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Antibacterial activity of sixteen plant species from Phalaborwa, Limpopo Province, South Africa

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Leaves of seventeen medicinal plants used *inter alia* to treat infections were collected from Phalaborwa, Limpopo Province, South Africa. Plant species used in this investigation were *Terminalia sericea, Diospyros mespiliformis, Cordia grandicalyx, Fluggea virosa, Cassia abbreviata, Colophospermum mopane, Xanthorcesis zambesiaca, Dichrostachys cineria, Helinus integrifolius, Schotia brachypetala, Berchemia discolor, Bridelia mollis, Psidium guavaja* and *Vangueria infausta.* The antibacterial activity of acetone leaf extracts was determined against twenty different bacterial species using a serial microplate dilution method to determine the minimal inhibitory concentrations (MIC). *C. abbreviata* and *X. zambesiaca* had the highest activity against both groups of bacteria with overall average MIC values of 0.113 and 0.285 mg/ml, respectively, while *C. grandicalyx* had poor inhibition against both Gram positive and Gram negative bacteria (overall MIC value of 1.677 mg/ml). Judging from average MIC values (mg/ml), it would appear that all extracts save *C. abbreviata* had better activity against Gram positive bacteria than Gram negative bacteria. *V. infausta, D. mespiliformis, F. virosa* and *T. sericea* had good activity against Gram positive bacteria. Judging from average MIC values, it would seem that *K. oxytoca* was the most sensitive bacterium to plant extracts, while *P. mirabilis* was the most resistant. Some of these plant species are used to treat ailments associated with one or another form of infection. These finding suggests that extracts of these plants exert their medicinal actions through inhibition of growth of the pathogen.

Key words: Antibacterial activity, minimal inhibitory concentration, total activity, *Cassia abbreviate*.

INTRODUCTION

South Africans increasingly suffer from a high prevalence of infection with the human immunodeficiency virus (HIV) (Karim, 2000; UNAIDS, 2006). Infections caused by fungi (Hamza et al., 2006) and bacterial strains (WHO, 2005) frequently accompany HIV infection in many patients. Repeated efforts to treat secondary infections with antibiotic drugs at ineffective doses may transform infectious pathogens into antibiotic resistant strains (Green et al., 2010). The rapid emergence of resistant pathogenic strains, together with escalating costs of antibiotics (Debruyne, 1997) and lack of healthcare facilities in some regions of South Africa (Shai et al., 2008) point towards an urgent need for cheaper and readily accessible alternative treatments. Such alternatives may be in the form of plant-derived traditional medicines (Green et al., 2010).

Medicinal plants have been used for centuries to treat a variety of ailments (Hamilton, 2004). The World Health

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Organization reported that about 80% of the population in developing countries continue to use plant-derived remedies for healthcare purposes (WHO, 2002). This trend exists in Africa, where traditional medicine remains the only option for most people (Matsheta and Mulaudzi, 2008). Drivers for the continuous use of traditional medicine include among others, unrestricted access to traditional healers, cultural beliefs and low costs of traditional medicines (Rinne, 2001; Otshudi et al., 2000).

Many plants have been confirmed to have a variety of activities, including antimicrobial activity (Shai et al., 2008; Mathabe et al., 2006; Samie et al., 2010), anticancer activity (Gurib-Fakim, 2006), antioxidant activity (Cheung et al., 2003) and antidiabetic activity (Tanko et al., 2011). Determining the antimicrobial activity of many plant species has become the subject of recent intensive investigations. Recent studies have investigated the antibacterial activities of several plant species (Masoko et al., 2005; Masoko and Eloff, 2005; Shai et al., 2008), with the aim of isolating and characterizing of many active compounds (Eloff et al., 2008).

The bioactive compounds have been isolated from many medicinal plant species. The screening of plant extracts for presence of compounds that have medicinal properties has become an important component of drug discovery (Rabe and van Staden, 1997). Early work on plant-based compounds led to discovery of many drugs currently in primary healthcare use (Newman et al., 2000; Butler, 2004). Investigating the potential of South Africa's plant species (either randomly collected or linked to ethnomedicinal use) as antimicrobials becomes more urgent in view of the increasing destruction of indigenous flora (Gates, 2000; Goldring, 1999). This study was aimed at determining the antibacterial activities of plants collected in Phalaborwa in Limpopo Province, South Africa against 20 different bacterial species.

MATERIALS AND METHODS

Plant collection and extraction

Plant material were collected in at Mashishimale village, near Phalaborwa, in woven sacks and dried at room temperature for 7 days. Plant materials were ground to powder using a mill. Five grams (5 g) of each plant material was extracted with 50 ml acetone. The extracts were filtered and dried under a stream of air. The dried extracts were dissolved in methanol to a concentration of 10 mg/ml. Acetone was selected to extract plant material because it is volatile (easily evaporated), extracts a combination of polar and non-polar compounds and is less toxic to microorganisms than other organic solvents (Eloff, 1998). It also extracts a larger number of different compounds than many extractants (Kotze and Eloff, 2002).

Antibacterial activity

Minimal inhibitory concentrations (MIC) were determined using the serial microplate dilution method described by Eloff (1998). Bacterial cultures were supplied by Mr. Frederick Els of the Microbiology Unit of the Department of Biomedical Sciences, Tshwane University of South Africa. Bacterial species were cultured in Mueller-Hilton broth for 14 h prior to use in the assay. Gram negative bacterial species used were: *Escherichia coli*, *Klebsiela pneumonia*, *Klebsiela oxytoca*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Salmonella typhi*, *Serratia marcescens*, *Enterobacter aerogenes*, *Proteus mirabilis*, *Citrobacter fruendii* and *Proteus vulgaris.* Gram positive bacterial species used were: *Staphylococcus aureus* (SA), *Staphylococcus epidermidis, Enterococcus faecalis, Bacillus stearothermophilus, Bacillus cereus, Streptococcus pyogenes, Micrococcus luteus* and *Lactobacillus acidophilus.*

RESULTS AND DISCUSSION

The acetone extract of *Diospyros mespiliformis* yielded the highest mass of extract (250 mg). *Dichrostachys cineria* extract had the lowest mass (Figure 1). The leaves of *Schotia brachypetala* had better extractability than the stem bark, while the leaves of *Helinus integrifolius* resulted in a larger mass of extract than the roots.

Many authors consider a MIC lower than 0.1 mg/ml as significant activity (Eloff, 2004). Nearly 9% of the extracts (29) had an activity of 0.080 mg/ml or lower against the bacteria tested (Table 2). In seven cases a MIC of 0.04 mg/ml and in four cases a MIC of 0.02 mg/ml was found. The values lower than 0.16 mg/ml were printed bold in Table 2. *Xanthorcesis zambesiaca* was by far the most interesting species to follow up because against nine bacteria there were activities of 0.08 mg/ml or lower. In this case, there appeared to be little selectivity and it may be possible that this extract contain a metabolic toxin, that is, a compounds that interferes with general metabolic processes and may be toxic to human cells as well.

To evaluate which plant extract was the most active against all the bacteria and which bacterial species was the most sensitive to all the extracts the average MIC values were calculated. For those extracts with a MIC of >2.5 mg/ml, a value of 2.5 mg/ml was allocated to make the calculations possible (Tables 2 and 3).

Among acetone extracts of plants tested for antibacterial activity in this study, *Cassia abbreviata* had high activity against all the bacterial species tested, with MIC values as low as 0.04 mg/ml against *S. pyogenes* and *P. fluorescens* (Tables 2 and 3). *X. zambesiaca*, like *C. abbreviata,* had good activity against both Gram positive and Gram negative bacteria. *X. zambesiaca* and *H. integrifolius* leaf extracts had their lowest MIC values (0.02 mg/ml) against *S. typhi. Cordia grandicalyx* was the least active of all the extracts against both Gram positive and Gram negative bacteria (Tables 2 and 3). With the exception of *C. abbreviata*, all the extracts were more active against Gram positive than Gram negative bacteria (Figure 2). This trend was clearer with *Vangueria infausta* and *Combretum krausii,* with average MIC values around 0.2 mg/ml against Gram positive bacteria and over 0.6 mg/ml against Gram negative bacteria (Figure 2).

The antimicrobial activities of some of these plant

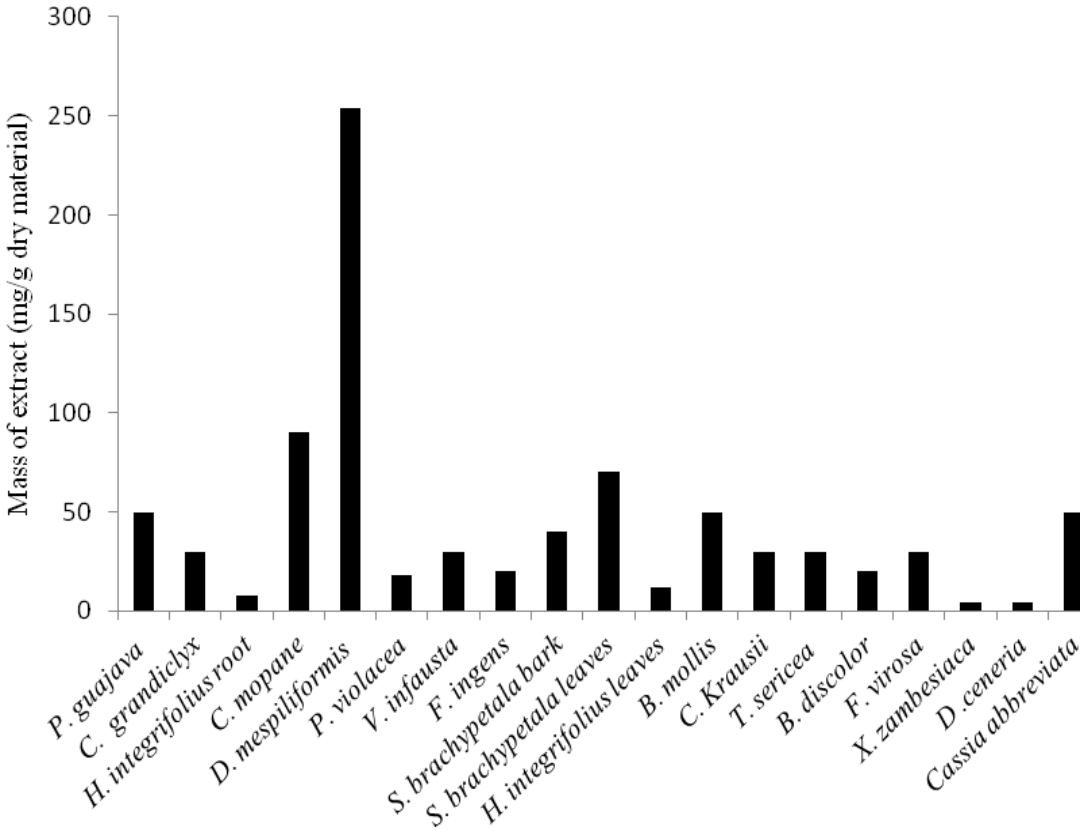

Figure 1. Mass of extracts from plant material.

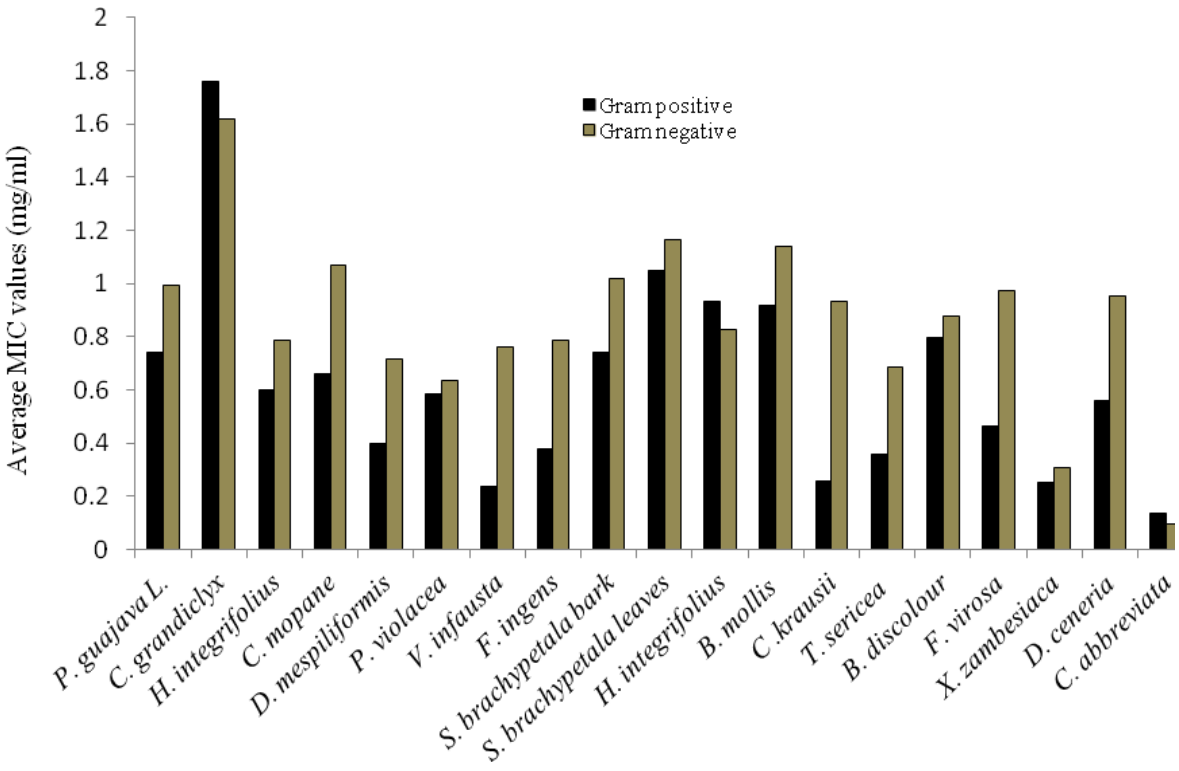

Figure 2. Average MIC values of plant extracts against all bacterial species investigated.

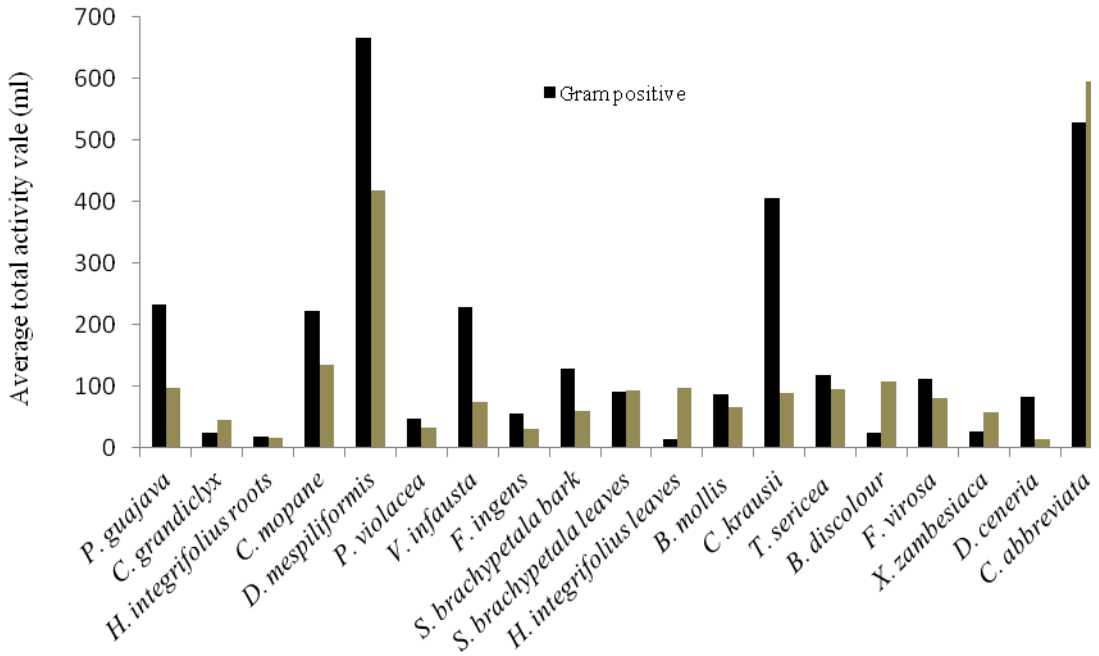

Figure 3. Average total activity of plant extracts against bacterial species tested.

species have been demonstrated in other studies. Mathabe et al. (2006) tested and confirmed antibacterial activity of *S. brachypetala* extracts against several bacterial species, with MIC values of the range 0.156-0.312 mg/ml. These MIC values are comparable to the values obtained in this study. McGaw et al. (2002) isolated fatty acids with activity against Gram negative and Gram positive bacteria from *S. brachypetala*. Our results also confirm the presence of antibacterial principles reported by Kambizi and Afolayan (2001). Furthermore, Green et al. (2010) demonstrated antimycobacterial activity of *Berchemia discolor* and *Terminalia sericea* extracts. Samie et al. (2010) reported the antifungal activities of *B. discolor, Bridelia mollis, D. mespiliformis* and *S. brachypetala* extracts. It is possible that the antifungal compounds may be responsible for the observed antibacterial activity of these extracts.

To determine which plant has the best potential to combat bacterial infections, not only is the MIC considered, but also the quantity extracted by the plant should be considered. The total activity for a plant can be calculated by dividing the mass (mg) extracted from 1 g with the MIC in mg/ml (Eloff, 2000) (Table 3). Total activity, first described by Eloff (2000), indicates the volume (ml) to which the extract (from 1 g dry plant material) can be diluted and still maintain its antimicrobial activity. The high total activity values of *C. abbreviata* (averaging over 500 ml) confirmed that extracts from this plant species had good potential to use against infections by both Gram positive and Gram negative bacteria, than any other plant species tested. *D. mespiliformis* had consistently high total activity values due to high extractability of the plant material with acetone (Tables 2 and 3). Average total activity values recorded confirmed the antibacterial activity of *C. abbreviata* stem bark extract (Figure 3). Also, these results support some traditional uses of some of the medicinal plants tested (Table 1). The identity of the bioactive compounds, safety as well as cytotoxicity of plant extracts and compounds must be confirmed. All the plants tested in the study, except *C. grandicalyx,* are used to treat one form of infection or another. *C. grandicalyx,* with no correlation to treatment of infections and ethnomedical use, had low activity against bacterial species tested. This finding suggests that ethnomedical leads are important for activity screening of plants against bacteria. These results also indicate which species should be useful for in-depth study to isolate the bioactive compounds, namely, *C. abbreviata* and *X. Zambesiaca*.

From the total activity values, it was also possible to calculate the average sensitivity of the different bacteria towards the plant extracts (Table 3). The generalization that Gram negative bacteria are more resistant to plant extracts than Gram positive bacteria did not always hold. The most sensitive was *K. Oxytoca* (Gram negative), followed by *B. stearothermophilus* (Gram positive).

Traditional methods of extraction use water as a solvent. The activity of extracts obtained using traditional methodology may be compared with extracts obtained using organic solvents such as methanol and acetone. These experiments will indicate the usefulness of these plant species to rural communities who almost exclusively rely on water as a solvent. Furthermore, the identity of the active ingredients may be important in

Table 1. Plant species selected for the study and their medicinal uses.

Plant species	Medicinal uses	References
Terminalia sericea Burch. ex DC. (COMBRETACEAE) PRU 113817	Bilharzias and stomach problems	Kokwaro (2009)
	Stomach ache and diarrhoea	Ribeiro et al. (2010)
Diospyros mespiliformis Hochst. Ex A.DC. (EBENACEAE) PRU 117185	Stomach ache	Kokwaro (2009)
	Male sexual dysfunction	Cheikhyoussef et al. (2011)
	Tuberculosis	Green et al. (2010)
Cordia grandicalyx	None listed in literature	-
Fluggea virosa (Roxb. Ex Wild) Voigt (PHYLLANTHACEAE) PRU 115081	Abscesses	Ribeiro et al. (2010)
	Pneumonia and contraceptive	Maroyi (2011)
Cassia abbreviata Oliv. (FABACEAE) PRU 113819	Eye wash, stomach ache, diarrhoea, malaria	Ribeiro et al. (2010)
	Aphrodisiac, abortion, constipation, gonorrhoea	Maroyi (2011)
Colophospermum mopane (Benth.) Leonard	Bleeding and dysentery	Ribeiro et al. (2010)
	Stomach ache and swollen legs	Cheikhyoussef et al. (2011)
Dichrostachys cineria (L.) Wight & Am (FABACEAE) PRU 113836	Skeletal disorders and laxative	Ribeiro et al. (2010)
Helinus integrifolius (Lam.) Kuntze RAMNACEAE PRU 117192	Leg pains and stroke	Cheikhyoussef et al. (2011)
Schotia brachypetala Sond. (CAESALPINACEAE) PRU 113839	Diarrhoea	Mathabe et al. (2006)
	Dysentery	Hutchings et al. (1996)
Berchemia discolor (Klotzsch) Hemsl. (RAMNACEAE) PRU 117188	Infertility and menorrhagia	Arnold and Gulumian (1984)
Bridelia mollis Hutch. (EUPHORBIACEAE) PRU 117187	Cough	Maroyi (2011)
Psidium guavaja L. (MYRTACEAE)	Cough, flu and fever	Maroyi (2011)
Vangueria infausta Burch (RUBIACEAE) PRU 113837	Diarrhoea	Maroyi (2011)
Xanthorcesis zambesiaca (Baker) Dumaz-le-Grand (FABACEAE) PRU 115078	Diarrhoea	Shai (2012) Personal communication after interviewing a traditional healer in Phalaborwa

Table 2. Minimal inhibitory concentrations of plant extracts against Gram positive and Gram negative bacteria.

Plant	Gram positive bacteria (MIC in mg/ml)									Gram negative bacteria (MIC in mg/ml)									
	BS	SP	BC	EF	SE	ML	SA	LA	EE	PM	KP	EC	PF	PA	PV	CF	ST	KO	SM
P. guajava L.	0.62	**0.08**	0.62	>2.5	1.25	0.16	**0.08**	0.62	1.25	0.62	2.5	0.94	1.25	0.62	0.16	1.25	0.94	0.16	1.25
C. grandicalyx	1.25	1.25	>2.5	>2.5	>2.5	1.25	0.31	>2.5	1.25	>2.5	>2.5	0.46	>2.5	0.62	>2.5	>2.5	0.31	0.16	>2.5
H. integrifolius roots	0.62	0.31	0.62	0.62	0.16	0.62	0.62	1.25	0.31	1.25	1.25	0.23	0.62	0.62	1.25	1.25	0.46	0.16	1.25
H. integrifolius leaves	0.31	0.62	1.25	ND	>2.5	0.62	0.62	0.62	1.25	1.25	0.31	1.25	1.25	0.04	2.5	0.62	0.02	0.31	0.31
C. mopane	0.31	0.31	0.31	0.31	>2.5	0.62	0.31	0.62	0.31	2.5	1.25	0.71	1.25	0.62	2.5	1.25	0.46	0.31	0.62

Table 2. Contd.

D. mespiliformis	0.08	0.31	0.31	0.62	0.62	0.62	0.31	0.31	0.62	1.25	0.62	0.94	0.62	0.62	1.25	0.62	0.39	0.31	0.62
V. infausta	0.08	0.04	0.16	0.31	0.31	0.31	0.08	0.62	0.62	2.5	0.62	0.19	0.62	1.25	0.16	1.25	0.23	0.31	0.62
F. ingens	0.08	0.31	0.62	0.62	0.31	0.31	0.16	0.62	1.25	1.25	0.62	0.71	0.62	1.25	0.62	0.62	0.62	0.31	0.62
S. brachypetala bark	1.25	0.16	0.31	0.31	2.5	0.62	0.16	0.62	>2.5	0.62	1.25	0.94	0.62	2.5	0.62	0.62	0.94	0.31	0.31
S. brachypetala leaves	0.31	0.62	0.62	0.62	2.5	0.62	0.62	2.5	2.5	1.25	0.62	0.94	1.25	2.5	1.25	0.62	1.25	0.31	0.31
B. mollis	0.31	0.62	1.25	1.25	0.16	>2.5	0.62	0.62	2.5	1.25	0.62	0.39	1.25	0.62	>2.5	1.25	1.25	0.31	0.62
C. krausii	0.31	0.04	0.62	0.08	0.62	>2.5	0.08	0.31	2.5	2.5	1.25	0.19	0.31	1.25	0.08	0.31	0.94	0.31	0.62
T. sericea	0.31	0.31	0.31	0.31	0.62	0.31	0.08	0.62	1.25	2.5	1.25	0.16	0.31	0.16	0.62	0.31	0.23	0.16	0.62
B. discolor	0.16	1.25	1.25	1.25	0.62	0.62	0.62	0.62	0.04	2.5	0.62	0.23	2.5	2.5	0.31	0.31	0.39	0.16	0.08
F. virosa	ND	0.62	ND	0.31	0.62	0.31	ND	ND	1.25	1.25	0.31	ND	0.62	ND	2.5	1.25	ND	0.31	0.31
X. zambesiaca	0.16	0.31	0.08	0.16	0.08	0.31	0.62	0.31	0.04	0.08	0.08	1.25	0.04	0.16	0.08	1.25	0.02	0.31	0.08
D. ceneria	0.08	0.16	0.02	0.16	>2.5	0.31	0.02	1.25	1.25	0.62	1.25	0.31	1.25	1.25	1.25	0.62	0.04	0.16	>2.5
C. abbreviata	0.08	0.04	0.08	ND	0.12	0.16	0.16	0.31	0.08	0.08	0.08	0.08	0.04	0.08	0.16	0.08	0.16	0.16	0.08
Gentamicin (µg/ml)	0.2	0.8	0.4	ND	0.4	0.2	0.8	0.2	ND	0.4	0.2	0.2	0.4	0.3	0.8	0.2	0.8	0.8	0.2

Table 3. Total activity of plant extracts against Gram positive and Gram negative bacterial species.

Plant	Gram positive bacteria (Total activity)									Gram negative bacteria (Total activity)									
	BS	**SP**	**BC**	**EF**	**SE**	**ML**	**SA**	**LA**	**EE**	**PM**	**KP**	**EC**	**PF**	**PA**	**PV**	**CF**	**ST**	**KO**	**SM**
P. guajava L.	81	625	81	20	40	313	625	81	40	81	20	53	40	81	313	40	53	312	40
C. grandicalyx	12	24	12	12	12	24	97	12	24	12	12	65	12	48	12	12	97	187	12
H. integrifolius roots	13	26	13	13	50	13	13	6	26	6	6	35	13	13	6	6	17	50	6
H. integrifolius leaves	10	19	10	ND	5	19	19	19	10	10	39	10	10	300	5	19	600	39	39
C. mopane	290	290	290	290	36	145	290	145	290	36	72	127	72	145	36	72	196	290	145
D. mespiliformis	819	819	819	410	410	410	819	819	410	203	410	270	410	410	203	410	651	819	410
P. violacea	58	14	58	14	113	29	58	29	58	14	29	23	29	29	29	29	29	58	29
V. infausta	188	750	188	97	97	97	375	48	48	12	48	158	48	24	188	24	130	97	48
F. ingens	32	65	32	32	65	65	125	32	16	16	32	28	32	16	32	32	32	65	32
S. brachypetala bark	129	250	129	129	16	65	250	65	16	65	32	43	65	16	65	65	43	129	129
S. brachypetala leaves	113	113	113	113	28	113	113	28	28	56	113	75	56	28	56	113	56	226	226
B. mollis	40	81	40	40	313	20	81	81	20	40	81	128	40	81	20	40	40	161	81
C .krausii	48	750	48	375	48	1500	375	97	12	12	24	158	97	24	375	97	32	97	48
T. sericea	97	97	97	97	48	97	375	48	24	12	24	188	97	188	48	97	130	188	48
B. discolor	16	16	16	16	32	32	32	32	500	8	32	87	8	8	65	65	51	125	250
F. virosa	ND	74	ND	148	74	148	ND	ND	37	37	148	ND	74	ND	18	37	ND	148	148

Table 3. Contd.

X. zambesiaca	50	13	50	25	50	13	7	13	100	50	50	3	100	25	50	3	200	13	50
D. ceneria	200	25	200	25	2	13	200	3	3	7	3	13	3	3	3	7	100	25	2
C. abbreviata	625	125	625	ND	417	313	313	16	625	625	625	625	1250	625	625	313	313	313	625
Average value	157	220	157	109	98	180	232	87	120	69	95	116	129	115	113	78	154	176	125

further investigations to establish the mechanisms used by some of these plants.

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