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Full Length Research Paper

Cultural importance and antibacterial activity of *Ziziphus mucronata* (Willd.) in the Umlazi community in Durban

R. M. Coopoosamy[1]*, K. K. Naidoo[1] and N. J. Ndlazi[2]

[1]Department of Nature Conservation, Mangosuthu University of Technology, P. O. Box 12363, Jacobs, 4026, Durban, KwaZulu-Natal, South Africa.
[2]Faculty of Natural Science, Mangosuthu University of Technology, P. O. Box 12363, Jacobs, 4026, Durban, KwaZulu-Natal, South Africa.

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The Zulu community of Umlazi, Durban makes extensive use of *Ziziphus mucronata* (Buffalo thorn) for predominantly cultural purposes. There are many superstitions and beliefs associated with the tree. A validation of its antimicrobial properties from leaf, bark and root extracts were attempted against three Gram positive and three Gram negative bacteria. In addition, an antifungal assay was attempted, using ethanol, aqueous and boiled aqueous extracts on six fungal species. Acetone and ethyl acetate samples showed greater inhibition of mostly Gram positive bacteria. Extracts from the leaves showed the greatest inhibition, while root extracts showed the least inhibition. Ethanol extracts were found to possess greater antifungal activity than aqueous and boiled aqueous extracts. Extracts were most effective against *Aspergillus flavus* and *Aspergillus glaucus* with little effect on *Candida albicans* and *Candida tropicalis*. It was evident that the bark and leaves of *Z. mucronata* possess greater antimicrobial properties than the root and can be used as a substitute for other extensively harvested species demonstrating similar properties.

Key words: *Ziziphus mucronata*, antimicrobial, cultural importance.

INTRODUCTION

In recent years, the interest of traditional medicine from different cultures has increased significantly in industrialized countries, with prescription drugs worldwide originating from tropical flora (Nelson-Harrison et al., 2002). Traditional medicine plays an important role in many areas of South Africa, where communities do not have access to proper healthcare facilities. In Southern Africa, it is estimated that 70% of the black population consults traditional healers for health hazard problems and utilize traditional medicines, most of which is derived from plant species indigenous to the region (Jager et al., 1995). One of such community, living in Umlazi, Durban makes extensive use of buffalo thorn for predominantly cultural purposes.

*Corresponding author. E-mail: rogercoopoosamy@gmail.com. Tel: +27 82 200 3342. Fax: +27 31 907 7665.

Africans have many beliefs and superstitions attached to this tree. Zulus use the buffalo thorn in connection with burial rites. It was once customary that when a Zulu chief died, the tree was planted on his grave as a reminder or symbol of where the chief lies, hence, the name umLahlankosi (that which buries the chief, *Pers. Comm*: Fakazi). A twig from the tree was and is still used to attract and carry the spirit of the deceased from the place of death to the new resting place. When a stock owner died and was buried, according to custom, within the cattle or goat kraal, some branches were placed on the grave so that the animals nibbled on leaves and twigs, and so understood that their master had died (Palmer and Pitman, 1972). In other parts, Africans drag a branch round the village to protect it from evil spirits, as it is believed to keep evil spirits away (*Pers. Comm*: Fakazi). In Botswana as well as most parts of South Africa, the residents believed the buffalo thorn to be immune against lightning; anyone standing under one in a storm would be

safe. It is also believed that if it is felled in summer, a drought, hail or lightning will certainly follow (*Pers. Comm*: Fakazi). A decoction of the glutinous roots is commonly administered as a sedative for all sorts of pains as well as dysentery (Nadembega et al., 2011). A concoction of the bark and the leaves is used for respiratory ailments and other septic swellings of the skin. Pastes of the root and leaves can be applied to treat boils, swollen glands, wounds and sores (Hutchings et al., 1996). Steam baths from the bark are used to purify and improve complexion (Palmer and Pitman, 1972). A study using root extracts from buffalo thorn showed activity against dermatophytes causing skin diseases (Adamu et al., 2006). Furthermore, aqueous and methanol extracts of the stem bark showed antifungal activity against *Candida albicans* (Gundidza, 1986). In East Africa, roots are used for treating snake bites (Hutchings et al., 1996). It has been reported that rural communities in Burkina Faso use a decoction of the root to prevent obesity and stave off hunger pangs (Nadembega et al., 2011).

Programmes for the screening of plant remedies are important for validating the traditional use of herbal remedies and for providing leads in the discovery of new active principles. It is evident that buffalo thorn is used sparingly by traditional healers in Umlazi, possibly because of its scarcity in the area as well as the fact that there is an abundance of other species exhibiting high medicinal value. Although, some work has shown antifungal properties of the plant, not much focus has been placed on the antibacterial properties. Validation of the antibacterial and antifungal properties of buffalo thorn may thus ease existing pressure on other intensively harvested medicinal plant species and intensify efforts to re-introduce this species in previously occupied habitats where it has become almost extinct due to overharvesting.

METHODOLOGY

Plants species were located with the assistance of Mr Fakazi and samples were collected from Umlazi, Durban, South Africa.

Antibacterial assay

One kilogram of dried material (leaf, root and bark) was crushed and placed in a 2 L conical flask containing one of the three mediums, that is, water, ethyl acetate and acetone, for extraction based on varying polarity of solvents. The media were left for 72 h in an orbital shaker at 20 shakes per minute. After 72 h, the extracts were filtered. The extracts were then used for further tests.

The plant extract was then tested for antibacterial properties against three strains of Gram positive (*Bacillus subtilis, Micrococcus kristinae* and *Staphylococcus aureus*) and three strains of Gram negative bacteria (*Escherichia coli, Proteus vulgaris* and *Enterobacter aerogenes*) for antibacterial activity using the cup-plate method. Each organism was prepared by diluting in 24 h old broth cultures with sterile nutrient broth. The cultures were then diluted 100 fold to give approximately 10^6 bacteria ml^{-1}. Cultures were incubated for 72 h at 60°C. Each treatment was done in triplicate and the mean values were calculated.

Antifungal assay

Buffalo thorn leaf, bark and root samples (approximately 1 kg of each) were cut into small pieces and crushed in a homogenizer. The plant materials were soaked in ethanol (95% v/v) and in distilled water in 2 L conical flasks for 3 weeks. The extracts (water and ethanol) obtained were evaporated at reduced pressure (45°C) to a residue. Extracts for testing ethanol and aqueous extract were prepared in three different concentrations. The stock solutions were prepared by dissolving 100 mg of dry extract in 1 ml of ethanol and water separately in order to obtain a concentration of 100 mg/ml dilutions (1:10, 1:100 and 1:500). These stock solutions were then used in phosphate buffer at pH 6.0 to evaluate the antifungal activity (Champion et al., 1992). The solutions were then tested for antifungal activity using the following fungal cultures: *Aspergillus flavus, Aspergillus glaucus, Candida albicans, Candida tropicalis, Trichophyton mentagrophytes, and Trichophyton rubrum.* Plates containing potato dextrose agar served as controls. All tests were done in triplicate and the mean values were calculated.

RESULTS AND DISCUSSION

Water did not serve as a good extraction medium with only bark extract samples inhibiting *B. subtilus* and *M. kristinae* (Table 1). Acetone and ethyl acetate samples showed greater inhibition of mostly Gram positive bacteria (Tables 2 and 3). Acetone extracts from leaves showed the greatest inhibition while extracts from roots showed the least inhibition (Table 2). All Gram positive bacteria and one Gram negative bacteria, that is, *E. coli* were inhibited by acetone extracts obtained from the leaves. *Staphylococcus aureus* was the only Gram positive bacteria not inhibited by acetone extracts from bark (Table 2).

All Gram positive bacteria were inhibited by ethyl acetate extracts from leaves and bark while extracts from roots inhibited only *S. aureus* (Table 3). In contrast, none of the Gram negative bacteria were inhibited by ethyl acetate extracts of the different plant parts (Table 3). The results of the present study are similar to findings obtained by other researchers who showed that antibacterial activity was more prevalent in Gram positive strains (Coopoosamy and Magwa, 2007; Grierson and Afolayan, 1999).

The mixed extracts of buffalo thorn showed similar activities to that of the leaves (Tables 1, 2 and 3). However, the mixed extract was less effective when compared with the leaf extract. This can be explained by the fact that these samples contained extracts from leaves, bark and roots; extracts from bark and root being less effective.

The antifungal activities (Table 4) of the ethanol extracts were found to be more effective than aqueous and boiled aqueous extracts. Extracts were most effective against *A. flavus* and *A. glaucus* with little effect on *C. albicans* and *C. tropicalis* (Table 4). No effects

Table 1. Minimal inhibitory concentration (MIC) of *Z. mucronata* in water extract.

Bacteria	Gram +/-	Plant part											
		Leave			Stem bark			Roots			Mixture of leaves, bark and roots		
B. subtilis	+	Na	Na	Na	4.0	5.0	Na	Na	Na	Na	6.0	4.0	Na
M. kristinae	+	Na	Na	Na	4.0	4.0	Na	Na	Na	Na	Na	Na	Na
S. aureus	+	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	6.0	Na
E. coli	-	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na
P. vulgaris	-	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na
E. aerogenes	-	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na

Na = No activity; all activities were done in triplicate.

Table 2. Minimal inhibitory concentration (MIC) of *Z. mucronata* in acetone extract.

Bacteria	Gram +/-	Plant part											
		Leaves			Stem bark			Roots			Mixture of leaves, bark and roots		
B. subtilis	+	3.0	4.0	3.0	2.0	3.0	Na	5.0	Na	Na	3.0	4.0	Na
M. kristinae	+	4.0	3.0	5.0	3.0	3.0	4.0	Na	Na	Na	4.0	Na	Na
S. aureus	+	2.0	4.0	4.0	Na	Na	Na	Na	Na	Na	4.0	5.0	Na
E. coli	-	3.0	3.0	3.0	Na	Na	Na	Na	Na	Na	Na	Na	Na
P. vulgaris	-	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na
E. aerogenes	-	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na

Na= No activity; all activities were done in triplicate.

Table 3. Minimal inhibitory concentration (MIC) of *Z. mucronata* in ethyl-acetate extract.

Bacteria	Gram +/-	Plant part											
		Leaves			Stem bark			Roots			Mixture of leaves, bark and roots		
B. subtilis	+	4.0	3.0	3.0	2.0	3.0	Na	5.0	Na	Na	4.0	4.0	2.0
M. kristinae	+	5.0	4.0	5.0	4.0	4.0	5.0	Na	Na	Na	4.0	Na	3.0
S. aureus	+	3.0	Na	4.0	3.0	3.0	4.0	5.0	3.0	3.0	3.0	2.0	2.0
E. coli	-	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na
P. vulgaris	-	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na
E. aerogenes	-	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na

Na = No activity; all activities were done in triplicate.

were recorded for *T. mentagrophtes* and *T. rubrum* (Table 4).

This contradicts a study by Adamu et al. (2006) where methanol extracts of root samples showed antifungal activity against *T. rubrum*, *T. mentagrophytes*, *Aspergillus fumigatus* and *Microsporum canis*. Unfortunately, those researchers did not test leaf nor bark samples based on their results on modifications of existing methods.

Growth inhibition (zone of inhibition) was recorded as very high (++++), high (+++), medium (++) and low (+),which indicated zones of inhibition between 41 to 50, 31 to 40, 21 to 30 and 11 to 20 mm, respectively.

These zones of inhibition were determined from the central point to the point where growth inhibition has occurred and measured, respectively. The ethanol extract and boiled aqueous extract of the leaves were noted to have more antimicrobial effects as compared to the aqueous extracts.

The high zones of inhibition noted in the ethanol extracts (using a 1:10 concentration) suggest further explanation of the possibility of using this plant against certain ailments caused by the aforementioned fungal

Table 4. Effect of ethanol, aqueous extracts and boiled aqueous extracts obtained from *Z. mucronata* on different fungal species (Tests were done in triplicate).

Fungal species	Ethanol extract			Aqueous extract			Boiled aqueous extract		
	1:10	1:100	1:500	1:10	1:100	1:500	1:10	1:100	1:500
A. flavus	++	++	++	++	-	-	+++	+	-
A. glaucus	+++	+	-	++	+	-	+++	-	-
C. albicans	+	-	-	-	-	-	++	-	-
C. tropicalis	+	-	-	-	-	-	-	-	-
T. mentagrophytes	-	-	-	-	-	-	-	-	-
T. rubrum	-	-	-	-	-	-	-	-	-

- = Negative antifungal activity; + = Positive antifungal activity (low inhibition); ++ = Positive antifungal activity (medium inhibition); +++ = Positive antifungal activity (high inhibition); ++++ = Positive antifungal activity (very high inhibition). Plates containing potato dextrose agar served as controls. Controls did not show any inhibition of any of the test fungal species.

organisms.

Conclusion

It was evident that *Ziziphus mucronata* does possess antimicrobial properties and can be used as a substitute for other extensively harvested species demonstrating similar properties. Although, the roots of the plant did not yield positive results, the study showed that leaves and bark possesses greater antimicrobial properties. Therefore, this study was important as it may help to sustain remnants of the existing population in Umlazi. However, further investigations are needed, including purification and identification of the active compounds present in the leaves and bark.

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