Journal of Medicinal Plants Research Vol. 4(11), pp. 999-1002, 4 June, 2010
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR09.243
ISSN 1996-0875© 2010 Academic Journals

Full Length Research Paper

The possible role of medicinal plants in tackling resistant microbial pathogens in Limpopo Province, South Africa

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Accepted 16 March, 2010

The present study was conducted to investigate the antibacterial activities of nine selected medicinal plants against metheciline resistance coagulase negative S*taphylococcus*, metheciline resistance *Staphylococcus aureus* and extended spectrum beta-lactamase producing *Escherichia coli, Enterobacter cloace* and *Pseudomonas aeroginosa*. The grounded plant materials were extracted with different extractants and screened for anti-microbial activity using the disk diffusion, micro-dilution techniques and bioautographic methods. Preliminary screening revealed that methanol extracts were most active against all assayed bacteria. Metheciline resistance coagulase negative S*taphylococcus* and metheciline resistance *S. aureus* were found to be susceptible to all crude methanolic extracts of all tested plants (100%), followed by *P. aeroginosa* and extended spectrum beta-lactamase producing *E. coli, E. cloace.* Contact biautography indicated that *Schotia brachypetala* extracts possess one major anti-microbial component against extended spectrum beta-lactamase producing *E. coli* , *E. cloace* and four components against *P. aeroginosa* The lowest minimum inhibitory concentration recorded for the different crude methanol extracts against metheciline resistance coagulase negative S*taphylococcus*, metheciline resistance *S. aureus* and extended spectrum beta-lactamase producing *E. coli*, *E. cloace* and *P. aeroginosa* were 500, 125 and 250 ug/ml respectively. This study also confirm the antimicrobial potential of investigated plants and their usefulness in treatment of resistance microorganisms both gram-negative and gram-positive

Key words: Metheciline resistance coagulase negative S*taphylococcus,* metheciline resistance *Staphylococcus aureus,* extended spectrum beta-lactamase producing *Escherichia coli, Enterobacter cloace*, *Pseudomonas aeroginosa.*

INTRODUCTION

Medicinal plants form an important aspect of the daily lives of many people, and are an important part of the Southern Africa cultural heritage. Plants contain chemical constituents that have great potential for medicinal use and both traditional healers and pharmaceutical drug companies make use of these plants (Coopoosamy and Magwa, 2006).

Many drugs used in western medicine today are derived from plants and other natural resources. Increasing resistance of bacterial isolates to antibiotics lead to find out the alternative antimicrobial agents. Many plants have been tried to evaluate the effectiveness of traditional medicines used in the treatment and management of bacterial, fungal infection and viral infection (Mathabe et al., 2006; Buwa and Van Staden, 2006; Rangasamy et al., 2007; Bessong and Obi, 2006). Much effort went into screening plants used medicinally in different regions of the world, e.g. Rwanda (Cos et al., 2002), in South Africa a lot of studies have been conducted in different Provinces in Kwazulu natal (Lin et al., 2002), in Limpopo (Samie et al., 2005; 2007). Several methods have been used for preparation of plant extracts, determination of antimicrobial activity, isolation and separation of bioactive compounds.

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These studies showed that traditional healers from different localities use different plants and different plants part for the treatment of various bacterial infections. A lot of studies have been done on many plants to investigate antimicrobial against bacterial strains to our knowledge. This is the first study to investigate the anti-microbial activities of medicinal plants against metheciline resistance coagulase negative S*taphylococcus, metheciline* resistance *Staphylococcus aureus* and extended spectrum beta-lactamase producing *Escherichia coli, Enterobacter cloace* and *Pseudomonas aeroginosa*

MATERIALS AND METHODS

Ethnobotanical survey

A survey was conducted by interviewing ten traditional healers in the Vhembe district finding about plants used and plants parts used for the treatment of septic wounds and bacterial related infections, preparation of medication and treatment administration.

Plant material

The following medicinal plants were collected from different region of Limpopo Province based on the information received from local communities and traditional practitioners on the basis of their popular usage against infections and diseases, *Capparis tomentosa, Carissa edulis*, *Dodonaea angustifolia, Erythrina lysistemon, Psidium guajava, Schotia brachypetala, Thesium hystriex, Typha capensis,* and *Zantedeschia aethiaopica.* Plant parts used were leaves, bark, and roots, ripe fruits, unripe fruits and twig tips. Collected plants were air-dried at room temperature and then ground into fine powder.

Strains used

Resistant bacterial strains used were metheciline resistance coagulase negative S*taphylococcus,* metheciline resistance *S. aureus* and extended spectrum beta-lactamase producing *E. coli, Enterobacter cloace*, and *P. aeroginosa,* this micro-organisms were obtained from South African institute for medical research laboratory in Petersburg. Vanillin, sulphuric acid and analytical reagents were purchased from Merck, Sigma and Aldrich. Aluminium backed Silica gel TLC plates were purchased from Macherey Nagel GmbH. Agar diffusion plastic plates ware purchased from Biddy Sterillin. Control disc were purchased from Davies diagnostics, Iodonitro tetrazolium violet (INT) was purchased from Sigma Chemical- Company.

Preparation of plants extracts

A 50 g sample of each ground material was soaked in 500 ml of methanol, acetone or hexane for at least 72 h with frequent shakings. The samples were suction filtered through Whatman No1 filter paper. The filtrate was evaporated to dryness under reduced pressure, collected in 10 ml of the solvent, placed in the tube and allowed to dry at room temperature. A stock solution of 0.2 g/ml in dimethyl sulfoxide (DMSO) was made for each extract. All the extracts were kept at 4°C in the dark until further use.

Anti-microbial assays

Antimicrobial assay of plant extracts

The disc diffusion method was used as described by Samie et al. (2005). Briefly; Mueller Hinton Agar (MHA) was supplemented with 0.01% Tween 80 to enhance the solubilisation of oils and extract. 100 µl of 18 h old culture of each test organism was spread on the agar plate and left for 30 min to dry. Whatman No. 1 paper was used to prepare discs of 6 mm diameter and sterilized by autoclaving. The blank sterile discs were deposited on top of the seeded MHA and 15 µl (3 mg) of each extract or essential oil was added on top of the disc. The plate was incubated at 37°C for 24 h. All tests were performed in triplicates using 10 µl of a 50 mg/ml gentamycin as a positive control and 15 µl (6%) of DMSO as negative control. Each test was repeated four times and the antibacterial activity was expressed as the mean of inhibition diameters (mm) produced by the plant extracts.

Determination of minimum inhibitory concentration

Serial dilutions of the extracts were made in microtiter wells with Mueller–Hinton broth (0.01% Tween 80) to cover the range 0.08 - 12 mg/ml for a volume of 100 µl with a final concentration of 2.5% DMSO (Merck, Germany). A McFarland No1 standard suspension of test bacteria was made in Mueller-Hinton broth, from which 100 µl of the final inoculum containing approximately 1.5 x 10^6 colony forming units (CFUs) was used to fill each well to a final volume of 200 µl. Inoculated plates were incubated at 37°C for 24 h. One hour before the end of incubation 40 µl of a 0.2% solution of Iodo-Nitro Tetrazolium (INT) (Merck, Germany) was added to the wells and the plate was incubated for another hour. Inhibition of growth was detected when the solution in the well was clear after incubation with INT. The assay was repeated thrice. The lowest concentration of each extract showing no visible growth was recorded as the minimum inhibitory concentration (MIC).

Thin layer chromatography (TLC)

Thin Layer chromatography (5 µl of a 100 mg extract /ml solution) was on Silica Gel 60 coated on glass plates (Merck TLC F254) with hexane/ethyl acetate 1/1(v/v) and dichloromethane /methanol/water 65/35/0.5 as eluants. The separated components were visualized under visible and ultraviolet light (254 and 360nm, Camag Universal UV lamp TL-600) or using spray reagents such as 5% anisaldehyde in 5% sulphuric acid in ethanol solution, vanillin and Dragendonorff (Eloff, 1998)

Bioautography

TLC plates (20 × 20 cm) were loaded with 20 µl of each the extract using the tip of Pasteur pipette.

Develop TLC plates were dried overnight in a sterile room. One colony of bacterial culture was inoculated into nutrient broth and incubated in the water bath for 3 - 4 h. After four hours of incubation the colony were adjusted to be equivalent to 0.5 McFarland standard the TLC were covered with a fine spray of the test bacteria (plates were covered by metheciline resistance coagulase negative S*taphylococcus,* metheciline resistance *S. aureus* and extended spectrum beta-lactamase producing *E. coli, E. cloace* and *P. aeroginosa*) the plates were sprayed until they were completely wet, and incubated for 18 - 24 h at 37°C in a chamber at 100% relative humidity and then sprayed a 0.2 mg/ml INT (*p*-iodonitro tetrazolium violet). Plates were incubated before they were completely dry at 37°C for 1 h in the dark for color development. Clear zones on the

Table 1. Inhibition zone diameter and Minimum inhibitory concentration of tested bacteria.

Plant	Plant part extracted	Extraction solvent	Extract yield (mg)	*MRSE*	*MRSA*	*E. coli*	*E. cloacae*
Capparis tomentosa	Bark	A	121	(19.0) 0.312	(23.0) 0.312	(5.0) 0.625	(7.0) 0.625
		H	8	(22.0) 0.156	(25.0) 0.156	(8.0) 0.625	(6.0) 0.625
		M	110	(26.0) 0.078	(30.0) 0.078	(0.0) N/A	(0.0) N/A
		W	15	(3.0) N/A	(6.0) N/A	(0.0) N/A	(0.0) N/A
Carissa edulis	Leaves	A	50	(17.0) 0.039	(21.0) 0.039	(17.0) 0.156	(13.0) 0.156
		H	35	(19.0) 0.156	(17.0) 0.156	(15.0) 0.625	(14.0) 0.625
		M	102	(26.0) 0.078	(30.0) 0.078	(35.0) 0.156	(37.0) 0.156
		W	33	(3.0) N/A	(6.0) N/A	(0.0) N/A	(0.0) N/A
Dodonaea angustifolia	Leaves	A	65	(18.0) 0.625	(20.0) 0.625	(12.0) 0.625	(9.0) N/A
		H	36	(9.0) N/A	(6.0) N/A	(0.0) N/A	(0.0) N/A
		M	88	(24.0) 0.312	(25.0) 0.312	(15.0) 0.625	(0.0) 0.625
		W	16	(0.0) N/A	(0.0) N/A	(0.0) N/A	(0.0) N/A
Erythrina lysistemon	Bark	A	123	(20.0) 0.312	(25.0) 0.312	(16.0) 0.625	(21.0) 0.625
		H	24	(19.0) 0.625	(15.0)0.625	(12.0) N/A	(13.0) N/A
		M	136	(35.0) 0.312	(28.0)0.312	(40.0)0.625	(38.0) 0.625
		W	28	(3.0) N/A	N/A	N/A	N/A
Psidium guajava	Leaves	A	179	(15.0) 0.039	(12.0) 0.039	(6.0) 0.312	(4.0) 0 .312
		H	59	(10.0) N/A	(12.0) N/A	(0.0) N/A	(0.0) N/A
		M	480	(20.0) 0.078	(22.0) 0.078	(10.0) 0.312	(13.0) 0.625
		W	56	(3.0) N/A	(6.0) N/A	(0.0) N/A	(0.0) N/A
Schotia brachypetala	Bark	A	200	(25.0) 0.156	(22.0) 0.078	(18.0) 0.625	(20.0) 0.312
		H	78	(18.0) 0.625	(15.0) 0.625	(16.0) 0.312	(22.0) 0.156
		M	380	(33.0) 0.156	(27.0) 0.078	(35.0) 0.625	(40.0) 0.312
		W	73	(3.0) N/A	(6.0) N/A	(0.0) N/A	(0.0) N/A
Reference:	Gentamicin (10 µg)			0.001	0.001	0.001	0.001

Extract: A, acetone; H, hexane; M, Methanol; W, Water .Bacteria: MRSE (Metheciline resistance *Staphylococcus epidermidis*), MRSA (Metheciline resistance *Staphylococcus aureus*), ESBL *E. coli* (Extended spectrum beta-lactamase *Escherichia coli*), and ESBL *E. cloacae* (Extended spectrum beta-lactamase *Enterobacter cloacae*)

chromatogens indicated inhibition of bacterial growth. Growth inhibition areas with the *R*f of the related spots on the TLC plate revealed with different

Reference strains

The following standard strains were included as controls, *E. coli* ATCC 25922 beta-lactamase producer; *Klebsiella pneumonia* ATCC 700603 ESBL producer were used

Statistical analysis

Statistical analyses were done using SPSS 11.0 for windows. The experimental data were subjected to analysis of variance for a completely random design to determine the least significant difference among mean at level of 0.05. After multiple comparisons, the means were plotted in the graphs and tables. The tables and graphs were plotted using sigma plot.

Antimicrobial activity testing

A number of selected resistant bacterial pathogens were used to screen selected medicinal plant extracts for antimicrobial activity.

RESULTS AND DISCUSSION

Results of the study clearly showed that the plants used for the study contained significant anti-microbial activity (Table 1). The results showed that of four solvents used for extraction, methanol extracts displayed a broader barks and leaves extracts from acetone and methanol tended to be more active (that is, have a lower MIC) than the hexane and water extracts. The results showed that leaf material could be useful for antimicrobial than roots and bark which could be used without any detrimental

Table 2. MIC values of active extracts

Botanical names (Family)	Vernacular names	Plant part used	Traditionally used to treat:
Capparis tomentosa Family: Capparaceae	Munzere (Venda)	Bark	Diarrhea and dysentery, sore throat
Carissa edulis Familiy; Apocynaceae	Murungulu (Venda)	Leaves	High blood pressures and gastrointestinal diseases
Dodonaea angustifolia Family: Sapindaceae	Muthathavhana (Venda)	Leaves	Dysentery, diarrhea, eye infections and wound infections
Erythrina lysistemon Family: Fabaceae/Leguminosae	Muvhale (Venda)	Bark	Wound infections, sepsis, Stomach troubles, cold and fever, babies food, diarrhea and wounds
Psidium guajava family: Myrtaceae	Mugwavha (Venda)	Leaves	Diarrhea, abdominal pains, gastrointestinal problems
Schotia brachypetala Family: Fabaceae	Muvhale (Venda)	Bark	Wound infections and sepsis, gastrointestinal problems

effect on the plant.

In the present investigation, the extracts were prepared serially with the dried and grounded plant material, the first extractant being hexane followed by other solvents of high polarity and finally by water. This led to fractionation of anti-microbial compounds of investigated plants. We observed that most compounds were isolated by polar extractent than non-polor. We also demonstrated that little amount of flavonoids separated by TLC are sufficient to produce an inhibitory effect on the growth of ESBL producing bacteria. MIC values of active extracts are shown in (Table 2). Among the plants tested *Dodonaea angustifolia, Erythrina lysistemon, Psidium guajava, Schotia brachypetala,* showed the best antibacterial activity. The methanolic and ethanolic extracts of *Schotia brachypetala* had the highest inhibitory activity against ESBL producing bacteria. *Carissa edulis* plant extracts showed the best MIC values for methanolic extracts where the extracts demonstrated activity against all tested bacteria.

In conclusion, the screening of crude extracts made from tested medicinal plants has demonstrated that most of the screened plats are potential rich sources of antibacterial agents. To determine the antibacterial activity of plants which are used by traditional healers is very much useful to 60-80% of people who use traditional medicine as their first line of health care services. Further studies need to be done to determine the structure of the compound.

ACKNOWLEDGEMENTS

We thank National Research Foundation for financial assistance and traditional healers for their help and willingness to share knowledge on traditional medicine.

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