Antibiotic Activity of Selected Botswana Medicinal Plants

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Abstract

A number of plant extracts from selected Botswana Medicinal Plants were tested against a battery of organisms for antibiotic activity. The antibiotic profile for most of these extracts seems to support their traditional use in treating ailments associated with microbial infections. Detailed chemical and biological investigation of some of these plants led to isolation of antimicrobial constituents.

Introduction

Knowledge about use of plants to treat diseases dates back to time immemorial. Transmission of this knowledge from one generation to another has been through oral tradition from the master to the apprentice - normally members of the family of the former.

Documented evidence shows that 80 percent of Botswana's rural population still use medicinal plants for their primary health care needs and most of these plants are used for treating parasitic infections (Hedberg & Staugard 1989a). This is not surprising as most of the common diseases are caused by parasites.

The need for new antibiotics is currently a pressing one especially with the emergence of resistant strains of bacteria that render a lot of the modern antibiotics ineffective. Most recently with the advent of HIV/AIDS a lot of interest has been shown on antifungal drugs especially those that can treat oral candidiatis shown by patients who have been immuno-compromised by the HIV virus. Tuberculosis has also been on the increase recently due to the HIV/AIDS epidemic.

It was with these concerns in mind that we undertook this study to assess the validity of those plants used traditionally to treat these microbial infections. It can be said without exaggeration that there is a salient fear that antibiotics will one day become obsolete, leaving us at the mercy of the microbial world. This means new antibiotics must be sought to replace those against which resistance is known.

Most traditional medicines are given as a single loading dose, and these doses are not standardized. While these drugs may indeed be effective medicines, toxicity is also a common complication, most patients presenting with acute renal failure. Long term consequences are unknown. There is therefore a need to establish the purported medicinal value in these plants as well as the minimum inhibitory concentrations (MIC), minimum bactericidal concentrations (MBC) and the content, if any, of toxic substances. Subsequently traditional doctors or herbalists could be consulted with on the basis of established scientific data.

Of additional concern is the fact that Botswana, with an estimated 1.3 million population has an annual budget of over P33 million. The need to carry out research and determine the utilisability of local resources and how to preserve them for sustained development is a well accepted ideal towards cost-containment.

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Materials and Methods

Mortar and pestle (or mill), ethanol, filter paper (Whatman No. 2), funnel, antibiotic sensitivity agar, 37° C incubator, inoculating loop, bunsen burner, 0.5 MacFarland bacterial suspension, sterile swabs.

Selection of Plants for Screening

The choice of plants selected for screening was based on an inquiry made through discussions with local people and traditional doctors. From these symptoms described to us we deduced the most probable group of diseases for which the medicine is used. From this group of diseases we selected those most likely to be caused by bacteria and fungi. An attempt was made to include the suspected culprit species in the battery of test organisms for that plant extract.

Preparation of Crude Extract

Fifteen grams of test plants were ground using either a mortar and pestle or a mill. The powder or paste was macerated in 50 ml ethanol. The mixture was allowed to stand for 5-10 minutes at room temperature with frequent stirring. This was filtered and the filtrate (a fixed amount giving a loading of about 20 mg/disc) was applied to a filter paper disc. The discs were air dried and stored at room temperature until used.

Screening for Antibacterial Activity

The study organisms were obtained from stock culture maintained by the Department of Medical Technology, Institute of Health Sciences. First the identity of the organisms was ascertained, with both cultural, morphological and biochemical tests carried out to confirm the species identity. The API 20E set of biochemical tests were used for enterobacteriaceae, the slide coagulase and cultural characteristics were used for Staphylococcus aureus and the gem test tube test was used for Candida species. Sensitivity to bacitricin and optochin were used for group A Streptococcus and Streptococcus pneumoniae respectively.

A 0.5 MacFarland bacterial standard of each organism was streaked on a sensitivity agar plate using a sterile swab. A piece of extract-impregnated paper was placed over the inoculum and pressed lightly to ensure adhesion to the agar surface. The labeled plates were incubated at 37° C for 18 hours. At the end of the incubation period, the plates were examined for possible growth inhibition. Inhibition was said to be present if a clear area of inhibition (absence of bacterial growth) was observed immediately around the filter paper disc.

Results and Discussion

Table 1. The plants tested and the result of the antibiotic activity tests.

Combretum heroroense (mokabi): The local name literally means "enlarged genitalia", a name derived from its medicinal use to treat swollen penile and vulval lesions. The activity profile of the root and leave extract showed similar antibiotic activity against *Staphylococcus aureus*, *Proteus mirabilis* and *Salmonella typhi*. We could not obtain organisms associated with genital inflammation.

Ximenia americana (moretologa wa pudi) and Ximenia caffra (moretologa wa kgomo): These two plants are traditionally prescribed for, among others, body sores, leprosy, sexually transmitted diseases, infertility in women, diarrhoea in children and sore eyes- all suggesting antibiotic activity. Antibiotic activity was confirmed against our test organisms

Table 1. Activity Profile of Microorganisms A-I to Selected Medicinal Plant Extracts.

Plant extract from	Organisms								
	A	B	C	D	E	F	G	H	I
Clerodendrun tertanum	+	-	-	+	-	-	-	±	-
Combretum hereroense (leaves)	+	-	±	+	-	-	+	+	±
Combretum hereroense (roots)	-	-	-	+	-	-	+	+	-
Elephantorrhiza spp.	±	+	+	+	+	-	+	+	+
Euclea undulata (roots)	+	-	+	+	+	-	+	+	+
Grewia flava (roots)	±	-	-	+	-	-	+	+	+
Sansevieria scabrifolia (leaves)	+	±	-	-	-	-	-	±	-
Sansevieria scabrifolia (roots)	-	±	-	-	-	-	-	±	-
Terminalia sericea (roots)	+	+	-	+	+	-	+	+	+
Urgenia sanguinea (fresh bulbs)	NT	+	NT	+	NT	NT	NT	NT	NT
Vahlia capensis (whole plant)	+	+	-	+	NT	+	+	NT	-
Ximenia americana (roots)	±	-	-	+	+	-	+	+	+
Ximenia caffra (roots)	±	±	-	+	-	-	+	+	-
Ziziphus mucronata (roots)	+	-	-	+	-	-	+	+	-
Ziziphus mucronata (leaves)	+	-	+	+	±	-	+	+	+

Key: + = sensitive, - = resistant, NT = not tested, ± = small zone of inhibition

A = Group A *Streptococcus*
B = *Escherichia coli*
C = *Candida spp.*
D = *Staphylococcus aureus*
E = *Shigella sonnei*
F = *Klebsiella pneumoniae*
G = *Proteus mirabilis*
H = *Salmonella typhi*
I = *Pseudomonas spp.*

commonly implicated in wound infections such as *S. aureus*, *P. mirabilis* and *Pseudomonas species*. Activity was also demonstrated against organisms that cause gastroenteritis such as *S. typhi* (both extracts) and *Shigella sonnei* (*X. americana* only).

Sanseviera scabrifolia (mosokelatsebeng): The fleshy leaves of the plant are warmed in a fire and the juice is squeezed into the ear. We confirmed antibiotic activity against b-hemolytic group A *Streptococcus* and some weak activity against *S. aureus* both of which are likely to cause otitis.

Grewia flava (moretlwa): Roots are used to treat intestinal problems. Activity was shown against group A *streptococcus*, *S. aureus*, *P. mirabilis*, *S. typhi* and *Pseudomonas* species.

Euclea undulata (motlhakola): Roots of this plant are popularly used for brushing teeth and the tongue is also coloured red in the process. The roots are known to contain quinoids (Hedberg and Staugard 1989b). Activity was found against group A *Streptococcus*, *S. aureus S. sonnei*, *P. mirabilis*, *S. typh*i and *Pseudomonas* species. Activity was also shown against *Candida* species- this natural toothbrush is also important in the prevention of oral candidiasis as well.

Clerodendrum tertanum (legonyana): Medicinal uses include application to wounds, treatment of venereal diseases, backache and menstrual pains. Since backache may signal upper urinary tract infections, investigations were carried out including members of the enterobacteriaceae. However, the only organism that responded satisfactorily to the extract was *S. aureus* which is a less common cause of UTI, but more frequent etiologic agent in wound infections and septicermia. The extract was also found to cause severe haemolysis of human red blood cells on blood agar medium. Although the physiologic relevance of this toxicity has not been determined, the severity of the haemolysis is sufficient to warrant further inquiry into the chemical nature of the haemolysin and its effects on the blood function.

Ziziphus mucronata (mokgalo): Roots are used to alleviate pain and to cure irregular menses. They may also be chewed and spat on a sore or open wound which suggests antibiotic or analgesic effect. Leaves were also investigated and *Table 1* data indicates a broader spectrum of antibiosis than roots. This is a plant where roots are used more often than leaves and yet the latter is more effective even against fungi. Therefore the use of leaves rather than roots should be encouraged as these are more abundant and also easier to obtain. This is also important from a conservation point of view as use of leaves is less harmful to the tree than removing roots.

Terminalia sericea (mogonono): Roots used as a remedy for diarrhoeal and venereal diseases. Due to fastidious nature of Neisseria gonorrhoeae we could not obtain the organism at the time of testing. The extract was therefore tested for its therapeutic properties as regards gastroenteritis. It showed activity against E. coli and could presumably be active against enterotoxigenic E. coli. Moreover the extract was active against *S. typhi* and *S. sonnei*, both being significant enteric pathogens.

Vahlia capensis (leetsane): Extract of powdered plant extract is used to treat sore eyes. The extract showed broad spectrum activity against both bacteria and fungi (*Table 1*). Detailed chemistry on this plant gave various compounds (Majinda *et al*, 1995) and the active components were found to be gallic acid and vahlia biflavone. The MIC for vahlia biflavone was found to be 15.3 µg/ml and 30.6 µg/ml for *S. aureus* and Bacillus subtilis respectively, while that for gallic acid was found to be 71.3 µg/ml for both organisms (Majinda *et al* 1997a).

Urginea Sanguinea (sekaname): Fresh bulbs of this plant are used to treat gonorrhoea and other sexually transmitted diseases. This plant showed weak antibacterial activity against *S. aureus* and *B. subtilis* at a loading dose of 500 mg/disc. Detailed chemistry gave some cardiac glycosides called bufadienolides, phloroglucinol and its 1-glucoside and some phenolic acids. The active components were found to be salicylic acid and 4-methyl-3-hydroxybenzoic acid (Majinda *et al* 1997b). However caution is needed in the use of this plant as it contains bufadienolides, a class of compounds that are a source of livestock poisoning in South Africa (Krenn *et al* 1993).

Elephantorrhiza spp. (mosetsane): Rhizomes extracts of this plant showed the greatest antibiotic spectrum against both Gram positive and Gram negative bacteria and fungi (see Table 1). Detailed chemical investigation yielded a lot of polyphenols and flavonoids. Work is in progress to determine the active compounds in this plant extract.

The results of the present study command respect for traditional healers. All the plants studied have shown some degree of antibiosis and thus justifying traditional use of these medicinal plants. These plants do indeed have therapeutic value and with proper use and

standardization they could serve as alternative remedies. However a lot of studies need to be carried out to assess the toxicity of these plants. Two of these plants, *Clerodendurm tertanum* (legonyana) and *Urginea sanguinea* (sekaname) need to be used with great caution since these contain toxic constituents. Toxicological studies need therefore be carried out alongside activity studies in order to gain maximum benefit from these plants. We also need to give feedback to people who use these medicines so that they can use them more effectively and safely.

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