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File: ■ Bulbine (*Bulbine latifolia* syn. *B. natalensis*, Xanthorrhoeaceae) ■ Antimicrobial Activity

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RE: South African Bulbine Tuber Has Broad-spectrum Antimicrobial Activity

Yakubu MT, Mostafa M, Ashafa AOT, Afolayan AJ. Antimicrobial activity of the solvent fractions from *Bulbine natalensis* tuber. *Afr J Tradit Complement Altern Med*. 2012;9(4):459-464.

Bacteria are becoming resistant to available antibiotics, so new compounds are needed. Bulbine (*Bulbine latifolia* syn. *B. natalensis*, Xanthorrhoeaceae) tuber is common in South Africa. Historically, it has been used as an antimicrobial agent; however, the potential antimicrobial activity of bulbine has not been evaluated in scientific assays. The purpose of this in vitro study was to assess the antimicrobial activity of solvent fractions of bulbine against pathogenic gram-positive bacteria, gram-negative bacteria, and fungi.

Bulbine was collected from a single population in Sikusthwana village near Alice, Eastern Cape, South Africa. The identity of the samples was authenticated by D.S. Grierson of the Department of Botany, University of Fort Hare in Alice, South Africa, and a voucher specimen was deposited at the Giffen Herbarium, University of Fort Hare. The tuber material was washed, oven-dried, powdered, and extracted with 70% ethanol for 24 h. The ethanol extract was freeze-dried, suspended with water, and partitioned between solvents to produce ethyl acetate, n-butanol, and water fractions. After drying, stock solutions of the solvent fractions and ethanol extract were prepared and diluted to the following concentrations: 10, 7.0, 5.0, 3.0, 1.0, 0.5, and 0.1 mg/mL.

The antimicrobial screening panel consisted of the following organisms: the gram-positive bacteria *Staphylococcus aureus* American Type Culture Collection (ATCC) accession 6538, *Streptococcus faecalis* (ATCC 29212), *Bacillus cereus* (ATCC 10702), and *Bacillus pumilus* (ATCC 14884); the gram-negative bacteria *Escherichia coli* (ATCC 8739), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 19582), *Pseudomonas aeruginosa* (ATCC 7700), *Klebsiella pneumoniae* (ATCC 10031), *K. pneumoniae* (ATCC 4352), *Serratia marcescens* (ATCC 9986), *Proteus vulgaris* (ATCC 6830), *Proteus vulgaris* (CSIR 0030), *Enterobacter cloacae* (ATCC 13047), *Acinetobacter calcoaceticus* (UP), and *Shigella flexneri* (batch no. 0.57); and the fungi *Aspergillus niger*, *Aspergillus flavus*, and *Candida albicans*. Antimicrobial activity was evaluated using the agar diffusion method with chloramphenicol and streptomycin as positive controls and the solvents as negative controls. Bacterial plates were incubated

at 37°C and examined at 24 h; complete suppression of growth was required for the sample to be deemed active. The fungal plates were incubated at 26°C for five days. The diameter of the fungal growth was measured and expressed as the mean of percentage growth inhibition. Three replicates of each assay were performed.

The minimum inhibitory concentration (MIC) of the positive control streptomycin was <2 µg/ml against all 16 bacteria. The ethanolic extract inhibited 75% of the gram-positive bacterial strains and 75% of the gram-negative strains at MICs of 1-10 mg/ml. The n-butanol fraction inhibited 87.3% of the bacteria (75% of the gram-positives and 91.6% of the gram-negatives) at MICs of 3-10 mg/ml. The ethyl acetate fraction inhibited all 16 bacteria at MICs of 1-5 mg/ml. The ethyl acetate fraction had the best (lowest) MIC value against most of the bacteria (the ethanol extract had a lower MIC of 1 mg/ml against *Acinetobacter calcoaceticus* and *Serratia marcescens*). Against the diarrhea-causing bacteria, ethyl acetate was the only fraction that inhibited *Staphylococcus aureus*, *Escherichia coli*, and *Shigella flexneri*, and it had the lowest MIC (1 mg/ml) against *Bacillus cereus*. The water fraction did not produce any bacterial inhibition. However, the water fraction did produce 100% inhibition of *Aspergillus niger* and *Aspergillus flavus* at a concentration of 0.1 mg/ml. The ethanolic extract, ethyl acetate fraction, and n-butanol fraction were active against *Aspergillus niger* and *Aspergillus flavus* in a dose-dependent manner, producing 50-65% inhibition at the 0.1 mg/ml concentration. None of the extracts were active against *C. albicans*. Antifungal positive controls were not used; as a point of comparison, the reported MIC of amphotericin B against *Aspergillus niger* is <0.2 µg/ml.

Bulbine ethanol, ethyl acetate, and n-butanol extracts showed broad-spectrum antimicrobial activity against the organisms tested, inhibiting gram-positive bacteria, gram-negative bacteria, and fungi (with the exception of *C. albicans*). The ethyl acetate fraction was the most potent, with MICs of 1-5 mg/ml. The authors conclude that bulbine may be a "useful candidate in the management of infectious diseases caused by gram-negative bacteria."

—Heather S. Oliff, PhD

Note: The article contained numerous misspellings of the microbe names as follows: Shigella is misspelled Shigelia, Shigellia, and Shigelli; Klebsiella pneumoniae is misspelled Kiebsiella pneumonia; marcescens is misspelled marscens; Aspergillus is misspelled Aspergilus; and calcoaceticus is misspelled calcaoceuticus. Also, the bacterium *Streptococcus faecalis* is erroneously listed as *Staphylococcus faecalis*; since 1984, the proper name for *Streptococcus faecalis* is *Enterococcus faecalis*.

Editorial Comment:
Based on the reported MICs, the antibacterial activity of the ethyl acetate fraction and antifungal activity of the water fraction are >1000-fold less potent than pharmaceutical antimicrobials. Nonetheless, the findings support the traditional use of this plant as a common, freely available medicine that can be used as a first line of defense in communities with limited access to pharmaceutical treatments. It is possible that activity-guided fractionation may lead to the identification of more potent fractions/compounds.

Referenced article can be accessed at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3746651/.