

Full Length Research Paper

Antibacterial activity of the stem bark extracts of *Acacia mearnsii* De Wild

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Antibacterial activity of four different extracts from the stem bark of *Acacia mearnsii* was measured against five Gram-positive and five Gram-negative bacterial strains: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Micrococcus kristinae*, *Streptococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Klebsiella pneumonia* and *Serratia marcescens*. The extracts assessed include hexane, ethyl acetate, dichloromethane and methanol. The hexane, ethyl acetate and methanol extracts showed some inhibitory effects on the selected bacteria. The hexane extract showed some activity against four Gram-positive and two Gram-negative bacterial strains, but was not active against one Gram-positive and three Gram-negative bacterial strains. The ethyl acetate extract was effective against all the bacterial strains used in this study. The methanolic extract was effective against all the Gram-positive bacterial strains but it was not active against the Gram-negative bacterial strains except *Escherichia coli*. The dichloromethane extract was not active at all against all the bacterial strains tested.

Key words: *Acacia mearnsii*, antibacterial activity, bacteria species.

INTRODUCTION

Globally, the attractions that have led to the study of medicinal plants as a basis of pharmacologically active compounds have improved and have become greater than ever before. In some developing countries, it is acknowledged that plants are the main medicinal sources to treat infectious diseases. The human environment in these countries is crowded while sanitation is poor; therefore diseases like diarrhoea and dysentery which are caused by bacterial enteropathogens are among the core causes of morbidity and death (Alanis et al., 2005). Plants still make an input that is very important to health care, even though there is great progress in modern

medicine. The reason for this is the growing appreciation of the value of traditional medical systems, mainly of Asian origin, and the recognition of medicinal plants from the native pharmacopoeias, which have important healing power (Adebolu and Oladimeji, 2005).

A lot of work has been done on antimicrobial and phytochemical constituents of medicinal plants and utilizing them for the management of microbial infections for both topical and systemic applications as likely alternatives to formally approved chemically artificial drugs to which many infectious microorganisms have become resistant (Akinpelu and Onakoya 2006). National

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and international policymakers are calling for associations linking modern and traditional remedies to bridge the gap in global public health.

Scientists on the other hand predicted that phytochemicals with sufficient antibacterial efficacy will be helpful for bacterial infections. As a result of this, there is an increase in the hunt for natural products from plants as the latest antimicrobial representatives in current times (Olajuyigbe and Afolayan, 2011).

While there is rapid growth in the arsenal of agents available to treat bacterial infections, the rise in the number of antibiotic resistant bacteria is growing faster and therefore remains beyond reach. Information from literature and accounts from ethnobotany suggest that plants are the sleeping giants of the pharmaceutical industry (Farnsworth and Morris, 1976). This is suggested because mainly through secondary metabolites, higher plants are thought to provide the natural basis of antimicrobial drugs that will produce lead compounds that may be engaged in controlling some infections worldwide (Akinpelu and Onakoya, 2006).

Fabaceae is regarded as the second largest family of medicinal plants, with over four hundred and ninety medicinal plant species of which the majority have been used as traditional medicine. Thirty one species of medicinal plants that belong to twenty genera are found in this family and these are explained in the Chinese Pharmacopeia, and a number of species are included in the Japanese Pharmacopoeia. Afterwards, it was found that these plant species have important medicinal properties, they have been used widely as pharmaceutical products (Gao et al., 2010). *Acacia*, a member of the family *Fabaceae* is a large genus with nine hundred species; about seven hundred of the species are indigenous to Australia. Other species of the same genus occur mainly in tropical and subtropical regions of Africa, Asia and America (Ahmad et al., 2011). *Acacia mearnsii* de Wild is a fast-rising leguminous tree, which is native to South Eastern Australia and was introduced to South Africa hundred and fifty years ago, primarily for the tanning industry (Fatunbi et al., 2009).

Given the effects of bacterial infestation (Faulde et al., 2001), colonization (Goldmann et al., 1978) and resistance to existing antibacterial agents (Lenski, 1998), and the risks they pose in clinics, hospitals and hospices (Frieden, 2013), we measured the antibacterial activity of the extracts of the stem bark of *Acacia mearnsii* De Wild, against bacterial species of nosocomial origin, all of which are common isolates from local hospitals.

Study area

OR Tambo District Municipality (ORTDM) is found in the Eastern Cape Province, where it occupies the eastern coastal portion of the province. This District Municipality is bordered by KwaZulu-Natal and by the Eastern Cape districts of Amatole, Chris Hani, UKhahlamba and Alfred

Nzo. The district covers 15,946.84 km² and includes seven local municipalities. It has a diversity of vegetation, from grasslands and thicket to forests and bushveld. ORTDM is believed to have the richest natural resources and the most fertile areas in the country, with good soils and climatic conditions (McCann, 2005). The inhabitants of this district municipality are about 1.7 million. About 93% of the inhabitants dwell in rural areas, whereas approximately 77% of the population is without jobs. The local language of the majority of the dwellers is isiXhosa, a Nguni language, whereas the rest of the people speak Afrikaans and English. The larger part of the region is rural with a large area of arable land. On the other hand, agriculture in the ORTDM is poorly developed and mainly subsistence (Bisi-Johnson et al., 2010).

MATERIALS AND METHODS

Hexane, ethyl acetate, methanol and dichloromethane were obtained from Sigma-Aldrich as analytical reagents. The orbital shaker used was an MRC with twelve positions for 250 ml erlenmeyer flasks. The Buchner funnel was a Corning and the filter paper was a Whatman No. 1. The rotary evaporator was a Buchi R-215 fitted with a vertical water cooled condenser. Nutrient agar was purchased from Arcos. The autoclave was an Optima B class from Prestige Medical. The water was triple distilled and passed through a deionising column. The ten bacterial strains were obtained from the National Health Laboratory Services. All nutrient media were from Arcos.

Plant collection

In a parallel study, the five plants that were most frequently mentioned and highly recommended by herbalists, traditional healers and rural dwellers were found to be *Acacia mearnsii*, *Psidium guajava*, *Teucrium kraussii*, *Strychnos henningsii* and *Xysmalobium undulatum*. This parallel study consisted of a questionnaire survey through which the data were collected on the plants that are traditionally used for the treatment of ailments of the gastro intestinal tract. The data collected included the names of the plants, the location of collection (study area), the plant parts used, the method of preparation, the method of administration, and the amounts used. In this parallel study, *A. mearnsii*, the plant species selected for the present study was reported to be the most commonly used by most traditional healers and other community members in the study area. The study also showed that the dried stem bark was the most frequently used part of the plant. The material is ground and boiled in water and the mixture may be stored for a week in a closed glass container at room temperature before use.

Acacia mearnsii was collected from Lusikisiki, in the month of July, 2011. A voucher specimen was prepared after identification and deposited at the Kei Herbarium at the Nelson Mandela Drive delivery site, Mthatha campus, Walter Sisulu University. The bark was stripped off the stem and dried in open air in the dark for at least a week, before grinding using a mortar and pestle. The ground material (2 kg) was placed in a closed glass jar and stored in the cold room at -5°C.

Extraction of plant material

Ground plant material (400 g) was sequentially extracted with 1 L of

Table 1. Test organisms used in the study; five Gram positive and five Gram negative bacteria.

Test organism	Gram strain	Diseases caused	Reference
<i>Staphylococcus aureus</i>	Positive	Atopic dermatitis, ritter' disease, endocarditis	Khalid et al., 2011
<i>Staphylococcus epidermidis</i>	Positive	Nosocomial sepsis Intravascular catheter-associated infection	Vuong et al., 2003
<i>Bacillus cereus</i>	Positive	Diarrhoeal syndrome and emetic syndrome	Rupp et al., 2001
<i>Micrococcus kristinae</i>	Positive	Catheter-related recurrent bacteremia	Granum and Lund, 1997
<i>Streptococcus faecalis</i>	Positive	Urinary tract infections	Basaglia et al., 2002
<i>Escherichia coli</i>	Negative	Involved in infections of the intestinal and urinary tracts of Humans	Kau et al., 2005
<i>Pseudomonas aeruginosa</i>	Negative	Wound infection in burnt patients	Darfeuille-Michaud and Colombel, 2008
<i>Shigella flexneri</i>	Negative	Bacillary dysentery	Khalid et al., 2011
<i>Klebsiella pneumonia</i>	Negative	Liver abscess	Sansonetti et al., 2000
<i>Serratia marcescens</i>	Negative	Nosocomial infections	Fung et al., 2011
			Sartor et al., 2000

Table 2. Test organisms used in the present study and in Olajuyigbe and Afolayan (2011).

Used only in the Present Study	Common to both present study and to Olajuyigbe and Afolayan (2011)	Used only in Olajuyigbe and Afolayan (2011)
<i>Staphylococcus epidermidis</i> (+)	<i>Staphylococcus aureus</i> (+)	<i>Bacillus subtilis</i> (+)
<i>Micrococcus kristinae</i> (+)	<i>Streptococcus faecalis</i> (+)	<i>Micrococcus luteus</i> (+)
<i>Serratia marcescens</i> (-)	<i>Bacillus cereus</i> (+)	<i>Shigella sonnei</i> (-)
	<i>Pseudomonas aeruginosa</i> (-)	<i>Salmonella typhi</i> (-)
	<i>Escherichia coli</i> (-)	<i>Enterobacter cloacae</i> (-)
	<i>Klebsiella pneumonia</i> (-)	<i>Proteus vulgaris</i> (-)
	<i>Shigella flexneri</i> (-)	

hexane, ethyl acetate, dichloromethane and methanol. All the extracts were filtered under vacuum through Whatman No. 1 filter paper. The solvent extracts were concentrated using a rotary evaporator under reduced pressure and temperature (Fawole et al., 2009). Each dry extract was later re-dissolved in its respective solvent, 0.93 g dichloromethane, 1.55 g ethyl acetate, 1.68 g methanol and 1.98 g hexane.

Test organisms

Five Gram-positive and five Gram-negative bacterial strains were used in this study (Table 1). The Gram-positive bacterial strains included *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Micrococcus kristinae* and *Streptococcus faecalis*. The Gram-negative bacterial strains included *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Klebsiella pneumonia* and *Serratia marcescens*. All the bacterial strains were obtained from the National Health Laboratory Services (NHLS).

The antibacterial activities of crude aqueous and ethanolic extracts of the stem bark of *A. mearsii* De Wild against Gram-positive and Gram-negative bacteria have been reported by Olajuyigbe and Afolayan (2011). The Gram-positive bacterial strains

included *S. aureus*, *S. faecalis*, *B. cereus*, *Bacillus subtilis* and *Micrococcus luteus*. The Gram-negative strains were *Pseudomonas aeruginosa*, *Shigella sonnei*, *Salmonella typhi*, *E. coli*, *Enterobacter cloacae*, *K. pneumonia*, *Proteus vulgaris*, and *Shigella flexneri*. Commonality with the present study includes seven test organisms: *S. aureus*, *S. faecalis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *E. coli*, *K. pneumonia* and *S. flexneri*. However, unique to the present study are three test organisms: *S. epidermidis*, *Micrococcus kristinae* and *Serratia marcescens*, while unique to the study by Olajuyigbe and Afolayan (2011) are six test organisms: *B. subtilis*, *M. luteus*, *S. sonnei*, *S. typhi*, *Enterobacter cloacae* and *Proteus vulgaris*. The comparison of the present study with that reported by Olajuyigbe and Afolayan (2011) in terms of the test organisms used is summarised in Table 2.

Antibacterial testing

The antibacterial activity was evaluated using the dilution-in-agar technique (Alanis et al., 2005). The bacterial species that were used were sub-cultured and preserved on nutrient agar that was contained in Petri dishes (Nkomo and Kambizi, 2009). Nutrient agar (60 ml) was prepared and sterilized using an autoclave at 121°C for

Table 3. Antibacterial activity of *A. mearnsii*.

Bacteria species	Gram +/-	Minimum inhibitory concentration(MIC) (mg/ml)			
		Hexane	Ethyl acetate	Dichloromethane	Methanol
<i>Staphylococcus aureus</i>	+	10	1	na	1
<i>Staphylococcus epidermidis</i>	+	10	5	na	5
<i>Bacillus cereus</i>	+	10	5	na	5
<i>Micrococcus kristinae</i>	+	10	10	na	1
<i>Streptococcus faecalis</i>	+	Na	10	na	5
<i>Escherichia coli</i>	-	10	10	na	5
<i>Pseudomonas aeruginosa</i>	-	10	10	na	na
<i>Shigella flexneri</i>	-	na	10	na	na
<i>Klebsiella pneumonia</i>	-	na	10	na	na
<i>Serratia marcescens</i>	-	na	10	na	na

Minimum inhibitory concentration (mg/ml); na: not active.

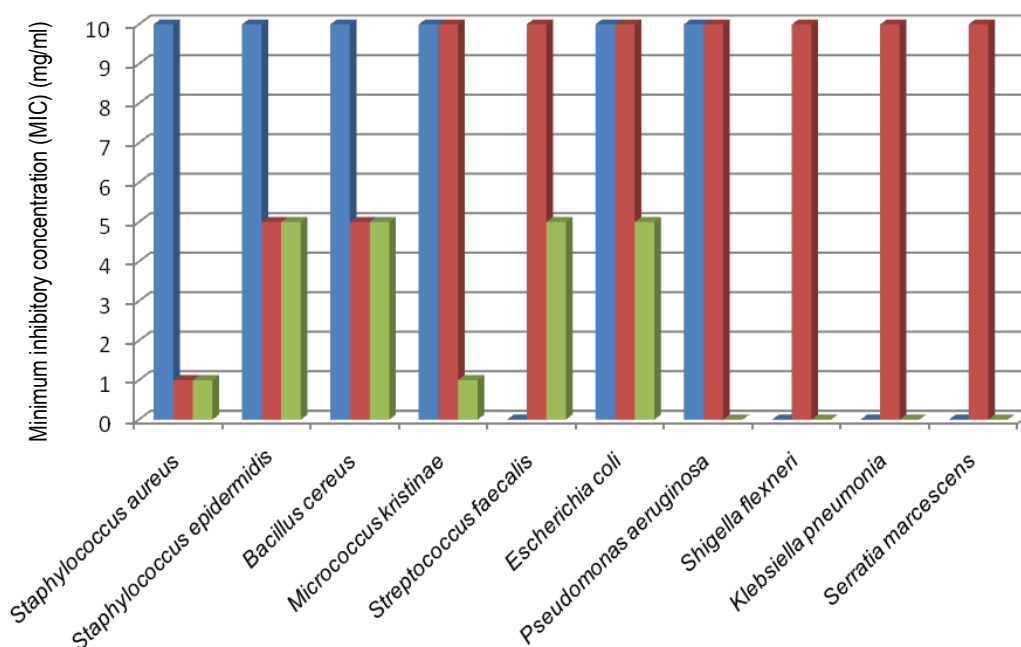


Figure 1. Antibacterial activity of the hexane (■), ethyl acetate (■) and methanol (■) extracts of the stem bark of *A. mearnsii* against ten test organisms.

15 min. The agar was distributed into 12 test tubes, after which, nine test tubes were mixed with the extract, ending up with final concentrations of 1.0, 5.0, and 10.0 mg of extract per ml of nutrient agar (Kambizi and Afolayan, 2008).

The relevant solvent was added to the remaining three test tubes containing nutrient agar and were used as controls (Nkomo and Kambizi, 2009). The control experiments that were set up consisted of inoculums without the plant extract (Banso, 2009). After this, the nutrient agar-extract and nutrient agar-solvent mixture were then poured into Petri dishes, allowed to cool and set. The test organism contained in sterilized nutrient broth was streaked in radial array on the agar plates and the plates were incubated at 37°C for 24 - 48 h; thus each treatment was replicated three times.

The concentrations of the extracts that were tested against the organisms, for suppression of growth were observed with the naked eye (Nkomo and Kambizi, 2009). The lowest concentration that

inhibited the growth of bacteria that can be visible with a naked eye was used as the minimum inhibitory concentration (MIC) (Alanis et al., 2005).

RESULTS

The results of the antibacterial activity are shown in Table 3 and are also illustrated in Figure 1. The MIC values of the dichloromethane extract are not shown in Figure 1, since the extract was not active against any of the bacterial strains. The hexane extract of the bark of *A. mearnsii* showed potent antibacterial activity against *E. coli* with MIC value of 10.0 mg/ml and the results were

almost similar to those of hexane extracts of the bark of *Ficus congensis* reported by Alaribe et al. (2011) with MIC values of 8.0 mg/ml.

The extract was not active against *K. pneumonia*. According to Alaribe et al. (2011) the same bacterial species was not inhibited by the extract. In this study, the hexane extract showed activity against four Gram-positive bacterial strains with MIC value of 10.0 mg/ml but was not active against *S. faecalis*. This extract also showed some activity against two Gram-negative bacterial strains, *E. coli* and *P. aeruginosa*, but was not active against the remaining three Gram-negative bacterial strains, *S. flexneri*, *K. pneumonia* and *S. marcescens*.

The ethyl acetate extract was effective against all bacterial strains used in this study with MIC values ranging from 1.0 and to 10.0 mg/ml. The methanolic extract was effective against all Gram-positive bacterial strains with MIC values of 1.0 and 5.0 mg/ml but it was not active against the Gram-negative bacterial strains except for *E. coli* which was susceptible at MIC value of 5.0 mg/ml. The extract from dichloromethane was not active at all against any of the bacterial strains tested. It can be concluded that dichloromethane was not a suitable solvent for extracting the active compounds in *A. mearnsii* as no active ingredients were extracted.

DISCUSSION

The inhibition of the growth of Gram-negative and Gram-positive bacteria may be dependent on several factors which include, but are not limited to: resistance to physical disruption, susceptibility to anionic detergents, the thickness of their peptidoglycan cell wall components; Gram-positive bacteria are characterised by a thick layer whereas Gram-negative are characterised by a thin layer with an impenetrable cell wall hence they are more resistant against antibiotics. Gram-negative and Gram-positive bacteria normally found in the gastrointestinal tract (GIT) can cause GIT diseases, and this is of particular interest to the present study. The organisms responsible for cholera and bubonic plague are Gram-negative. The MIC for the test microorganisms varied widely against the degree of their vulnerability. The antimicrobial with a low activity against an organism has a high MIC while a highly active antimicrobial gives a low MIC (Banso, 2009).

The methanol extract was the most active, although mainly against Gram-positive bacterial strains. The ethyl acetate extract on the other hand showed the widest spectrum of antibacterial activity, although it was relatively weaker in comparison to the methanolic extract activity. The hexane extract showed no anti-bacterial activity stronger than MIC = 10 mg/ml. The inactivity of the methanol extract on the remaining gram negative bacteria might be due to the composition of these

bacterial cell walls, their resistance to disruptions and the nature of the anionic compounds present in the extract. However, more detailed work is ongoing to ascertain this. The fact that the average activity is positively correlated with the hydrophilicity of the solvent, suggests that the active compounds are hydrophilic or ionic (Darout et al., 2000). The Gram-positive bacteria were clearly more susceptible to the extracts than the Gram-negative bacteria. This may be seen as a justification for the traditional use of the bark extracts of *A. mearnsii* De Wild for the treatment of GIT diseases, such as diarrhoea and stomach ache.

Furthermore, according to Ajali and Okoye (2009), wound ulcers can be infected by *S. aureus* and *Pseudomonas auriginosa* and in their study, these organisms were found to be very sensitive to the root bark extracts of *Olax viridis*. Even in the present study, *S. aureus* and *P. auriginosa* were found to be sensitive to the bark extracts of *A. mearnsii*.

The important activity of the extracts against some enteric organisms like *E. coli* invokes curiosity. This may provide an explanation about the ethnomedicinal use of *A. mearnsii* in the management of diarrhoea (Ajali and Okoye, 2009). The observations of antibacterial activity that were shown by the stem bark extracts of *A. mearnsii* could be due to the amount of one or more compounds present in the plant material. It is also possible that different compounds may be responsible for the activity observed (Fawole et al., 2009). The traditional healers rarely use a single plant in their prescriptions. Using plant mixtures is beneficial in most cases, where different plant parts are used in combination or in series. There is no uniformity in traditional medicine with respect to the harvesting of unrefined materials, technique of production and in quality control of the finished product. Hence there is a need for pharmacological screening of medicinal plants to provide a scientific foundation for the sustained traditional use of plants to provide society with potential sources of new, effective and safe drugs (Eldeen et al., 2005).

Conclusion

The higher potency of the bark extracts of methanol and ethyl acetate are indicative of the extracts that should be used to isolate active compounds. Although, many workers have reported that water is a poor extractor of antibacterial compounds from plant materials (Ibekwe et al., 2001; Karaman et al., 2003), the results of Olajuyigbe and Afolayan (2011) suggested that water may be a good extraction medium and its extract may be as potent as alcoholic solvent extracts. 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). However, the

inclusion of anionic components is likely to be minimised in the ethyl acetate extract. It is therefore unlikely that the potency of the ethyl acetate extract is due to the presence of anionic components, and more likely, it is due to the presence of active organic compounds. These organic compounds can be identified using the bio-autography method, followed by isolation, structure determination and structure-activity studies.

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