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Antibacterial activity of *Waltheria indica* Linn (Sterculiaceae), collected from Blouberg area, Limpopo Province, South Africa

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Mature roots of *Waltheria indica* were collected from Blouberg area, Limpopo Province, republic of South Africa. Water, ethanol and methanol extracts were investigated for antibacterial activity at 5 mg/ml against 10 human pathogenic strains using disc diffusion method. Ethanol extract exhibited potent antibacterial activity against selected strains while methanol extract exhibited largest zone of inhibition of 15.2±2.18 mm against *Bacillus pumilus*. Extracts were further investigated for antibacterial activity using minimum inhibitory concentration (MIC) assay. Lowest MIC of 0.65 mg/ml was exhibited by methanol extract against *Acinetobacter calcaoceuticals anitratus*. Water extract exhibited good MIC of 2.08 mg/ml against *Escherichia coli* while ethanol extract showed lowest MIC of 1.04 mg/ml against *Enterobacter cloacae* and 6.25 mg/ml against *Klebsiella* spp., *Serratia marscens* and *Staphylococcus epidirmidis*. Moreover, ethanol extract possessed a total activity of 433 mL/g against *Enterococcus faecalis*, meaning that the extract can be diluted to 433 mL and would still inhibit growth of these bacteria. These results in a way validate the use of *W. indica* L in the treatment of variety of infections, especially urinary tract infecting bacterial strains.

Key words: *Waltheria indica* L, minimum inhibitory concentration (MIC), disc diffusion, total activity, antibacterial.

INTRODUCTION

Family Sterculiaceae comprise of species of mainly trees and some herbs indigenous to the tropical rain-forest African region (Sonibare et al., 2009). Several members of this family in Africa have been reported to possess antimicrobial properties, anti-inflammatory activity, high levels of COX-1 inhibition and variety of compounds which includes alkaloids, tannins and cardiac glycosides (Reid et al., 2005; Babalola et al., 2012; Agyare et al., 2012, Hossain et al., 2013, Saradha et al., 2013).

Waltheria indica, commonly known as "*Mokhutesela*", is an erect perennial shrublet or a herb of up to ± 500 mm high, stalked leaves with margins shallowly and irregularly toothed (van Wyk and Malan, 1998). Its flowers are yellow and occur in clusters. Globally, its distribution and habitat is mostly in subtropical and tropical zones and in scrub forests, inundated savannas, riverbanks, and sandy

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or clay soils and in disturbed or impoverished soils (Saunders, 2007). Within Blouberg area in Limpopo Province, its roots are used to treat sexually transmitted infections, urinary tract infections, infant illnesses and heart problems. Elsewhere, roots are reported to treat ailments such as diarrhoea, fever, wounds, stomach ache and neurological disorders (Ayantunde et al., 2009; Rodrigues, et al., 2006; Romeiras et al., 2012). The whole plant is reportedly used in the treatment of diarrhoea while leaves are used to treat peptic ulcer (Mathabe et al., 2006; Oluranti et al., 2012). Moreover, sap from the stems of this plant may be used to treat wounds caused by syphilis (Hedimbi and Chinsembu, 2012) while decoction of its leaves combined with those of *Terminalia catappa* may be taken to treat anaemia (Gbadamosi et al., 2012). Phytochemically, a tiliroside compound known as Kaempferol 3-O-β-D-(6"-O-coumaroyl) glucopyranoside has been isolated from this plant (Calderón-Montaño et al., 2011). Moreover, extracts from this plant are reported to possess analgesic, anti-inflammatory and central nervous system depressant activity (Garcia et al., 2010; Mohammed et al., 2007; Hamidu et al., 2008). This paper was aimed at investigating the water, ethanol and methanol extracts from *W. indica* root against nosocomial hospital-acquired bacterial strains which may result in variety of diseases in humans. *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella spp*, *Staphylococccus epidermidis* and *Enterobacter cloacae* have been frequently isolated from patients with urinary tract infections in tertiary health care facilities world-wide and are some strains were reportedly resistant to gentamycin, penicillin, amoxylin, ciprofloxacin and tetracycline amongst other commonly used drugs in developing countries (Sabir et al., 2014; Drago et al., 2001; Jacobsen et al., 2008; Pallett and Hand, 2010).

MATERIALS AND METHODS

Plant materials

Mature roots of *W. indica* L. were collected from William Show farm, Blouberg area, Limpopo Province-Republic of South Africa. Collected specimen were washed with tap water, and then rinsed with distilled water repeatedly until soil debris was removed. Roots were cut into small pieces and dried on the bench. Voucher specimen was collected and identified by National Biodiversity Institute (NBI) in Pretoria, Republic of South Africa.

Extraction

All the chemicals used including solvents were of AR grade and were obtained from Sigma-Aldrich Co. Ltd. Small pieces of *W. indica* roots were ground into thin powder (2mm mesh size) and extracted (1:3 w/v) twice with boiling water, ethanol and methanol respectively. Organic solvents were evaporated using rotary evaporator, while water extract was freeze dried. Resulting residues were weighed and kept in a refrigerator.

Selected bacterial strains

A combination of ATCC, clinical isolates and multi-resistant strains were obtained from Department of Biochemistry and Microbiology, University of Zululand. Five Gram negative strains namely *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 13047), *Klebsiella* spp. (317302), *Acinetobacter calcaoceuticals anitratus* (CSIR) and *Serratia marscens* (ATCC 9986) and five Gram positive strains such as *Staphylococcus aureus* (ATCC 6538), *Staphylococcus aureus* (P12702), *Bacillus pumilus* (ATCC 14884) and clinical isolates of *Enterococcus faecalis* and *Staphylococcus epidirmidis* were selected for this study. All organisms were maintained on Mueller Hinton agar plates.

Antibacterial tests using disc diffusion method

Water, ethanol and methanol extracts from *W. indica* 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 15 ml of fresh sterile saline broth in a test tube which was vortexed thoroughly and incubated overnight at 37°C. Turbidity was then adjusted to that of 0.5 McFarland's standard using spectrophotometer (Spec. 20). About 100 µl of the inoculum was aseptically transferred to a labelled 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% dimethyl sulfoxide (DMSO) and gently placed individually on the seeded agar. Plates were allowed to dry for one hour and later incubated in an inverted position at 37°C in over night.

Zones of inhibition were measured using a ruler, including the diameter of sterile paper disc. Streptomycin and penicillin (10 µg/disc) were used as positive controls. Negative controls were performed using paper discs loaded with 10 µl of 5 % DMSO (Merck, RSA). Each experiment was repeated.

Minimal inhibitory concentrations (MIC) using micro dilution assay

Extracts showing activity in Disc Diffusion were chosen to assay the minimal inhibitory concentration (Eloff, 1998) using the micro plate broth dilution with slight modification. The 24 h old culture was diluted 1:100 with freshly prepared Muller-Hinton 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 1 h at the same temperature. Streptomycin sulphate was used as control. The MIC was defined as the lowest concentration of the extract to inhibit bacterial growth. To compare the activity of different extracts, the total activity in mL/g was calculated by dividing the total mass in mg extracted from 1 g of plant material by the MIC value in mg/ml (Eloff, 2000).

RESULTS AND DISCUSSION

Due to higher resistance of microorganisms to synthetic drugs, there is a need to screen medicinal plants for antibacterial activity, a first step towards finding the proper substitutes. Nosocomial infections increase the cost of medical care, extend hospital stay and reflect on

Table 1. Antibacterial activity of extracts from *Waltheria indica* Linn.

Bacteria	Water	Ethanol	Methanol	Streptomycin 10 µg	Penicillin 10 µg
E. coli	13.6 ± 0.38	11.1 ± 0.18	10.1 ± 0.85	27.0 ± 0.0	12.7 ± 0.88
E. cloacae	R	10.0 ± 0.0	10.0 ± 0.0	16.7 ± 0.88	8.7 ± 0.33
Klebsiella spp.	9.9 ± 0.57	12.7 ± 0.41	12.0 ± 0.0	16.0 ± 0.67	16.3 ± 0.67
S. marcescens	R	9.9 ± 0.57	9.9 ± 0.57	17.3 ± 0.33	11.3 ± 0.33
Acinetobacter calcaoceuticals anitratus	11.7 ± 1.32	10.0 ± 0.0	10.0 ± 0.0	22.0 ± 0.0	12.0 ± 0.0
S. aureus	R	12.2 ± 0.67	R	17.7 ± 1.53	11.3 ± 0.33
S. aureus (P12702)	9.9 ± 0.57	11.7 ± 0.88	R	24.3 ± 1.16	16.3 ± 0.67
B. pumilus	12.6 ± 1.06	11.7 ± 0.88	15.2 ± 2.18	18.7 ± 0.67	11.3 ± 0.88
E. faecalis	9.9 ± 0.57	10.5 ± 0.82	13.5 ± 0.71	12.7 ± 1.16	11.7 ± 1.52
S. epidirmidis	R	11.0 ± 1.26	R	18.3 ± 0.33	14.3 ± 0.67

R, resistant. Zones of inhibition (mm) were reported as mean of three replicates ± SEM.

the morbidity and mortality of the admitted patients (Melaku et al., 2012). Results for antibacterial activity of three extracts of *Waltheria indica* are shown in Table 1. Methanol extract showed activity against all selected gram negative bacterial strains, with zones of inhibition ranging from 9.9±0.57 mm (*S. marcescens*) to 12.0±0.00 mm (*Klebsiella* spp.), hence narrow spectrum. Besides being involved in neonatal sepsis and causing bacterial pneumoniae in adults, *Klebsiella* spp. may primarily attack immunocompromised patients mostly suffering from mellitus diabetes and chronic pulmonary infections resulting in various fatalities if untreated (Podschun and Ullmann, 1999), while *S. marcescens* is known to produce different enzymes including chitinase and lipase resulting in devastating bacteremia, respiratory tract infections, meningitis and infective endocardia amongst other infections if not treated (Hejazi and Falkiner, 1997).

Ethanol extract exhibited largest zone of inhibition of 15.2±2.18 mm against *Bacillus pumilus*, which is greater than that of penicillin (11.3±0.80 mm) against similar organism. Moreover, it exhibited activity against all Gram positive and Gram negative strains, hence broad spectrum. Similar trend has been reported elsewhere (Mukhtar and Ghori, 2012). Besides exhibiting good DPPH free radical scavenging activity, ethanol extract of leaves this plant reportedly exhibited activity against *B. subtilis* and *E. coli*, with zones of inhibitions ranging from 7 to 25 mm (Garba et al., 2012). Else where, 95 % ethanol extract of this plant exhibited activity of 13 mm (zone of inhibition) against *Escherichia coli*, *Staphylococcus aureus* and *Enterobacter aerogens* (Olajuyigbe et al., 2011). Elsewhere, the *n*-hexane extract from shoots of this plant exhibited MIC of 500 µg/ml against *S. aureus* (Maregesi et al., 2008). It is difficult to compare these results with the current study because of differences in nature and types of bacteria used, locality or geographical area, plant part used and solvent type.

Although water extract was the least active, it exhibited activity of 13.6±0.38 mm against *E. coli,* 12.6±1.04 mm against *B. pumilus* and 11.7±1.32 mm against *A. calcaoceuticals anitratus*. *E. coli* is by far the most common cause of nosocomial or urinary tract infection among hospitalised patients (Shilpi et al., 2012; Wilson and Gaido, 2004). Moreover, it may produce plasmid-mediated AmpC β-lactamases (PABLs) which may be difficult to detect and might interfere with infection control processes (Lee et al., 2009). Elsewhere, aqueous extract was reported to possess trypanocidal activity against *Trypanosoma brucei brucei* (Bala et al., 2009).

S. marscens and *E. cloacae* were amongst four strains found resistant to water extract. These organisms may become resistant to variety of cephalosporins and monobactams by overproducing their chromosomal AmpC β-lactamases (Pitout et al., 2010). *S. aureus* and *S. epidirmidis* were resistant to both water and methanol extract. These organisms are reported to be the most common causes of medical-device associated infections, including septicemic loosenings of orthopaedic implants (Krimmer et al., 1999). Selected strains were more susceptible to streptomycin than penicillin.

MIC values of *W. indica* are recorded in Table 2. Although water extract did not show good inhibition against selected strains, it exhibited MIC of 2.08 mg/ml against *E. coli,* while methanol extract exhibited MIC of 4.17 mg/ml against *E. cloacae, Klebsiella spp.* and *S. aureus.* Furthermore, Ethanol extract exhibited MIC between 1.04 (*E. faecalis*) and 6.25mg/ml against *Klebsiella spp*, *S. marscens* and *S. epidirmidis*. All the selected strains were susceptible to streptomycin sulphate.

Total activities were calculated to validate the quality of the extracts tested in Table 2. Ethanol extract exhibited highest total activity of 433 mL/g against *Enterococcus faecalis*. Compared to water and methanol extracts, ethanol extract exhibited potent total activity against selected bacterial strains. According to Makhafola and Eloff (2012), total activity determines quality of an extract (how much can an extract be diluted and still kill bacteria). However, it is dependent upon quantity of the

Table 2. Minimal Inhibitory concentrations (MIC) of extracts from *W. indica* (mg/ml) and total activity (mL/g) of *Waltheria indica* extracts

Bacteria	Water	Ethanol	Methanol	Streptomycin sulphate
Minimal Inhibitory concentrations (MIC) of extracts from *W. indica* (mg/ml)				
E. coli	2.08	3.65	6.25	0.04
E. cloacae	6.25	4.12	4.17	0.32
Klebsiella spp.	12.5	6.25	4.17	0.04
S. marcescens	4.17	6.25	12.5	0.32
Acinetobacter calcaoceuticals anitratus	4.17	1.90	0.65	0.08
S. aureus	6.25	5.21	4.17	0.08
S. aureus (P12702)	6.25	5.21	12.5	0.32
B. pumilus	6.25	2.08	3.65	0.08
E. faecalis	12.5	1.04	1.30	0.03
S. epidirmidis	2.60	6.25	12.5	0.08
Total activity (mL/g) of *Waltheria indica* extracts				
E. coli	39	123	22	
E. cloacae	13	109	33	
Klebsiella spp.	6.5	72	33	
S. marcescens	20	72	11	
Acinetobacter calcaoceuticals anitratus	13	236	212	
S. aureus	6.5	86	33	
S. aureus (P12702)	13	86	11	
B. pumilus	13	216	38	
E. faecalis	13	433	106	
S. epidirmidis	31	72	11	

plant material extracted from dried plant material and MIC of extract. Earlier, the methanol extract of the root was reported active against *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Proteus vulgaris* revealing 3.13, 0.65 and 2.08 mg/ml respectively while ethanol extract exhibited minimum bactericidal concentration of 2.08 and 4.17 mg/ml against *P. vulgaris* and *P. aeruginosa* respectively (Mongalo et al., 2012). In the current study, the clinical isolate of *E. faecalis* was susceptible to all the selected extracts with MIC ranging from 1.04 to 12.5 mg/ml. The MIC of 3 mg/ml is referred to as most potent (Mongalo et al., 2013; Aliyu et al., 2008), thereby validating the potency of methanol and ethanol extracts which are thus the most potential candidates for bioassays in trying to find new therapeutic compounds used to treat human infections which may be resistant to current antibiotics. These results and the currently reported data, validates the ethnomedicinal use of *W. indica* root in the treatment of variety of infections, especially urinary tract infections as majority of these organisms are the major causative agents of such infections.

Besides various fractions showing activity against *E. coli*, *P. aeruginosa* and *Salmonella typhi*, aqueous extract of the root revealed the presence of tannins, saponins, steroids and Cardiac glycosides (Zailani et al., 2010). Tannins may prevent development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable (Prasad et al., 2008) and may hasten the healing of wounds and inflamed mucous membrane (Njoku and Akumefula, 2007). Saponins have detergent properties and serve as lytic agents and exhibit anti-inflammatory properties (Abukakar et al., 2008) while cardiac glycosides are known to work by inhibiting the (Na^+/K^+) pump, thereby increasing the amount of Ca^{2+} ions available for the contraction of heart muscles which improves cardiac output and reduces distensions of heart, thus used in the treatment of congestive heart failure and cardiac arrhythmia (Ngbede et al., 2008).

Conclusions

Ethanol extract from *W. indica* exhibited potent anti-bacterial activity against selected bacterial strains. In a way, this work validates the use of *W. indica* in the treatment of nosocomial infections. However, there is a need to investigate the antimicrobial activity of this plant against microbes belonging to the traditional sphere of sexually transmitted infections. Biological activity exhibited by ethanol and methanol extracts in this work makes them the potential candidates for individual compounds isolation. Such compounds should also be investigated for various biological activities.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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