

ANTIMICROBIAL ACTIVITY OF THE SOLVENT FRACTIONS FROM *BULBINE NATALENSIS* TUBERM. T. Yakubu<sup>1</sup>, M. Mostafa, A. O. T. Ashafa<sup>2</sup> and A. J. Afolayan\*

\*Centre for Phytomedicine Research, Department of Botany, University of Fort Hare, Alice 5700, South Africa., <sup>1</sup>Phytomedicine, Toxicology and Reproductive Biochemistry Research Laboratory, Department of Biochemistry, University of Ilorin, Ilorin, Nigeria, <sup>2</sup>Department of Plant Sciences, University of the Free State, Qwagwa Campus, Private Bag X13, Phuthaditjhaba, 9866, South Africa.

\*Email: [Aafolayan@ufh.ac.za](mailto:Aafolayan@ufh.ac.za)**Abstract**

*Bulbine natalensis* Baker has been acclaimed to be used as an antimicrobial agent in the folklore medicine of South Africa without scientific evidence to substantiate or refute this claim. In view of this, the *in vitro* antimicrobial activity of solvent fractions (ethanol, ethyl acetate, n-butanol and water) from *Bulbine natalensis* Tuber against 4 Gram positive and 12 Gram negative bacteria as well as 3 fungal species were investigated using agar dilution. The ethanolic extract, n-butanol and ethyl acetate fractions inhibited 75, 87.5 and 100% respectively of the bacterial species in this study. The ethanolic, n-butanol and ethyl acetate fractions produced growth inhibition at MIC range of 1-10, 3-10 as well as 1 and 5 mg/ml respectively whereas the water fraction did not inhibit the growth of any of the bacterial species. Again, it was only the ethyl acetate fraction that inhibited the growth of *Shigella flexneri*, *Staphylococcus aureus* and *Escherichia coli*. The ethanolic, ethyl acetate and n-butanolic fractions dose dependently inhibited the growth of *Aspergillus niger* and *A. flavus* whereas the water fraction produced 100% growth inhibition of the *Aspergillus* species at all the doses investigated. In contrast, no growth inhibition was produced on *Candida albicans*. The growth inhibition produced by the solvent fractions of *B. natalensis* Tuber in this study thus justifies the acclaimed use of the plant as an antimicrobial agent. The ethyl acetate fraction was the most potent.

**Keywords:** *Bulbine natalensis*, ethylacetate fraction, antimicrobial agent, asphodelaceae

**Introduction**

The continuous evolution of bacterial resistance to currently available antibiotics has necessitated the search for novel and effective antimicrobial compounds. Globally, plant extracts are explored for their antibacterial, antifungal and antiviral activities (Fagbemi et al., 2009). However, the therapeutic potentials of some of these botanicals have not been scientifically evaluated (Havagiray et al., 2004). It would be interesting therefore to search for plants with antimicrobial activities that could be used against infectious diseases.

*Bulbine natalensis* Baker also known as *Bulbine latifolia* (Asphodelaceae) which is known as ibhucu (Zulu), rooiwortel (Afrikaans) and ingcelwane (Xhosa) is widely distributed in the eastern and northern parts of South Africa (van Wyk et al., 1997). The chemical investigation of the tuber of *B. natalensis* revealed the presence of tannins, anthraquinones, cardiac glycosides, saponins and alkaloids (Yakubu and Afolayan, 2009). The leaf sap is widely used in the management of wounds, burns, rashes, itches, ringworms and cracked lips. The infusion of the root as well as the stem is taken orally to quell vomiting, diarrhoea, convulsion, venereal diseases, diabetes and rheumatism (Pujol, 1990). Recently, the acclaimed folkloric use of the stem as an aphrodisiac and sexual invigorator was scientifically validated in male Wistar rats (Yakubu and Afolayan, 2009).

Despite the acclaimed folkloric use of the plant as an antimicrobial agent, there is dearth of information in the open scientific literature that has substantiated or refuted the effect of some of the solvent fractions (ethanol, ethyl acetate, n-butanol and water) of *B. natalensis* tuber on some bacteria and fungi. Therefore, this study was aimed at investigating the antimicrobial activity of some solvent fractions of *Bulbine natalensis* tuber on some of the common pathogenic bacteria and fungi.

**Materials and methods****Plant material and authentication**

Samples of the plant, collected from a single population in Sikuthwana village near Alice, Eastern Cape, were authenticated by Prof DS Grierson of the Department of Botany, University of Fort Hare, South Africa. A voucher specimen of the plant (Yakmed. 2008/1) was deposited at the Giffen Herbarium of the University.

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## Drugs and chemicals

Chloramphenicol and streptomycin used in this study as reference antibiotics were products of Sigma-Aldrich, Inc., St. Louis, USA. Nutrient broth and Potato Dextrose Agar (PDA) were products of Biolab Diagnostic (Pty) Ltd., Wadeville, Gauteng, South Africa. All other chemicals used were products of Merck Chemicals (Pty) Ltd, Bellville, South Africa.

## Microorganisms

Four Gram-positive bacteria [(*Staphylococcus aureus* (ATCC 6538), *Streptococcus faecalis* (ATCC 29212), *Bacillus cereus* (ATCC 10702), *Bacillus pumilus* (ATCC 14884)], and twelve Gram-negative bacteria [(*Escherichia coli* (ATCC 8739), *E. coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 19582), *P. aeruginosa* (ATCC 7700), *Klebsiella pneumoniae* (ATCC 10031), *K. pneumoniae* (ATCC 4352), *Serratia marcescens* (ATCC 9986), *Proteus vulgaris* (ATCC 6830), *P. vulgaris* (ATCC 0030), *Enterobacter cloacae* (ATCC 13047), *Acinetobacter calcoaceticus* (UP), and *Shigella flexneri* (batch no. 0.57)] as well as *Aspergillus niger*, *A. flavus* and *Candida albicans* were obtained from the Department of Biochemistry and Microbiology, University of Fort Hare. The organisms were revived for bioassay by sub-culturing in fresh nutrient broth for 24 h before use.

## Preparation of ethanolic extract and solvent fractions

The plant tuber was washed under running tap water, cut into pieces, oven-dried at 40 °C and then pulverized. Powdered material (755.00 g) was extracted in aqueous ethanol (70% ethanol) for 24 h on an orbital shaker (Stuart Scientific Orbital Shaker, UK). The extract was filtered and concentrated at 45 °C using rotary evaporator (Laborota 4000-efficient, Heidolph, Germany). This was then freeze-dried to give aqueous ethanolic extract (236.00 g) which was suspended in water and partitioned against ethyl acetate. The aqueous part of the ethanolic fraction (after partition in ethyl acetate) was further partitioned with n-butanol. Finally, the ethyl acetate and n-butanol fractions were concentrated at 45 °C using a rotary evaporator while the aqueous extract was freeze-dried using Savant Refrigerated Vapor Trap (RVT4104, USA). The yields of ethylacetate, n-butanol and water fractions were 20.66 g (2.73%), 29.30 g (3.88%) and 154.60 g (20.48%) respectively. Each fraction was re-constituted in their respective solvents to give the stock solution of 50 mg/ml with the exception of the butanol fraction which was constituted in aqueous methanol (50:50 v/v), because butanol was found to inhibit the growth of microorganisms used in this study. This was then diluted to the required concentrations of 0.1, 0.5, 1.0, 3.0, 5.0, 7.0 and 10 mg/ml that was used for the antibacterial experiment.

## Antimicrobial activity

### Antibacterial test

The antibacterial activity of the ethanolic extract and its fractions from *B. natalensis* tuber was evaluated using agar dilution method described by Meyer and Afolayan (1995). Briefly, nutrient agar was prepared by autoclaving and allowing to cool to 55°C before the addition of the ethanolic extract and its solvent fractions. The agar medium containing the extract and the solvent fractions at final concentrations of 0.1, 0.5, 1.0, 3.0, 5.0, 7.0 and 10 mg/ml were poured into Petri dishes, swirled gently until the agar began to set, and left over night for solvent evaporation. Agar plates containing ethanol, ethyl acetate, water and aqueous methanol (for the butanol fraction) served as controls. Organisms were streaked in radial pattern on the agar plates. The inoculum size of each test strain was standardized at  $5 \times 10^5$  cfu/ml using McFarland Nephelometer Standard as described by the National Committee for Clinical Laboratory Standards (NCCLS, 2003). The plates were incubated under aerobic conditions at 37°C and examined after 24 h. Each treatment was performed in triplicate and complete suppression of growth at a specific concentration of the extract and the fractions was required for it to be declared active (Mathekgga et al., 2000). Chloramphenicol and streptomycin (standard antibiotics) were used as positive controls in this experiment.

### Antifungal test

All the fungal cultures were maintained on PDA and recovered for testing by sub-culturing on PDA for 4 days at 25°C. PDA plates were prepared by autoclaving before the addition of the extract and its fractions. They were vortexed with molten agar at 45 °C to final concentrations of 0.1, 0.5, 1.0, 5.0, and 10 mg/ml and thereafter poured into the Petri dishes. Plates containing only PDA with the respective solvent served as controls. The prepared plates were inoculated with plugs (5 mm in diameter) obtained from the actively growing portions of the mother fungal plates and incubated at 25 °C for 5 days. The diameter of the fungal growth was measured and expressed as means of percentage growth inhibition (Quiroga et al., 2001; Lewu et al., 2006). Due to the nature of *Candida albicans*, the organism was streaked radially like the bacteria.

## Statistical analysis

Data were expressed as means  $\pm$  SD of three replicates. Statistical analysis was done using Student's t-test at  $P < 0.05$ .

**Table 1:** Antibacterial activity of ethanolic extract and the solvent fractions of *Bulbine natalensis* tuber

Bacteria	Gram +/-	MIC (mg/ml)			(µg/ml)		
		Ethanol	Ethylacetate	n-Butanol	Water	Chloramphenicol	Streptomycin
<i>Staphylococcus aureus</i> ATCC 6538	+	10	5	5	na	<2	<2
<i>Staphylococcus faecalis</i> ATCC 29212	+	na	5	na	na	<2	<2
<i>Bacillus cereus</i> ATCC 10702	+	10	5	10	na	<2	<2
<i>Bacillus pumilus</i> ATCC 14884	+	10	1	5	na	<2	<2
<i>Escherichia coli</i> ATCC 8739	-	7	1	10	na	<2	<2
<i>Escherichia coli</i> ATCC 25922	-	1	1	3	na	<2	<2
<i>Pseudomonas aeruginosa</i> ATCC 19582	-	1	1	10	na	<10	<2
<i>Pseudomonas aeruginosa</i> ATCC 7700	-	1	1	3	na	<10	<2
<i>Enterobacter cloacae</i> ATCC 13047	-	5	1	3	na	<2	<2
<i>Kiebsiella pneumonia</i> ATCC 10031	-	10	1	5	na	<2	<2
<i>Kiebsiella pneumonia</i> ATCC 4352	-	na	1	5	na	<2	<2
<i>Proteus vulgaris</i> ATCC 6830	-	na	1	10	na	<2	<2
<i>Proteus vulgaris</i> CSIR 0030	-	5	1	5	na	<2	<2
<i>Serratia marscens</i> ATCC 9986	-	1	5	3	na	<2	<2
<i>Acinetobacter calcaoeuticus</i> UP	-	1	5	5	na	<0.5	<2
<i>Shigelia flexneri</i> (batch no. 0.57)	-	na	1	na	na	<2	<2

MIC = Minimum inhibitory concentration, na = not active at 10 mg/ml (highest concentration tested)

## Results

The minimum inhibitory concentration (MIC) of the ethanolic extract and its fractions (ethyl acetate, n-butanol and water) from *Bulbine natalensis* tuber against 16 bacterial strains is depicted in Table 1. The ethanolic extract inhibited 75% of the test bacterial strains at MIC ranging from 1-10 mg/ml. The same extract also exhibited significant inhibition against gram-negative bacteria. Similarly, the n-butanol fraction also inhibited 87.5% of the test organisms at the MIC range of 3-10 mg/ml. While there was no growth inhibition of all the organisms by the water fraction of *B. natalensis* stem. The ethyl acetate fraction produced 100% growth inhibition of the test bacteria at MIC of 1 and 5 mg/ml. Most of the bacteria (68.8%) were inhibited by the ethyl acetate fraction at 1 mg/ml while the remaining 31.25% were inhibited at 5 mg/ml. Among the three diarrhoeal causing bacteria (*S. flexneri*, *S. aureus* and *E. coli*) investigated in this study, it was only the ethyl acetate fraction that produced 100% growth inhibition on these organisms. In addition, the growth inhibition by the ethyl acetate fraction produced the most profound MIC values (Table 1).

The ethanolic extract and the solvent fractions were active against *A. niger* and *A. flavus* (Table 2). The growth inhibition was dose dependent on the *Aspergillus* species except for the water fraction which produced 100% inhibition at all the doses investigated. In contrast however, the ethanolic extract as well as the solvent fractions of *B. natalensis* tuber at all the doses investigated did not inhibit the growth of *Candida albicans* (Table 2).

## Discussion

Infectious diseases of microbial origin caused by *Staphylococcus aureus*, *Bacillus cereus*, *Shigella spp* constitute the major causes of morbidity and or mortality in several countries (Kloos and Zein, 1993). Such microbial infection and pathophysiology in water and electrolyte transport could lead to diarrhoea.

**Table 2:** Antifungal activity of the ethanol extract and its ethylacetate, n-butanol and water fractions of *Bulbine natalensis* tuber.

		Growth inhibition (%) / Concentration (mg/ml)					
Organisms	Fractions	Control	0.1	0.5	1.0	5.0	10.0
<i>A. niger</i>	Ethanol	0.00 <sup>a</sup>	51.67 ± 0.83 <sup>b</sup>	58.61 ± 2.09 <sup>b</sup>	63.61 ± 0.48 <sup>c</sup>	68.89 ± 1.73 <sup>c</sup>	73.70 ± 1.15 <sup>d</sup>
	Ethylacetate	0.00 <sup>a</sup>	61.11 ± 0.48 <sup>b</sup>	67.50 ± 2.20 <sup>b</sup>	69.72 ± 0.47 <sup>bc</sup>	71.67 ± 2.20 <sup>c</sup>	77.31 ± 1.31 <sup>c</sup>
	n-Butanol	0.00 <sup>a</sup>	55.00 ± 2.06 <sup>b</sup>	65.56 ± 1.27 <sup>c</sup>	68.33 ± 2.20 <sup>c</sup>	75.56 ± 1.27 <sup>d</sup>	81.31 ± 1.09 <sup>e</sup>
	Water	0.00 <sup>a</sup>	100.00 ± 0.00 <sup>b</sup>	100.00 ± 0.00 <sup>b</sup>	100.00 ± 0.00 <sup>b</sup>	100.00 ± 0.00 <sup>b</sup>	100.00 ± 0.00 <sup>b</sup>
<i>A. flavus</i>	Ethanol	0.00 <sup>a</sup>	65.83 ± 2.00 <sup>b</sup>	72.78 ± 2.46 <sup>c</sup>	73.33 ± 5.77 <sup>c</sup>	76.11 ± 2.09 <sup>c</sup>	76.20 ± 4.04 <sup>c</sup>
	Ethylacetate	0.00 <sup>a</sup>	66.94 ± 2.74 <sup>b</sup>	69.17 ± 1.44 <sup>b</sup>	77.78 ± 1.27 <sup>c</sup>	80.56 ± 0.83 <sup>c</sup>	90.28 ± 0.47 <sup>c</sup>
	n-Butanol	0.00 <sup>a</sup>	54.72 ± 3.37 <sup>b</sup>	68.33 ± 1.20 <sup>c</sup>	69.44 ± 0.96 <sup>c</sup>	73.33 ± 0.50 <sup>c</sup>	85.00 ± 0.68 <sup>d</sup>
	Water	0.00 <sup>a</sup>	100.00 ± 0.00 <sup>b</sup>	100.00 ± 0.00 <sup>b</sup>	100.00 ± 0.00 <sup>b</sup>	100.00 ± 0.00 <sup>b</sup>	100.00 ± 0.00 <sup>b</sup>
<i>C. albicans</i>	Ethanol	0.00	0.00	0.00	0.00	0.00	0.00
	Ethylacetate	0.00	0.00	0.00	0.00	0.00	0.00
	n-Butanol	0.00	0.00	0.00	0.00	0.00	0.00
	Water	0.00	0.00	0.00	0.00	0.00	0.00

n = 3, means of percentage growth inhibition ± SD. Values across the row carrying superscripts different from the control are significantly different at p < 0.05.

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Several workers had used MIC as an index for measuring the efficacy of antibacterial agents (Kabir et al., 2005; Ushimaru et al., 2007; Fagbemi et al., 2009). Therefore, the activity of the ethanolic extract and the various fractions of *B. natalensis* tuber with the exception of water on the different bacterial strains used in this study explain the antibacterial activity of the plant. The differences in their activity appeared to be related to qualitative and quantitative diversity of compounds that was extracted by the different solvents (Geyid et al., 2005). The stronger extraction capacity of organic solvents has earlier been documented, which could have produced a greater number of phytoconstituents responsible for the observed antibacterial activity in this study (Parekh and Chanda, 2006). For example, tannins have been reported to possess antimicrobial properties by means of different mechanisms including enzyme inhibition, reduction in oxidative phosphorylation and iron deprivation among others (Parekh and Chanda, 2007). Similarly, steroids and saponins have also been implicated to exhibit antibacterial activities (Soetan et al., 2006). Although, the phytoconstituents of each solvent extracts was not determined in this study, it is possible that the ethyl acetate fraction might have extracted the most phytoconstituents responsible for antibacterial activity, hence the most outstanding antimicrobial activity of the solvent extract.

According to several workers, water extract usually show little or no activity against bacteria (Koduru et al., 2006; Lewu et al., 2006). This study has also complemented such findings as the water fraction was not active at all the doses against all the bacterial species investigated. Gram-negative bacteria are frequently reported to have developed multi-drug resistance to many of the antibiotics currently available in the market of which *E. coli* is the most prominent (Alonso et al., 2000; Sader et al., 2002). Therefore, it is noteworthy that the ethanolic extract and other fractions (except water) from the plant can be useful candidate in the management of infectious diseases caused by gram-negative bacteria. The inhibition of growth of the bacteria that cause gastroenteritis is a very assuring addition to reported possible antibacterial activity of this plant. It is also interesting to note that food poisoning bacteria, *S. aureus* as well as *S. flexneri* that cause dysentery were inhibited by the plant fractions. Diarrhoea causing organisms such as *S. flexneri*, *S. aureus* and *E. coli* were also inhibited by the ethanolic extract from the plant and its fractions. Therefore, the use of the ethanolic extract and its fractions from *Bulbine natalensis* tuber might assist in reducing cases of diarrhoea.

The importance of investigating the fungicidal activity of botanicals cannot be over emphasized in view of the fact that fungal infections of the skin, nails and hair are a major source of concern/morbidity throughout the world (Abebe et al., 2003). The genus, *Aspergillus* is known to be responsible for a group of diseases referred to as aspergillosis. *A. flavus* produces aflatoxin which is both a toxin and a carcinogen. Similarly, the fungus has also been implicated in cases of immuno-compromised patients that frequently develop opportunistic and superficial mycosis (Portillo et al., 2001). Therefore, the susceptibility of *A. flavus* to the ethanolic extract and fractions of *B. natalensis* tuber is noteworthy, as it can be explored in the management of some aspergillosis. The ability of the ethanolic extract and its fractions from *B. natalensis* tuber to inhibit the growth of several bacteria and fungi species in this study is an indication of the broad spectrum antimicrobial potential of the plant. This study thus lends scientific support to the folkloric use of *Bulbine natalensis* in traditional medicine of South Africa, as an antimicrobial agent. The ethyl acetate fraction appeared to be one with the most potent antimicrobial agent.

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