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Antibacterial and antifungal activities of methanol extracts of *Desmodium adscendens* root and *Bombax buonopozense* leaves

Bolanle A. ADENIYI [1], Kingsley C. IZUKA [1], Bamidele ODUMOSU [1] and Olapeju O. AIYELAAGBE [2*]

[1]*Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan, 200284 Nigeria.*
[2]*Department of Chemistry, University of Ibadan, Ibadan, 200284 Nigeria.*
[*] *Corresponding author, E-mail: oaiyelaagbe@yahoo.com*

ABSTRACT

In-vitro antibacterial and antifungal activities of methanol extracts of *Desmodium adscendens* (Sw.) DC root and *Bombax buonopozense* P. Beauv. leaves against some bacterial and fungal isolates implicated in oro-dental, urogenital and other opportunistic infections were investigated using standard microbiological methods. The extracts of *D. adscendens* exhibited significant inhibitory action against all twelve organisms tested at a concentration of 2.00 mg/ml, while that of *B. buonopozense* inhibited nine organisms at the same concentration and conditions. The minimum inhibitory concentration (MIC) exhibited by extract of *D. adscendens* ranged between 0.0625 and 0.25 mg/ml while that of *B. buonopozense* ranged between 0.125 and 0.50 mg/ml. Phytochemical analysis of the two plant extracts revealed the presence of tannins and saponins while *D. adscendens* has alkaloids and flavonoids in addition. Between 92 and 100% of *Staphylococcus aureus* SA 1199 were killed within 120 minutes of contact time with a concentration of 0.25 - 0.50 mg/ml, while 98 - 100% of *Candida albicans* ATCC 90029 were killed within the same period and concentration by the same extract (*D. adscendens*). Therefore, this study justified the antimicrobial properties of these plants, hence the plants could be recommended for use as source of new drugs for fungal and bacterial infections.

Keywords: Desmodium adscendens, Bombax buonopozense, antibacterial, antifungal, Minimum Inhibitory Concentration

INTRODUCTION

Desmodium adscendens (Sw.) DC. belongs to the family "Fabaceae" and to the genus *Desmodium* which comprises a total of 350 to 450 species. The plant is commonly called Beggar- lice, Beggar weed, Tick Clover or Tick trefoil (Taylor, 2005). *D. adscendens* is a weedy, perennial herb that grows to 50 cm tall and produces numerous light-purple flowers and green fruits in small, beanlike pods. It is indigenous to many tropical countries and grows in open forests, pastures and along the road sides (Taylor, 2005). Traditionally, *D. adscendens* has been used to treat asthma, bronchitis and central nervous system disorders (Taylor, 2005); vaginal infections (Barreto et al., 2002), promote lactation in women, treat wounds and sores, malaria and diarrhea (Addy, 1997), ovarian and uterine problems (Evans, 2002) by various ethnic groups. Chemical constituents of *D. adscendens* include flavonoids,

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alkaloids, saponins, and astragalin which is highly antimicrobial in action.

Bombax buonopozense belongs to the family *Bombacaceae*. It is a large tropical tree that grows to 40 m in height with large buttress roots. The bark is covered in large, conical spines which are later shed off to some degree as the plant ages. There have been some claims that *B. buonopozense* is used to treat some viral infections, venereal diseases, constipation and swelling. Mann et. al. (2011) also reported the antimicrobial activity of the flowers of Nigerian *B. buonopozense* against *Staphylococcus aureus, Escherichia coli* and *Aspergillus niger*. In addition the flower and fruits are used as food (Beentje et al., 2001). Some chemical constituents found in this plant include tannins and saponins. In spite of the work done on the biological potentials of these plants; as far as we know, there is no report on the antimicrobial activities of the roots of *D. adscendens* and leaves of *B. buonopozense* on all the opportunistic pathogens investigated. In this study, the *in-vitro* antimicrobial activities of crude extracts of *D. adscendens* root and *B. buonopozense* leaves against some bacteria and fungi that are implicated in various opportunistic infections were investigated.

MATERIALS AND METHODS

Plant material and preparation of extracts

Fresh roots of *D. adscendens* (FHI 108301) and leaves of *B. buonopozense* (FHI 108415) were collected from Ibadan, Nigeria, in October 2008 and authenticated at the Herbarium of the Forestry Research Institute of Nigeria (FRIN) Ibadan. The plant materials were later air-dried, pulverized using an electric blender and stored in airtight containers for further use. Exactly 500 g each of the pulverized root of *D. adscendens* and 100 g of *B. buonopozense* leaves were successively hot extracted using soxhlet apparatus with hexane and methanol for eight hours. After this, the solvents were recovered, extracts dried in-vacuo, aseptically measured and stored in a refrigerator until assayed.

Preparation of isolates for the experiment:

Standard strains of *Candida albicans* ATCC 90029, *Candida parapsilosis* ATCC 22019, *Candida krusei* ATCC 6825, *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* SA 1199, *Staphylococcus aureus* ASXY 212, *Enterobacter cloacae* ATCC 49141, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 35657, *Pseudomonas aeruginosa* ATCC 27853 and *Acinetobacter baumannii* ATCC 747, were obtained from the laboratory stock and subcultured in Mueller Hinton broth and agar.

Phytochemical analysis of the plant extract

Small portions of the dried extract were used for preliminary phytochemical screening test for the presence of tannins, saponins, alkaloids and flavonoids (Harborne, 1998).

In-vitro assay

The antibacterial and antifungal sensitivity assay of the crude extracts were determined using agar-well diffusion method (Adeniyi et al., 1996). The bacteria and fungi isolates used in this study were first grown for 18 h (for bacteria) and 48 h (for fungi) in Mueller Hinton broth and standardized to 0.5 McFarLand standards (10^6 CFU/ml). Bacterial seeded plates were prepared by inoculating 200 µl of the standardized cell suspensions into molten agar bottles maintained at 45 ^oC after which it was mixed thoroughly, poured and allowed to set while fungal carpeted plates were prepared by spreading 200 µl of the standardized cell suspensions and allowed to dry. Wells were then bored into the agar using a sterile 7 mm cork borer. Approximately 100 µl of the crude extracts at concentration of 2.00 mg/ml were introduced into the wells, allowed to stand at room temperature for about 2 h and then incubated

at 37 ^{o}C and 25 ^{o}C for bacteria and fungi respectively. The plates were observed for zones of inhibition after 24 h and 48 h for bacteria and fungi respectively. The effects were compared with that of gentamicin at a concentration of 12.5 µg/ml and ketoconazole for fungi at a concentration of 4 mg/ml.

Determination of the minimum inhibitory concentrations (MIC)

The MICs of the extracts were determined using agar dilution method. 19 ml of a sterile molten Mueller Hinton agar maintained at 45 ^{o}C was added to 1 ml volume of the dissolved extract of the following concentration 2.00 mg/m1, 1.00 mg/m1, 0.50 mg/m1, 0.125 mg/m1, 0.0625 mg/m1, 0.03125 mg/m1 and 0.015625 mg/ml. They were properly mixed for even distribution of the extracts within the agar medium. The mixtures were poured and allowed to set. The plates were then dried to remove steam. 100 µl of the cell suspensions were inoculated to each concentration of the solidified agar-extract mixture in duplicates. Controls were prepared by inoculating plates without the extracts with the cell suspensions. The plates were then examined for the presence of colonies after the incubation period of 24 h and 48 h at 37 ^{o}C and 25 ^{o}C for bacteria and fungi respectively. The least concentration that gave no visible colonies was taken as the minimum inhibitory concentration of the extract for the particular dilution of the organism.

Determination of the Minimum bactericidal / fungicidal concentration (MBC/MFC)

The (MBC/MFC) of the plant extracts were determined by a modification of the method of Adeniyi et al. (2000). To a 0.5 ml extract at different concentrations as used in the MIC assay that showed no visible growth on the agar plates, was added 0.5 ml of test organism in tubes. These were incubated at 37 ^{o}C and 25 ^{o}C for 24 h and 48 h for bacteria and fungi respectively. After the incubation period, the mixture in the tubes were streaked out from the tubes on to Nutrient agar, Sabourand dextrose agar and Mueller Hinton agar to determine the minimum concentration of the extract required to kill the organisms. These concentrations were indicated by failure of the organisms to grow on transfer to these media plates. The lowest concentration that prevented bacterial/fungal growth after days of incubation was recorded as the minimum bactericidal/fungicidal concentration (MBC/MFC). All tests were performed in duplicates to ensure accuracy. Agar plates without extracts and another agar plate without any inoculated organism were also incubated serving as positive and negative control plates respectively.

Determination of the bactericidal/ fungicidal activity of the plant extract

This was determined using the viable counting technique. 0.5 ml of each culture was subcultured into a warm (37 ^{o}C) 4.5 ml. Tryptone Soy broth and incubated for 90 min using a Gallenkamp orbital incubator to give a logarithmic phase culture. 0.1 ml of the logarithmic phase culture was then inoculated into a warm 4.9 ml of Tryptone Soy broth containing the tested compound to give 1 in 50 dilution of the culture (equivalent to approximately 1x 10^{7} colony forming units) and the required concentration of the extract. An appropriate quantity of the test sample (extract culture mixture) was withdrawn immediately, diluted out in normal saline and two drops of each dilution plated into an oven dried Mueller Hinton agar to give control time 0 minute count. Samples were taken at an interval of 30, 60, 120, 180, 240 and 360 minutes respectively. The procedure was carried out in duplicates to ensure reproducibility. Plates were incubated at 37 ^{o}C for 24 - 48 h (for bacteria) and at 25 ^{o}C for 48 - 72 h (for fungi) before counting the colonies. Control plates for negative and positive were also incubated. The number of colony forming unit were counted after the period of incubation. The numbers of surviving bacterial cells per ml were calculated by taking into consideration the diluting factor

and the volume of the inoculums. All the procedure was repeated for 1/4x MBC, 1/2x MBC, MBC and 2 MBC respectively.

RESULTS

All the crude extracts of the three plants tested showed varying degrees of antimicrobial activities against the tested bacterial and fungal species (Tables 1, 2 and 3). The antimicrobial activities of the plant extracts compared favorably with that of control drugs (gentamicin for bacteria and ketoconazole for fungi) and appeared to be broad in nature as its activity were independent on Gram reaction. The order of antimicrobial (in terms of broad spectrum) activities of the extracts is *D. adscendens* > *B. buonopozense*. The MIC of *D. adscendens* extract ranged between 0.0625 and 0.125 mg/ml while that of *B. buonopozense* ranged between 0.125 and 0.50 mg/ml each (Table 3).

Phytochemical analysis of the plants revealed the presence of tannins, saponins, steroids and alkaloids (Table 4).

The MIC and MBC of various antibiotics on susceptible bacteria are presented in Table 5. Figures 1 and 2 show the kinetic kill curve of *Desmodium adscendens* on *Staphylococcus aureus* SA 1199 and *Candida albicans* ATCC 90029, respectively.

Table 1: The antimicrobial activities of methanol extracts of *D. adscendens* and *B. buonopozense.*

Test Organism	Zone of Inhibition (mm) at 2 mg/ml		
	D. adscendens	***B. buonopozense***	**Control***
S. aureus ATCC 29213	15±1.30	0±0.00	19±0.00
S. aureus SA 1199	17±0.00	0±0.00	25±1.00
S. aureus ASXY 212	25±0.10	0±0.00	20±0.00
P. aeruginosa ATCC 27853	20±0.00	16±0.50	15±0.50
E. cloacae ATCC 49141	12±0.50	12±1.00	23±0.00
E. faecalis ATCC 29212	35±0.00	18±0.00	21±0.00
E. coli ATCC 25922	15±1.50	12±0.00	25±0.00
C. parapsilosis ATCC 22019	15±0.00	12±1.00	17±0.50
C. albicans ATCC 90029	15±1.50	12±0.00	18±0.00
C. krusei ATCC 6825	18±2.00	13±0.50	20±0.50
K. peumoniae ATCC 35657	10±0.50	16±0.00	25±0.00
A. baumannii ATCC 747	12±0.50	15±0.50	12±0.00

Values are mean; ± = standard error of the mean. Diameter of cork borer = 7 mm.

* Gentamicin ,12.5 µg/ml for bacteria and Ketoconazole 4 mg/ml for fungi.

Table 2A: Susceptibility of the standard strains microorganisms to various antibiotics.

Test Organism	Diameter zone of inhibition (mm) Gram Negative Disc						
	AMX 25µg	**COT 25µg**	**NIT 300µg**	**GEN 10µg**	**NAL 30µg**	**OFL 30µg**	**TET 30µg**
S. aureus ATCC 29213	0±0.00	10±1.00	21±2.50	15±0.00	15±0.50	17±0.50	0±0.00
P aeruginosa ATCC 27853	0±0.00	11±0.50	19±1.00	0±0.00	0±0.00	30±0.50	10±0.5
E. cloacae ATCC 49141	0±0.00	17±0.50	16±0.00	17±1.50	16±0.50	30±0.00	10±2.50
F. faecalis ATCC 29212	0±0.00	15±1.50	24±0.50	10±1.00	0±0.00	20±0.00	0±0.00
E. coli ATCC 25922	0±0.00	16±1.00	15±0.50	15±2.00	20±3.50	30±1.20	10±1.50
C. parapsilosis ATCC 22019	0±0.00	0±0.00	20±2.00	10±1.50	0±0.00	20±2.60	0±0.00
C. albicans ATCC 90029	0±0.00	14±1.50	20±2.00	18±2.5	0±0.00	26±0.50	15±0.00
C. krusei ATCC 6825	0±0.00	20±1.50	21±2.50	15±1.00	13±0.5	30±2.50	10±2.00
K. pueumoniae ATCC 35657	0±0.00	20±1.50	18±0.00	18±0.50	20±2.50	25±0.00	10±0.00
A.. baumannii ATCC 747	0±0.00	20±1.50	18±0.50	8±1.70	15±0.00	28±1.00	6±0.50
S aureus SA1199	0±0.00	18±1.00	18±0.00	17±1.50	0±0.00	30±3.50	10±0.00
S. aureus ASXY 212	0±0.00	0±0.00	16±1.00	0±0.00	0±0.00	0±0.00	0±0.00

Values are mean; ± standard error of the means; diameter of antibiotic disc= 4 mm.

AMX: amoxicilline; COT: co-trimoxazole; NIT: nitrofurantoin; GEN: Gentamicin; NAL: nalidixic acid; OFL: Ofloxacin; TET: Tetracycline.

Table 2B: Susceptibility of the standard strains microorganisms to various antibiotics.

Test Organism	Diameter zone of inhibition (mm) Gram Positive Disc							
	AUG 30µg	AMX 25µg	ERY 5µg	TET 10µg	CXC 5µg	GEN 10µg	CHL 30µg	COT 25µg
S. aureus ATCC 29212	0±0.00	0±0.00	0±0.00	17±0.00	0±0.00	16±0.00	22±2.00	26±3.00
P. aeruginosa ATCC 27853	0±0.00	0±0.00	0±0.00	15±0.50	0±0.00	19±1.50	25±2.50	20±1.00
E. cloacae ATCC 49141	15±1.50	0±0.00	0±0.00	18±0.50	0±0.00	20±0.50	26±2.50	25±0.50
E. faecalis ATCC 29212	18±0.50	20±0.5	14±0.50	20±0.00	12±0.50	24±0.50	18±0.00	23±2.50
E. coli ATCC 25922	10±0.00	10±1.5	0±0.00	20±0.50	0±0.00	21±1.50	24±3.50	0±0.00
C. parapsilosis ATCC 22019	16±1.50	25±0.5	0±0.00	0±0.00	0±0.00	20±0.00	12±0.00	0±0.00
C. albicans ATCC 90029	0±0.00	0±0.00	0±0.00	8±0.50	0±0.00	20±0.00	0±0.00	0±0.00
C. krusei ATCC 6825	15±2.50	18±0.5	0±0.00	0±0.00	0±0.00	18±0.50	14±0.00	0±0.00
K. pueumoniae ATCC35657	12±1.50	0±0.00	0±0.00	18±0.00	0±0.00	23±2.00	25±2.50	26±0.00
A. baumannii ATCC 747	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	19±1.50	0±0.00	0±0.00
S. aureus SA 1199	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	16±0.00	0±0.00	0±0.00
S. aureus ASXY 212	20±2.20	22±3.70	27±2.00	15±0.50	0±0.00	18±0.50	24±3.80	22±0.50

AMX: amoxicilline; COT: co-trimoxazole; AUG: Augmentin; GEN: Gentamicin; ERY: erythromycin; CXC: cloxacillin; TET: Tetracycline; CHL: chloramphenicol.

Table 3: The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal /Fungicidal Concentration (MBC/MFC) of the crude plant extracts.

Test Organism	*Concentraton (mg/ml)*			
	D. adscendens		*B. buonopozense*	
	MIC	MBC	MIC	MBC
S. aureus ATCC 29213	0.25	0.25	0.00	0.00
S. aureus SA 1199	0.25	0.25	0.00	0.00
S. aureus ASXY 212	0.25	0.25	0.00	0.00
P. aeruginosa ATCC 27853	0.25	0.25	0.25	0.25
E. cloacae ATCC 49141	0.125	0.50	0.25	0.25
E. faecalis ATCC 29212	0.062	0.125	0.125	0.25
E. coli ATCC 25922	0.125	0.25	0.50	1.00
C. parapsilosis ATCC 22019	0.125	0.25	0.50	1.00
C. albicans ATCC 90029	0.125	0.125	0.50	1.00
C. krusei ATCC 6825	0.125	0.25	0.25	0.25
K. peumoniae ATCC 35657	0.125	0.50	0.125	0.125
A. baumannii ATCC 747	0.125	0.50	0.125	0.125

Table 4: Preliminary phytochemical screening of the two plants.

Test	*D. adscendens*	*B. buonopozense*
Alkaloids	++	++
Saponins	++	++
Anthaquinones	++	++
Tannins	++	++
Flavonoids	++	+

++ = strongly positive reaction; + = positive reaction.

DISCUSSION

Phytochemical analysis of the plants revealed the presence of tannins, saponins, steroids and alkaloids. These compounds are known to posses antimicrobial activities. Tannins for example have been found to form irreversible complexes with proline-rich proteins (Shimada, 2006) resulting in the inhibition of the cell protein synthesis. Also, tannins react with proteins to provide the typical tanning effect which is crucial for the treatment of inflamed or ulcerated tissues (Parekh and Chanda, 2007). Herbs that have tannins as their principal component are astringent in nature and are used for treating gastritis, diarrhea and dysentery (Dharmananda, 2003). Tannins are reported to possess broad antimicrobial properties by means of different mechanisms that include enzyme inhibition, oxidative phosphorylation reduction and iron deprivation, among others (Parekh and Chanda, 2007). These observations thus support the use of these plants in herbal remedies. Reports on antimicrobial and physiological effects of saponins, alkaloids, steroids and flavonoids have also been given (Quinlan et al., 2000; Kam and Liew, 2002; Neumann et al., 2004; Evans, 2005). Therefore, the presence of these compounds in these plants strongly aided the antimicrobial potentials observed. The weak antimicrobial activities of *B. buonopozense* compared to *D. adscendens* was observed and this may be due to the relative concentrations of each of these antimicrobial phytochemicals present in each of the plants.

Different concentrations of the *Desmodium adscendens* extract exhibited significant bactericidal effects on *Staphylococcus aureus* SA1199 and *Candida albicans ATCC 90029*. At the concentration of 0.0625 mg/ml of *D. adscendeus* extract, about 17% of the *S. aureus* (SA 1199) cells were killed within 30 min of contact time with the extract, while the percentage killed increased to 46% within 120 min of the cells interaction with the extract (Figure 1). When the concentration of the extract was increased to 0.25 mg/ml, the percentage of the cells death was approximately 100% within 120 min. A similar trend of reaction occurred when the *Candida albicans* ATCC 90029 was tested with the *D. adscendens* extract (Figure 2). Approximately 39 to 98% of cells were killed in 120 min at the different concentrations of the extracts.

Table 5: MIC and MBC of various antibiotics on susceptible bacteria.

Organisms	**(µg/ml)**	**COT**	**NIT**	**TET**	**OFL**	**NAL**	**AMX**	**CHL**	**ERY**	**AUG**	**GEN**
S. aureus	MIC	7.81	31.25	ND	1.56	7.81	ND	1.17	ND	ND	3.13
ATCC 29213	MBC	125.00	62.50	ND	3.13	62.50	ND	9.38	ND	ND	3.13
P. aeruginosa	MIC	3.91	7.81	1.95	ND	ND	ND	4.68	ND	ND	ND
ATCC 27853	MBC	125.00	15.62	3.91	ND	ND	ND	18.75	ND	ND	ND
E .cloacae	MIC	15.62	31.25	0.98	0.20	3.91	ND	4.68	ND	125.00	1.56
ATCC 49141	MBC	125.00	125.00	250.00	0.40	3.91	ND	150.00	ND	125.00	25.00
E. faecalis	MIC	15.62	15.62	ND	1.56	ND	18.75	15.62	15.62	62.50	1.56
ATCC 29212	MBC	62.50	15.62	ND	3.13	ND	18.75	31.25	31.25	62.50	1.56
E. coli	MIC	62.50	7.81	1.95	1.56	0.98	9.38	4.68	ND	7.81	1.56
ATCC 25922	MBC	125.00	15.63	15.63	1.56	0.98	9.38	18.75	ND	7.81	1.56
K. peumoniae	MIC	15.62	62.50	3.91	1.56	15.62	ND	18.75	ND	62.50	0.78
ATCC 35657	MBC	31.25	250.00	31.25	1.56	15.62	ND	18.75	ND	62.50	0.78
A. baumannii	MIC	ND	62.50	31.25	0.78	1.95	ND	ND	ND	ND	0.78
ATCC 747	MBC	ND	250.00	62.50	1.56	3.91	ND	ND	ND	ND	1.56
S. aureus	MIC	31.25	250.00	1.95	1.96	ND	ND	75.00	ND	ND	0.78
SA 1199	MBC	125.00	250.00	31.25	6.25	ND	ND	150.00	ND	ND	12.50
S. aureus	MIC	ND	250.00	ND	ND	ND	1.95	ND	125.00	1000.00	ND
ASXY 212	MBC	ND	250.00	ND	ND	ND	9.81	ND	1000.00	1000.00	ND

ND= Not Determined (not active during screening with disc and hence not used).

AMX: amoxicilline; COT: co-trimoxazole; AUG: Augmentin; GEN: Gentamicin; ERY: erythromycin; TET: Tetracycline; CHL: chloramphenicol; NIT: nitrofurantoin; NAL: nalidixic acid; OFL: Ofloxacin;

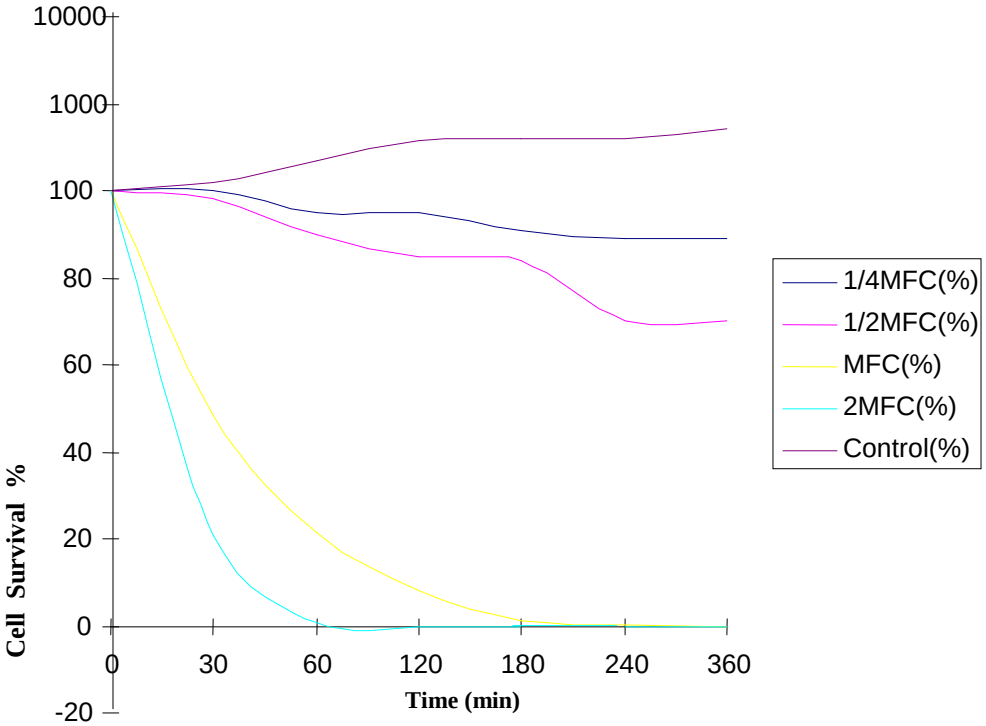

Figure 1: Kinetic kill curve of *Desmodium adscendens* on *Staphylococcus aureus* SA 1199.

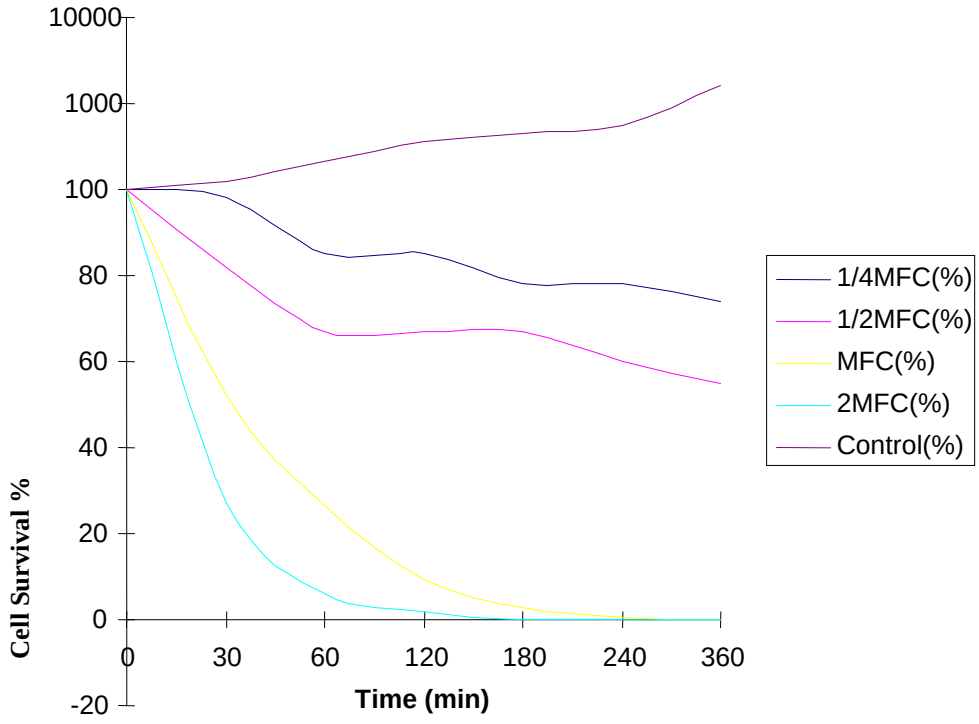

Figure 2: Kinetic kill curve of *Desmodium adsendens* on *Candida albicans* ATCC 90029.

In this study, the antimicrobial activities of the extracts were observed to increase with an increase in the concentration of the extracts. This finding is in agreement with the report of Banso et al. (1999), which states that higher concentration of antimicrobial compounds induced reasonable growth inhibition. The zone of inhibition produced by the plant extracts is an indication of susceptibility of the tested microorganisms to the plant. Also the diameters of the zones of inhibition were observed to vary from one organism to another and from one plant to another. These differences in the zones of inhibition observed could be explained according to Prescott (2005) that the effect of an antimicrobial agent varies with the target species. Pelczar et al., (2002), and Hugo and Russell (2007) also reported that the position of the zone edge (diameter of inhibition zone) is a function of the initial population density of the organism, their growth rate, their physiological state, nature and the rate of diffusion of the antimicrobial agent.

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