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Full Length Research Paper

Antibacterial activity of root and leaf extracts of *Jatropha zeyheri* Sond (Euphorbiaceae)

Mongalo NI[1]*, Opoku AR[2] and Zobolo AM[1]

[1]Department of Botany, University of Zululand, Private Bag x1001, Kwadlangezwa 3886, Republic of South Africa.
[2]Department of Biochemistry and Microbiology, University of Zululand, Private Bag x1001, Kwadlangezwa 3886, Republic of South Africa.

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Resistance of human pathogenic bacterial strains results in selective pressure against known antibiotics. *Jatropha zeyheri* is used by traditional medicine practitioners in the treatment of sexually transmitted and urinary tract infections. Acetone, methanol and ethyl acetate extracts of both leaves and roots of *Jatropha zeyheri* collected from Capricorn District, Limpopo Province, South Africa were investigated for antibacterial activity, against 14 human pathogenic bacterial strains, using disc diffusion method. Ethyl acetate extracts exhibited activity against 11 of the selected strains and showed zone of inhibition of 10.7 mm against *Klebsiella pneumoniae.* Largest zone of inhibition of 12 mm was obtained with the acetone leaf extract against *Enterobacter cloacae* and *Acinetobacter calcaoceuticals* and methanol extract of the leaf against *Enterococccus faecalis*. Acetone extract of the root exhibited minimal inhibitory concentration (MIC) of 0.39 mg/ml against *Salmonella* spp. followed by methanol and acetone extracts of the root (0.78 mg/ml) against *Serratia marcescens*. Methanol extract of the leaf exhibited MIC of 3.13 mg/ml against *Staphylococcus aureus*. This study validates the use of *Jatropha zeyheri* in the treatment of various illnesses.

Key words: Inhibition zone, antibiotics, *Jatropha zeyheri*, disc diffusion, MIC.

INTRODUCTION

Microorganisms are frequently developing resistance to common drugs and antibiotics and this pose an enormous threat to the treatment of a wide range of serious infections (Taylor et al., 2002; Sibanda and Okoh, 2007). Since penicillin and mutation resistant strains are on the increase, there is a need to search for new compounds (that are not penicillin based) that inhibit microbial growth. Medicinal plants have been a basic source of antibiotics against a variety of illnesses over the years in most communities. South African medicinal plants extracts have been screened for antimicrobial activities (Samie et al., 2010; Buwa and Afolayan, 2009; Van Vuuren, 2008). *Jatropha zeyheri*, indigenously known as "*Sefapabadia*" amongst Sotho tribe, (root) is used by traditional medicine practitioners in the treatment of sexually transmitted infections and urinary tract infections. It is also used to treat menstrual pains, irregular periods, and to ensure a strong foetus during pregnancy (Van Wyk and Gericke, 2007). Genus *Jatropha* consists of approximately 172 species with significant economic importance (Bhagat and Kulkarni, 2010), distributed mainly in the tropical and subtropical regions of America and Africa and have been reported to possess a variety of biological activities. Crude aqueous extract of ground seeds of *Jatropha curcus* and crude extract of *Jatropha elliptica* were reported to possess molluscidal activity (Devappa et al., 2010), while *J. curcus* and *J. podagrica* were reported to possess antimicrobial activities (Aiyelaagbe et al., 2000; Essiett and Ajibesin, 2010; Aiyelaagbe et al., 2007). Aqueous extract of *Jatropha zeyheri* root combined with *Warburgia*

*Corresponding author. E-mail: nmongalo@pan.uzulu.ac.za. Tel: +27359026112. Fax: +27866395217.

salutaris bark and *Pentanisia prunelloides* has been reported to possess MIC of >2 mg/ml against *Bacillus subtilis* and *Staphylococcus aureus* (Jager, 2003). Genus *Jatropha* is known to produce diterpenes which mostly belongs to rhamnofolane, daphnane, lathyrane, tigliane, dinorditerpene, deoxy preussomerin and pimarane skeletal structures (Devappa et al., 2011). A compound, jaherin, has been isolated from *Jatropha zeyheri* root and has been reported to possess MIC of 8 mg/ml against *Streptococcus pyogenes* and 16 mg/ml against *Microsporum canis*, *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Sporotrichum schenkii* (Dekker et al., 1987). Besides antibacterial activity, dichloromethane and methane extracts of the root have been reported to possess both anti-inflammatory and mutagenic effects (Luseba et al., 2007). This paper aimed at investigating the antibacterial activity of root and leaf extracts from *Jatropha zeyheri* against pathogenic strains, mostly causative agents of urinary tract infections.

MATERIALS AND METHODS

Chemicals used

Unless otherwise stated, all the chemicals used including solvents were of AR grade and were obtained from Sigma-Aldrich Co. Ltd.

Plant material and extraction

Roots and leaves of *Jatropha zeyheri* were collected on April, 2008, from the Pickum farm-Blouberg area within Capricorn District, Limpopo Province Republic of South Africa. Collected plant specimens were separately washed with distilled water to remove the adhering soil, cut into small pieces, dried in the shade and ground into thin powder (2 mm mesh) using hammer mill (Perten Instruments 3100, Sweden). Each dry powder was separately extracted (1:5w/v) with acetone, methanol, and ethyl acetate by incubating the mixture on a mechanical shaker (Merck, South Africa) at 100 rpm for 24 h at room temperature. Extracts were filtered through Whatman No1 paper and the organic solvent extracts were concentrated using rotary evaporator. Dry extracts were weighed and kept in refrigerator at 4°C until needed.

Selected bacterial strains

14 bacterial strains, seven Gram negative strains viz, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 7700, *E. cloacae* ATCC13047, *K. pneumoniae* ATCC 10031, *S. marscens* ATCC 9986, and reference clinical isolates of *Shigella flexineri* and *Samonella spp*. and seven Gram positive strains viz, *S. aureus* ATCC 6538, *B. cereus* ATCC 10702, *B. pumilus* ATCC 14884, and reference clinical isolates of *E. faecalis*, *S. epididirmis, Acinetobacter calcaoceuticals* and *B. subtilis* were used in this study. The microorganism were obtained from the Department of Biochemistry and Microbiology, University of Zululand, and maintained on Muller-Hinton agar (MHA) (Oxoid). Clinical isolates were isolated from hospital patients of various illnesses within KwaZulu-Natal Province.

Antibacterial tests using disc diffusion method

Plant extracts were tested for antibacterial activity by the disc diffusion method according to National Committee for Clinical Laboratory Standard guidelines (NCCLS, 2001). A single colony of the respective organism was aseptically transferred with an inoculating loop to a 20 ml of fresh sterile saline broth in a test tube which was vortexed thoroughly and incubated overnight at 37°C. Turbidity was then spectrophotometrically adjusted to that of 0.5 McFarland's standard. About 100 µl of the inoculum was aseptically transferred to a labeled disposable Petri-dish containing 15 ml Muller-Hinton agar and spread thoroughly using sterile glass spreader. Sterile paper discs of 5 mm (Mast Disks, UK) were impregnated with 10 µl of 5 mg/ml plant extract dissolved in 5% dimethylsulfoxide (DMSO) and gently placed individually on the seeded agar. Plates were allowed to dry for 1 h and later incubated in an inverted position at 37°C over night. Zones of inhibition were measured in millimeters, including sterile paper disc. Neomycin (10 µg/disc) was used as positive control. Negative controls were performed using paper discs loaded with 10 µl of 5% DMSO. Each experiment was repeated.

Minimal inhibitory concentrations (MIC) using microdilution assay

Extracts showing activity in Disc Diffusion were chosen to assay the minimal inhibitory concentration (Eloff, 1998) using the micro plate broth dilution assay. The 24 h old culture was diluted 1:100 (McGaw and Eloff, 2005) with saline broth. About 100 µL of extracts (50 mg/ml in 5 % DMSO) were added to multi well plate containing 100 µL of freshly prepared broth and serially diluted, yielding 12.5 mg/ml in the first well. Plates were then incubated over night at 37°C. About 40 µl of 2 mg/ml freshly prepared iodo-nitro-tetrazolium chloride were added to each well and incubated for 30 min at the same temperature. Metronidazole was used as the control. The MIC was defined as the lowest concentration of the extract to inhibit bacterial growth.

RESULTS AND DISCUSION

Results for the antibacterial activity (zones of inhibition) of *Jatropha zeyheri* are shown in Table 1 and ranged from 7.0 to 12 mm. Although, *S. aureus* continue to become a global threat to antimicrobial chemotherapy (Tacconelli, 2011; Zetola et al., 2005), it was the only strain susceptible to all the three root extracts in our current study. Largest zone of inhibition (12 mm) was exhibited by acetone extract of the leaf against *E. cloacae* and *A. calcaoceuticals*, and methanol extract of the leaf against *E. faecalis*. *S. epidirmidis* and *B. subtilis* were resistant to all extracts except ethyl acetate root extract while *Salmonella spp* was resistant to all leaf extracts. Ethyl acetate extract of the root showed best activity against all selected mocroorganisms except *Salmonella spp.* and *Serratia marscens*, hence had broad spectrum. Although root extracts generally shows higher activity compared to the leaf extracts (Darabpour et al., 2011), methanol extract of the leaf in our study revealed activity against *S. aureus*, *B. cereus*, *E. faecalis, E. coli* and *P. aeruginosa*, while methanol extract of the root was inactive against *B. cereus* and *E. faecalis,* suggesting that leaf may be a substitute for root; a conservation effort. No zone of inhibition observed on negative control.

P. aeruginosa was resistant to both acetone and ethyl acetate leaf extracts and methanol extract of the root,

Table 1. Antibacterial activity of *Jatropha zeyheri* leaf and root extracts (zones of inhibition in mm and n=3).

Bacteria	Leaf			Root			Neomycin (10 µg) disc
	Methanol	Acetone	Ethyl acetate	Methanol	Acetone	Ethyl acetate	
Staphylococcus aureus	10.7	na	na	8.3	9.0	9.0	20.3
Staphylococcus epidirmidis	na	na	na	na	na	7.3	20.7
Bacillus pumilus	na	10.7	na	8.7	na	8.0	22.7
Bacillus subtilis	na	na	na	na	na	9.7	24.3
Bacillus cereus	8.7	na	9.7	na	7.0	8.3	21.7
Enterococcus faecalis	12	na	na	na	na	9.7	21.7
Escherichia coli	11.7	na	na	8.0	na	7.0	29.3
Pseudomonas aeruginosa	10.7	na	na	na	8.7	8.0	24.0
Enterobacter cloacae	na	12	na	na	na	na	23.3
Klebsiella pneumoniae	na	10.7	na	na	na	0.7	21.0
Serratia marscens	na	10.3	9.7	8.3	7.7	na	27.7
Shigella flexineri	na	na	9.0	7.0	na	9.7	20.3
Salmonella spp	na	na	na	8.7	7.3	na	23.0
Acinetobacter calcaoceuticals	na	12	10.3	8.7	na	8.0	26.7

Results were reported as mean of three replicates;na, not active.

while *E. cloacae* resisted all extracts except acetone leaf extract. *E. cloacae* have recently been reported to cause infections among burn victims, immunocompromised patients and patients with malignancy and may cause bacteremia, urinary tract and pulmonary infections (Musil et al., 2010), while *P. aeruginosa* which mainly infects the pulmonary tract and urinary tract is amongst the most difficult to treat with conventional antibiotics (Mathekga and Meyer, 1998). *E. cloacae* are also reported to resist expanded-spectrum cephalosporins and such resistance may be caused by stable derepression of the chromosomal Ampler class C β-lactamase (Kartali et al., 2002). Only acetone extract of the leaf and ethyl acetate extract of the root inhibited *K. pneumoniae* with a zone of inhibition of 10.7 mm. *K. pneumoniae* has been associated with nosocomial infections and may produce extended spectrum β-lactamases (ESBLs), which renders it resistant to carbapenems (Falagas et al., 2007).

Results for MIC values of *Jatropha zeyheri* root and leaf extracts are presented in Table 2 and ranged from 0.78 to ≥ 12.5 mg/ml. Ethyl acetate extract of the root exhibited good minimal inhibitory concentration of 1.56 mg/ml against *B. pumilus*, *B. cereus* and *E. faecalis*. Methanol and acetone extracts of the root exhibited activity against some gram negative bacterial strains and showed low minimal inhibitory concentration of 0.78 mg/ml against *S. marcescens,* which compares well to that of metronidazole (0.63 mg/ml) against similar organism. Acetone extract of the root exhibited potent MIC value of 0.39 mg/ml against *Salmonella spp*. According to Kruger et al. (2004), *Salmonella spp* producing extended-spectrum beta-lactamases are becoming prevalent in some hospitals in South Africa and are resistant to variety of expensive drugs.

Although, Luseba et al. (2007), reported the MIC of 90% methanolic extract of *Jatropha zeyheri* root at 0.63 mg/ml against *Esherichia coli* and 2.5 mg/ml against both *S. aureus* and *P. aeruginosa*, it is difficult to compare these results to our study due to differences in variables such as solvent type, extraction procedure and other environmental conditions. To our knowledge, there is no reported previous investigation on antibacterial effects of *Jatropha zeyheri* leaf extracts. Methanol extract of the leaf showed MIC value of 3.13 and 4.16 mg/ml against *S. aureus* and *E. faecalis* respectively. According to Aliyu et al. (2008), MIC of 3 mg/ml is of high potency. Acetone and ethyl acetate extracts of the leaf exhibited moderate activity of 6.25 mg/ml against *A. calcaoceuticals*. These results , in a way, validates the use of *Jatropha zeyheri* in the treatment of various ailments including urinary tract infections.

Traditional medicine is an important source of products for majority of developing countries in treating a variety of bacterial infections, thus mitigating many of the side effects that may be associated with synthetic antimicrobials. Bacterial strains such as *S. aureus*, *Enterococcus*, *P. aeruginosa*, *Acinetobacter*, *E. coli*, *Klebsiella* are amongst microbes causative agents of hospital acquired infections, with stethoscope being a possible vector (Randrianirina et al., 2010; Killic et al., 2011) and may cause urinary tract infections cohabiting with *Proteus* and *Serratia* species. The results of the antibacterial screening of *Jatropha zeyheri* root and leaf extracts against 14 bacteria species indicate the

Table 2. Minimal inhibitory concentrations of Jatropha zeyheri leaf and roots extracts (n=3).

Bacteria	Leaf			Root			Metronidazole
	Methanol	Acetone	Ethyl acetate	Methanol	Acetone	Ethyl acetate	
Staphylococcus aureus	3.13	-	-	≥12.5	≥12.5	3.13	0.32
Staphylococcus epidirmidis	-	-	-	-	-	≥12.5	0.08
Bacillus pumilus	-	6.25	-	≥12.5	-	≥12.5	0.08
Bacillus subtilis	-	-	-	-	-	1.56	0.63
Bacillus cereus	6.25	-	6.25	-	≥12.5	1.56	0.63
Enterococcus faecalis	4.16	-	-	-	-	1.56	0.63
Escherichia coli	≥12.5	-	-	6.25	-	3.13	0.08
Pseudomonas aeruginosa	≥12.5	-	-	-	4.16	4.16	0.08
Enterobacter cloacae	-	4.16	-	-	-	-	0.63
Klebsiella pneumoniae	-	6.25	-	-	-	≥12.5	0.08
Serratia marscens	-	≥12.5	6.25	0.78	0.78	-	0.63
Shigella flexineri	-	-	4.16	≥12.5	-	6.25	0.08
Salmonella spp	-	-	-	3.13	0.39	-	0.04
Acinetobacter calcaoceuticals	-	6.25	6.25	≥12.5	-	≥12.5	0.08

Results were reported as mean of three replicates; -, not done.

bioactivity of the plant. In general, gram negative strains were more resistant to extracts than gram positives. Plant extracts are generally known to be more effective on Gram-positive bacteria than on the Gram-negative ones (Parekh and Chanda, 2006; Chan et al., 2008) and this may be attributed to possible reasons such as permeability barrier provided by presence of cell wall with multilayer structure in Gram negative bacteria or the membrane accumulation mechanisms or presence of enzymes in periplasmic space, which are able to break down foreign molecules introduced from outside (Motamedi et al., 2010).

Conclusion

Selected bacterial strains are among the most common pathogens which cause a variety of infections in human. Activity of these extracts validates the use of *Jatropha zeyheri* against urinary tract infections and other human pathogens. Moreover, there is a need to screen extracts of this plant against organisms belonging to the traditional sphere of sexually transmitted infections.

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