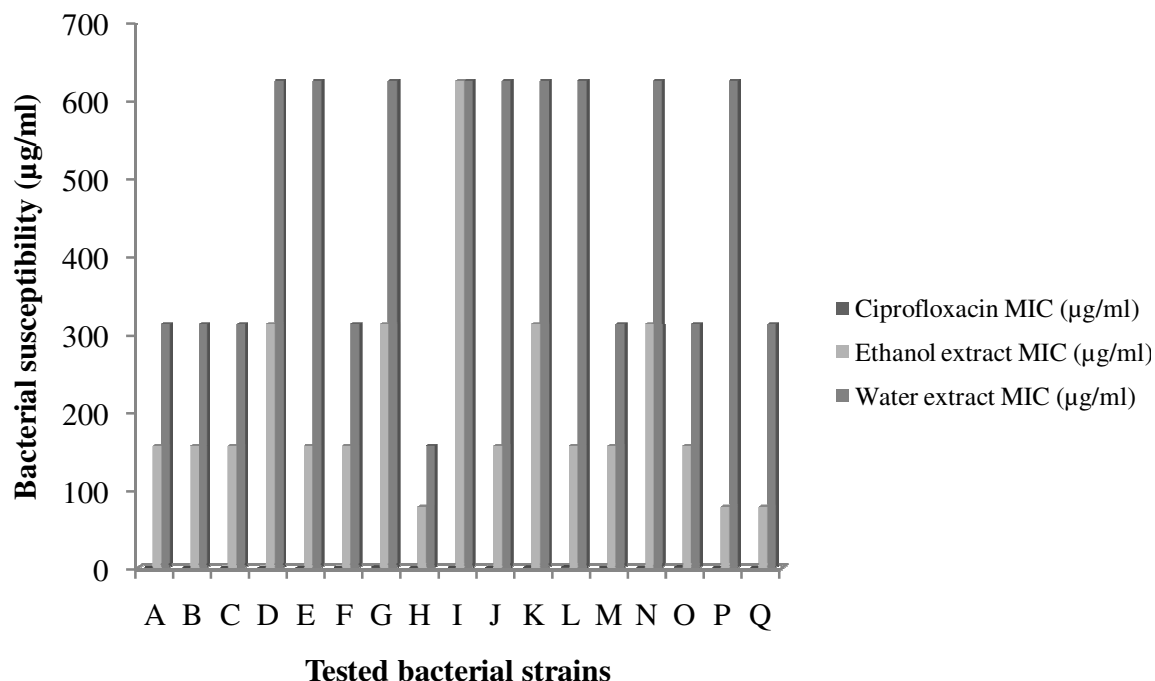
Figure 3. Comparative antibacterial effects of ciprofloxacin, ethanol and water extracts of *A. mearnsii* on tested bacterial strains. A, *P. aeruginosa* (ATCC 19582); B, *S. sonnei* (ATCC 29930); C = *S. typhi* (ATCC 13311); D, *Escherichia coli* (ATCC 25922); E, *Enterobacter cloacae* (ATCC 13047); F, *K. pneumoniae* (KZN); G, *P. vulgaris* (KZN); H, *S. flexneri* (KZN); I, *S. mercescens* (ATCC 9986); J, *K. pneumonia* (ATCC 4352);   K, *S. aureus* (ATCC 6538); L, *S. faecalis* (ATCC 29212); M, *B. cereus* (ATCC 10702); N, *B. subtilits* (KZN); O, *S. aureus* (OK2a); P = *M. luteus;* Q = *S. aureus*(OK1).

extract mainly due to the better solubility of the active compounds in the organic solvents. Though MIC_{index} greater than 1 was considered as bacteriostatic, none of the MIC_{index} was equal to or greater than16.0. Hence, the extracts were considered effective against the organisms.

Many pharmacologically bioactive compounds such as alkaloids, flavanoids, tannins, anthraquinones and phenolic compounds have been implicated in the antibacterial activities of many plants (Hostettman et al., 1995; Edeoga et al., 2005; Nawrot et al., 2007). The antibacterial activities of both extracts of *A. mearnsii* may be attributed to the presence of the pharmacologically important compounds such as flavonoids which are soluble in ethanol and water. It may also be due to the presence of broad spectrum antibacterial compounds or metabolic toxins in addition to other pharmacologically active compounds in the plant. While the antibacterial activity of the extracts depended on the plant chemotype, extract preparation, solvent used and the sensitivity of the bacteria (Loziene et al., 2007), the variation of the MIC values among the isolates depended on the presence of intrinsic levels of tolerance to antimicrobials in the tested microorganisms (Ahmad and Aqil, 2007).

The antibacterial activities of ethanol and water extracts had earlier been reported (Farrukh et al., 2008; Sharma et al., 2010). In this study, ethanol extract showed greater antibacterial activity as compared to water extract. The

stronger extraction capacity of ethanol could have produced greater active constituents responsible for the antimicrobial activity. This is similar to the general reports of many workers (Aliero and Afolayan, 2006; Annamalai et al., 2007; Parekh and Chanda, 2007; Khond et al., 2009). However, contrary to previous reports indicating that water extract could be ineffective or have low antibacterial activity against microbial agents (Paz *et al.,* 1995; Vlietinck et al., 1995; Jayaraman et al., 2008), water extract of *A. mearnsii* exhibited a significant antibacterial activity against all bacteria tested.

Although many workers have reported that water is a poor extractor of antibacterial compounds from plant (Ibekwe et al., 2001; Karaman et al., 2003), this study has indicated that water may be a good extraction medium and its extract may be as potent as alcoholic extract. This potency may, however, be due to the presence of anionic components such as thiocyanate, nitrate, chloride and sulphates along with other water soluble antibacterial compounds present in the plant material (Darout et al., 2000). With the exception of *S. mercescens* and *S. flexneri* where the MIC_{index} equaled 8, the inhibitory effect of the water extract was significant. The ability of the water and ethanol extracts to inhibit the organisms at relatively low concentrations has justified the use of *A. mearnsii* by the rural communities in the treatment of some infections.

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