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Full Length Research Paper

Antibacterial and antioxidant activity of the extracts of *Waltheria indica* Linn. collected from Capricorn District, Limpopo Province, South Africa

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***Waltheria indica* L., a member of Sterculiaceae family, is widely used traditionally to treat a variety of infections in humans. Roots of *W. indica* were collected from William Show farm, Blouberg Area-Limpopo Province, South Africa. Water, ethanol, methanol and acetone extracts were tested for antibacterial activity. Zones of inhibition ranged from 8.9 ± 0.79 to 20.2 ± 0.57 and were dose dependent. Methanol extract exhibited lowest of 0.52 mg/ml against *Bacillus cereus.* Ethanol extract exhibited lowest minimum bactericidal concentrations against *B. cereus* at 0.65 mg/ml. Methanol extract was also tested for antioxidant activity using 2, 2-diphenyl-1-picryhydrazyl (DPPH) radical scavenging assay and exhibited 75.45 ± 2.76 at a concentration of 0.75 mg/100 ml. DPPH inhibition was also found to be dose dependent. These biological activities observed in the selected extracts validate ethnomedicinal use of *W. indica*.**

Key words: *Waltheria indica* L, antibacterial, antioxidant, ethnomedicine.

INTRODUCTION

Herbal medicines still remain the mainstay of about 75 to 80% of the whole population in developing countries, for primary health care because of cultural acceptability (Parekh and Chanda, 2006). Each culture or community within an area, whether large or small, has its own ethnobotanical perspective which differs from one another. Within Capricorn District of the Limpopo province in South Africa, *Waltheria indica* is indigenously called "*Mokhutesela*" and is used for the treatment of sexually transmitted infections, urinary tract infections, and a variety of infant illnesses. Elsewhere, its roots' extracts are reported to treat ailments such as diarrhoea, wounds and stomach ache (Ayantunde et al., 2009), while leaves are used as purgatives (Ganesan et al., 2009). Whole plant may be used to treat cough, haemorrhage, fever, and malaria amongst others (Olowokudejo et al., 2008; Diallo et al., 1999).

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W. indica L. belongs to the family Sterculiaceae. It is an erect perennial shrublet 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, in scrub forests, inundated savannas, riverbanks, sandy or clay soils, and in disturbed or impoverished soils (Saunders, 2007).

Roots extracts have been reported to be highly active against *Escherichia coli*, *Pseudomonas aeruginosa, Salmonella typhi* (Zailani et al., 2010), and trypanosome parasites (Bala et al., 2011). Flavonoids such as epicatechin, quercetin, and tiliroside were isolated from whole plant extract and dose independently inhibits production of inflammatory mediator nitric oxide (NO), cytokines (TNF)-α, and interleukin (IL)-12, in lipopoly-saccharide and interferon activated murine peritoneal macrophages, without any cytotoxicity (Rao et al., 2005). Ethanolic extracts of stems, roots, and leaves have been reported to possess potent activity against a variety of gram negative strains, with the largest zone of inhibition of 15 mm against *Citrobacter freundii* (Olajuyigbe et al.,

2011).

Free radicals like reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive chlorine species (RCS) are produced *in vivo* from various biochemical reactions including respiratory chain. They are also introduced into the body from outside harmful chemicals in the environment [such as ultra violet (UV) light, radiation, smoking, and air pollution], unhealthy foods, stress, certain drugs and others. Currently, available synthetic antioxidants like butylated hydroxyl anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinone and gallic acid esters have been suspected to cause or prompt negative health effects (Mon et al., 2011); hence, there is a need to find medicinal plants with antioxidant properties. This paper aims at investigating the antibacterial activity and free radical scavenging activity of *W. indica* L.

MATERIALS AND METHODS

Plant materials and extraction

W. indica roots were collected from William Show farm within Capricorn District, Limpopo Province, Republic of South Africa. Voucher specimen NI 005 was collected and identified at South African Biodiversity Institute in Pretoria with GENSPEC NO. 1850, where voucher specimen is kept. Roots were washed with distilled water to remove the adhering soil, cut into small pieces, dried in the shade, and ground into powder (2 mm mesh) using hammer mill. The dry powder was separately extracted (1:5 w/v) with boiled tap water, methanol, ethanol, and acetone by incubating the mixture on a mechanical shaker (60 rpm) for 24 h at room temperature. Extracts were filtered through Whatman No. 1 paper and the organic solvent extracts were concentrated using rotary evaporator while aqueous extract was freeze dried. Dry extracts were kept refrigerated at 4°C until needed.

Bacterial strains used

A combination of ATCC, clinical isolates and multi-resistant strains were obtained from Department of Biochemistry and Microbiology, University of Zululand and Lancet Laboratory, Richards Bay. Five gram negative strains namely *Pseudomonas aeruginosa* (T3374), *Klebsiella pneumoniae* (517298), *Proteus vulgaris* (clinical isolate), *Shigella flexineri* (clinical isolate) and *Salmonella spp* (clinical isolate), and five gram positive strains namely *Enterococcus faecalis* (clinical isolate), *Bacillus cereus* (ATCC 10702), *Bacillus subtilis* (clinical isolate), *Streptococcus viridans* (517141) and *Staphylococcus aureus* (B10808) were selected for this study. All organisms were maintained on Muller Hinton agar plates.

Antibacterial test using disc diffusion

Plant extracts were tested for antibacterial activity by the disc diffusion method as stipulated in the 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 adjusted to that of 0.5 McFarland's standard.

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 filter paper discs of 5 mm were impregnated with 10 µl of 5, 10 and 20 mg/ml plant extracts 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 overnight.

Zones of inhibition, including sterile paper disc, were measured using caliper. Streptomycin (10 µg) 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) and minimal bactericidal concentrations (MBC)

Extracts showing activity in disc diffusion at 10 mg/ml were selected for the minimal inhibitory concentration assay using the micro plate broth dilution assay (Eloff, 1998) with slight modification. The 24 h old culture was diluted 1:50 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 overnight at 37°C. About 40 µl of 0.2 mg/ml freshly prepared iodo-nitro-tetrazolium chloride (Fluka) were added to each well and incubated for 30 min 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. In the MBC, a loopful of the bacteria from wells showing little or no growth in the MIC were further subcultured on petri plates containing freshly prepared Muller Hinton Agar for 24 h at 37°C. MBC was defined as the lowest concentration that showed no bacterial growth in the subcultures (N'guessan et al., 2007).

2, 2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging activity

DPPH scavenging activity of the methanol extract of the plant was carried out according to the method previously described (Opoku et al., 2002). Decolourisation of DPPH (purple) upon addition of the extract indicated radical scavenging activity, and this was measured after 30 to 60 min at 514 nm. Ascorbic acid (Merck) was used as a positive control. Percentage of inhibition was calculated as:

$$\% \text{ Scavenging Inhibition} = [1-A_t/A_0] \times 100$$

Where A_t represent the absorbance of the test sample, while A_0 represent absorbance of fully oxidized solution

RESULTS AND DISCUSSION

Antibacterial activity

Antibacterial activity of *W. indica* extracts is shown in Tables 1 to 3. Lowest zone of inhibition was exhibited by 5.0 mg/ml water extract at 8.9 ± 0.79 mm against *S. aureus* while methanol extract at 20 mg/ml showed largest zone of inhibition of 20.2 ± 0.57 mm against *E. faecalis*. Water extracts showed least activity against the selected bacterial strains. It is apparent that traditional healers (who normally use water as solvent for their preparations) could be missing out some of the active compounds that are present in the plant (Kelmanson et al., 2000). All extracts were active against *E. faecalis* and

Table 1. Antibacterial activity of *W. indica* L. using disc diffusion method, (n = 3).

Concentration (mg/ml)	*Pseu aeru*	*Kleb pneu*	*Shig flex*	*Salm spp*	*Prot vulg*	*Ente faec*	*Bacc cere*	*Bacc subt*	*Stre viri*	*Stap aure*
Water										
5	na	na	na	na	na	9.9 ± 0.57	na	na	na	8.9 ± 0.79
10	na	11.8 ± 0.63	na	na	na	11.2 ± 0.84	na	na	na	11.1 ± 0.81
20	na	12.2 ± 0.81	9.1 ± 0.64	na	na	12.0 ± 0.66	12.6 ± 1.06	13.8 ± 2.46	12.2 ± 0.42	10.4 ± 0.46
Methanol										
5	10.2 ± 1.02	9.9 ± 0.73	11.1 ± 0.18	na	10.0 ± 1.29	13.5 ± 0.71	9.7 ± 0.72	11.0 ± 1.26	na	10.9 ± 0.89
10	11.3 ± 1.17	10.9 ± 0.93	11.9 ± 0.44	13.6 ± 0.38	13.2 ± 1.13	12.3 ± 0.68	9.9 ± 1.38	10.8 ± 0.25	na	11.4 ± 1.76
20	12.1 ± 1.67	11.9 ± 1.11	13.4 ± 0.44	15.5 ± 0.82	15.2 ± 2.18	20.2 ± 0.57	14.9 ± 1.20	17.0 ± 1.74	na	12.5 ± 1.50
Ethanol										
5	11.4 ± 0.72	9.4 ± 1.0	10.8 ± 0.80	12.2 ± 0.67	na	10.5 ± 0.82	10.8 ± 0.20	11.5 ± 0.79	na	12.9 ± 0.26
10	11.2 ± 1.10	9.9 ± 0.34	12.0 ± 0.47	12.6 ± 0.80	na	11.3 ± 0.84	11.7 ± 1.32	14.5 ± 2.35	na	12.4 ± 0.43
20	12.7 ± 0.41	10.8 ± 0.26	12.0 ± 0.87	15.4 ± 0.49	16.3 ± 1.47	13.9 ± 0.95	17.2 ± 0.90	15.6 ± 1.66	11.4 ± 1.05	14.4 ± 1.12
Acetone										
5	10.4 ± 0.15	10.4 ± 0.90	na	na	na	10.4 ± 0.03	na	na	na	10.1 ± 0.85
10	10.9 ± 0.67	11.4 ± 0.98	na	12.3 ± 0.56	11.0 ± 1.53	11.3 ± 0.74	14.4 ± 1.07	12.4 ± 0.82	na	10.5 ± 0.44
20	13.1 ± 0.68	11.6 ± 0.62	14.2 ± 0.60	13.4 ± 0.09	13.8 ± 0.68	15.9 ± 1.99	14.2 ± 0.60	13.2 ± 1.21	na	11.6 ± 0.55
Streptomycin (10 µg disc)	13.1 ± 0.68	14.8 ± 0.32	12.3 ± 1.20	21.7 ± 1.67	17.6 ± 0.49	12.7 ± 0.88	20.6 ± 0.81	16.3 ± 1.20	16.5 ± 1.41	14.0 ± 0.39

Results were recorded as mean of three replicates ± SE. Key: *Pseu aeru-Pseudomonas aeruginosa, Kleb pneu-Klebsiella pneumoniae, Shig flex-Shigella flexineri, Salm spp-Salmonella spp , Prot vulg-Proteus vulgaris, Ente face- Enterococcus faecalis, Bacc cere- Baccilus cereus, Bacc subt- Baccilus subtilis, Stre viri-Streptococcus viridans , and Stap aure-Staphylococcus aureus.*

S. aureus at three tested concentrations. These organisms are known to cause infective endocarditis which is a serious complication of bacteremia (Kamalakannan et al., 2007). *Enterococcus spp.* and *P. aeruginosa* were reported to contribute 8.5 and 10.7% of infections in hospitals, respectively (Hryniewicz et al., 2001). Such infections may lead to increase in resistance among urinary tract pathogens to conventional drugs and is a major health concern. These resistances may lead to local communities resorting to medicinal plants. Although *S. viridans* was the most resistant organism, it showed water extract activity at 20 mg/ml. Although *S. viridans* is mostly prevalent in oral cavity, it may reside in the upper respiratory tract and can lead to life threatening diseases which include endocarditis and pneumonia (Tunkel and Sepkowitz, 2002; Refoua et al., 2005). Methanol extract showed significant activity against selected human pathogens and this may be attributed to the presence of soluble phenolic and polyphenolic compounds (Igbinosa et al., 2009). Ethanol extract at 20 mg/ml exhibited best activity against all the

Table 2. Minimal concentrations (mg/ml) of *W. indica* L. Root (n = 3).

Extract	*Pseu aeru*	*Kleb pneu*	*Shig flex*	*Salm* spp.	*Prot vulg*	*Ente faec*	*Bacc cere*	*Bacc subt*	*Stre viri*	*Stap aure*
water	-	2.60	-	-	-	5.21	-	-	-	> 10
Methanol	3.13	0.65	3.65	> 10	2.08	1.30	0.52	1.30	-	4.17
Ethanol	6.25	1.04	4.12	6.25	-	1.04	0.65	1.04	-	6.25
Acetone	> 10	1.82	-	> 10	1.90	1.30	1.56	1.82	-	6.25
Streptomycin sulphate	0.02	0.01	0.03	0.02	0.03	0.03	0.02	0.03	0.04	0.04

Results were recorded as mean of three replicates. Key: *Pseu aeru-Pseudomonas aeruginosa, Kleb pneu-Klebsiella pneumoniae, Shig flex-Shigella flexineri, Salm spp-Salmonella spp , Prot vulg-Proteus vulgaris, Ente face- Enterococcus faecalis, Bacc cere- Baccilus cereus, Bacc subt- Baccilus subtilis, Stre viri-Streptococcus viridans , and Stap aure-Staphylococcus aureus.*

Table 3. Minimum bactericidal concentrations (mg/ml) of *Waltheria indica* L. root (n = 3).

Extract	*Pseu aeru*	*Kleb pneu*	*Shig flex*	*Salm spp*	*Prot vulg*	*Ente faec*	*Bacc cere*	*Bacc subt*	*Stre viri*	*Stap aure*
Water	-	4.17	-	-	-	6.25	-	-	-	>10
Methanol	4.17	6.25	6.25	>10	2.08	4.17	2.08	1.30	-	4.17
Ethanol	4.17	4.17	6.25	4.17	-	1.90	0.65	6.25	-	6.25
Acetone	>10	4.17	-	>10	6.25	6.25	1.56	4.17	-	>10
Streptomycin sulphate	0.02	0.02	0.05	0.03	0.03	0.02	0.02	0.04	0.02	0.04

Results were recorded as mean of three replicates. Key: *Pseu aeru-Pseudomonas aeruginosa, Kleb pneu-Klebsiella pneumoniae, Shig flex-Shigella flexineri, Salm spp-Salmonella spp , Prot vulg-Proteus vulgaris, Ente face- Enterococcus faecalis, Bacc cere- Baccilus cereus, Bacc subt- Baccilus subtilis, Stre viri-Streptococcus viridans , and Stap aure-Staphylococcus aureus*

selected organisms, hence broad spectrum. There were no zones of inhibition in negative controls; *K. pneumoniae* is the most susceptible gram negative bacteria. Generally, *W. indica* extracts, inhibits a variety of bacterial strains in a dose dependent manner and similar pattern has been reported elsewhere (Pandey et al., 2011). In all tested extracts, maximum inhibition was mostly shown at highest concentration of 20 mg/ml and at 10 mg/ml the moderate inhibition, while 5 mg/ml exhibited minimum inhibition. All the selected organisms were susceptible to streptomycin, with zones of inhibition ranging from 12.3 ± 1.20 (*Shigella flexineri)* to 21.7 ± 1.67 (*Salmonella* spp.).

MIC of selected extracts ranged from 0.52 (*B. cereus*) to ≥ 10 mg/ml against selected organisms (Table 2).

Plant extracts showing MIC values ranging from 1.25 to 10 mg/ml has high potent (Gango'ue-Pieb'oji, 2009). In our results, methanolic and ethanolic extracts exhibited lowest MICs compared to 1.25 mg/ml against *K. pneumoniae* and *B. cereus*. Moreover, ethanolic extract exhi-bited MIC value of 1.04 mg/ml against both *E. faecalis* and *B. cereus*. All selected extracts showed good MIC values against *K. pneumoniae* and *E. faecalis*. MICs tested ranged from 0.65 to > 10 mg/ml. Lowest MBC was exhibited by ethanolic extract against *B. cereus*. Although MBC values are significant at 0.45 to 1.00 mg/ml (Maji et al., 2010), methanolic extract in our study showed good MBC value of 2.08 mg/ml against both *P. vulgaris* and *B. cereus*. These findings, in a way, validate the use of *W. indica* against variety of infectious diseases. All the selected organisms were susceptible to streptomycin sulphate.

Free radical scavenging activity of methanolic extract shows that *W. indica* has good inhibition against DPPH (Table 4). At lowest concentrations (0.06 to 0.25 mg/100 ml), methanol extract shows better or comparable inhibition compared to

Table 4. DPPH free radical scavenging activity of *W. indica* L. root (n = 3).

Extract concentration (mg/100 ml)	Methanol extract (%) scavenging activity	Ascorbic acid
0.06	10.71 ± 1.35	9.9 ± 1.74
0.08	17.17 ± 2.55	14.6 ± 2.32
0.13	28.1 ± 3.40	18.6 ± 1.95
0.25	37.07 ± 1.46	38.2 ± 2.06
0.50	65.71 ± 2.32	100 ± 0.0
0.60	70.28 ± 3.91	100 ± 0.0
0.75	75.41 ± 2.76	100 ± 0.0
1	100 ± 0.0	100 ± 0.0

ascorbic acid Although ascorbic acid completely inhibits DPPH at concentration of 0.5 mg/100 ml, methanolic extract inhibit 65.71 ± 2.32 mg/100 ml at similar concentration.

Some major secondary metabolites detected in the aqueous and powdered root extracts of *W. indica* include tannins, saponins, and cardiac glycosides (Zailani et al., 2010). Furthermore, these compounds may account to both antibacterial and free radical scavenging activity of the plant as reported in this paper. These classes of compounds are known to possess antimicrobial activity. Tannins may selectively inhibit HIV replication, and are widely known to make trees and shrubs a difficult meal for caterpillars due to its astringent taste (Ishikawa et al., 2008). Furthermore, tannins may prevent development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable (Prasad et al., 2008). Moreover, it 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). 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).

Conclusion

Although current results validates the use of *W. indica* to treat variety of human infections, hence showing good free radical scavenging activity against DPPH, there is a need to investigate its cytotoxicity, antioxidant activity using other methods, and its biological activity against agents of sexually transmitted infections.

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REFERENCES

Abukakar MG, Ukwuani AN, Shehu RA (2008). Phytochemical screening and antibacterial activity of *Tamarindus indica* pulp extract. Asia. J. Biochem. 3(2):134-138.

Ayantunde AA, Hiernaux P, Briejer M, Udo H, Tabo R (2009). Uses of local plant species by Agropastoralists in South-western Niger. Ethnobot. Res. Appl. 7:053-066.

Bala AY, Adamu T, Abubakar U, Ladan MJ (2011). Inhibition of *Trypanosoma brucei brucei* by extracts from *Waltheria indica* L. (Sleepy morning). Res. J. Paras. 6(1):53-59.

Diallo D, Hveem B, Mahmoud MA, Berge G, Paulsen BS, Maiga A (1999). An ethnobotanical survey of herbal drugs of Gourma District, Mali. Pharm. Biol. 37(1):80-91.

Eloff JN (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Plant Med. 64:711-713.

Ganesan S, Ponnuchamy M, Kesavan L, Salvaraj A (2009). Floristic compositin and practices on the selected sacred groves of Pallatty village (Reserved forest), Tamil Nadu. Ind. J.Trad. Knowl. 8(2):154-162.

Gango'ue-Pieb'oji J, Eze N, Djintchui AN, Ngameni B, Tsabang N, Pegnyemb DE, Biyiti L, Ngassam P, Koulla-Shiro S, Galleni M (2009). The *in-vitro* antimicrobial activity of some traditionally used medicinal plants against beta-lactam-resistant bacteria. J. Infect. Dev. Ctries 3(9):671-680.

Hryniewicz K, Szczypa K, Sulikowska A, Jankowski K, Betlejewska K, Hryniewicz W (2001). Antibiotic susceptibility of bacterial strains isolated from urinary tract infections in Poland. J. Antimicrob. Chemother. 47:773-780.

Igbinosa OO, Igbinosa EO, Aiyegoro OA (2009). Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn). Afr. J. Pharm. Pharmacol. 3(2):058-062.

Ishikawa K, Kato ETM, Yoshida M, Kaneko TM (2008). Morphoanatomic aspects and phytochemical screening of *Plinia edulis* (Vell.) S obral (Myrtaceae). Braz. J. Pharm. Sci. 44(3): 515-520.

Kamalakannan K, Pai RM, Johnnson LB, Gardin JM, Saravolatz LD (2007). Epidermiology and clinical outcomes of infective endocarditis in haemodialysis patients. Ann. Thorac. Surg. 83:2081-2086.

Kelmanson JE, Jager AK, van Staden J (2000). Zulu medicinal plants with antibacterial activity. J. Ethnopharmacol. 69:241-246.

Maji S, Dandapadat P, Ojha D, Maity C, Halder SK, Das Mohapatra PK, Pathak TK, Pati BR, Samanta A, Mondal KC (2010). *In Vitro* antimicrobial potentialities of different solvent extracts of ethnomedicinal plants against clinically isolated human pathogens. J. Phytol. 2(4):57-64.

Mon MM, Maw SS, Oo ZK (2011). Quantitative determination of free

radical scavenging activity and anti-tumor activity of some Myanmar herbal plants. World Acad. Sci. Eng. Tech. 75:524-530.
NCCLS, National Committee for Clinical Laboratory Standard guidelines (2001). Performance standards for anti-microbial susceptibility testing: 11th Informational Supplement, Document M100.
Ngbede J, Yakubu RA, Nyam DA (2008). Phytochemical screening for active compounds in *Canarium schweinfurthii* (Atile) leaves from Jos North, Plateau State Nigeria. Res. J. Biol. Sci. 3(9):1076-1078.
Njoku PC, Akumefula MI (2007). Phytochemical and nutrient evaluation of *Spondias mombin* leaves. Pak. J. Nutr. 6(6):613-615.
N'guessan JD, Dinzedi MR, Guessennd N, Coulibaly A, Dosso M, Djaman AJ, Guede-Guina F (2007). Antibacterial activity of the aqueous extract of *Thonningia sanguinea* against Extended-Spectrum-β-Lactamases (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* strains. Trop. J. Pharm. Res. 6(3):779-783.
Olajuyigbe OO, Babalola AE, Afolayan AJ (2011). Antibacterial and phytochemical screening of crude ethanolic extract of *Waltheria indica* L. Afr. J. Micr. Res. 5(22):3760-3764.
Olowokudejo JD, Kadiri AB, Travih VA (2008). An ethnobotanical survey of herbal markets and medicinal plants in Lagos. Ethnobot. Leaf 12:851-865.
Opoku AR, Maseko NF, Terblanche SE (2002). The *In Vitro* antioxidative activity of some traditional Zulu medicinal plants. Phytol. Res. 16:S51-S56.
Pandey P, Mehta A, Hajra S (2011). Evaluation of Antimicrobial activity of *Ruta graveolens* stem extracts by disc diffusion method. J. Phytol. 3(3):92-96.
Parekh J, Chanda S (2006). *In-vitro* antimicrobial activities of extracts of *Launaea procumbens* Roxb. (Labiateae), *Vitis vinifera* L. (Vitaceae) and *Cyperus rotundus* L. (Cyperaceae). Afr. J. Biol. Res. 9:89-93.
Prasad RN, Viswanathan S, Devi JR, Nayak V, Swetha VC, Archana BR, Parathasarathy N, Rajkumar J (2008). Preliminary phytochemical screening and microbial activity of *Samaea saman*. J. Med. Plant Res. 2(10):268-270.
Rao YK, Fang S, Tzeng Y (2005). Inhibitory effects of the flavonoids isolated from *Waltheria indica* on the production of NO, TNF-α and IL-12 in activated macrophages, Biol. Pharm. Bull. 28(5):912-915.
Refoua Y (2005). A study of *Streptococccus viridans* in the maxillofacial region. J. Dent. 2(4):174-177.
Saunders JG (2007). Sterculiaceae of Paraguay II. *Waltheria*. Bonpl.,16(1-2):143-180.
Tunkel AR, Sepkowitz KA (2002) Infections caused by *Viridans Streptococci* in patients with neutropenia. Clin. Infect. Dis. 34:1524-1529.
Van Wyk B, Malan S (1998). Field guide to the wild flowers of the Highveld. Struik publishers. Capetown, p. 178.
Zailani AH, Jada SM, Wurochekke UA (2011). Antimicrobial activity of *Waltheria indica*. J. Am. Sci. 6(12):1591-1594.