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Full Length Research Paper

Screening of traditional utilized *Haworthia limifolia* for antibacterial and antifungal properties

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Haworthia limifolia **is often used by traditional healers as a spiritual remedy to ward off evil as well as a treatment as blood purifiers and cures against coughs, skin rashes, sun burns, burns, etc. *H. limifolia* exhibits similar morphological characteristics as *Aloe* species. A validation of the use of *H. limifolia* crude extracts from the leaves was successful against five Gram-positive and four Gram-negative bacteria. Extracts from the leaves also exhibited antimicrobial activities in all treatments against the selected bacterial and fungal strains.**

Key words: *Haworthia limifolia*, antimicrobial, traditional medicines.

INTRODUCTION

South Africa has a very rich plant diversity, many of which are medicinally useful (Afolayan and Adebola, 2004). This rich resource is decreasing at an alarming rate as a result of indiscriminate and unsustainable harvesting. Demands for plant derived medicines have created a trade in indigenous plants in South Africa conservatively estimated to be worth approximately R270 million a year (Mander, 1998). Mander (1998) estimates that there are 27 million consumers of traditional medicine and its supporting industry in South Africa. Population growth coupled with rapid urbanization is creating an ever increasing demand for traditional medicine. This together with the high rate of unemployment is forcing many people to turn to gathering and selling medicinal plants to eke out a meagre living. This has resulted in the exploitation of some species, in certain instances, almost to the brink of extinction (Mander, 1998). Several plant species have been so greatly exploited that they are seldom found in unprotected areas (Dold and Cocks, 2002)

More than 1020 plants are used for traditional medicine purposes in KwaZulu-Natal, of which approximately 450 species are sold in large volumes in markets (Mander, 1998). Nine plant specimens make up approximately one-fifth of the traditional plant market in KwaZulu-Natal (Mander, 1997). The low socio economic standing of a great majority of people in KwaZulu-Natal, especially in rural areas, suggests that many use traditional methods of healthcare (Hirst, 1990). To this end, traditional health practitioners play a crucial role in providing health care to the majority of the population. The role of natural products, herbal medicine, tribal and traditional medicines is increasingly appreciated for the prevention and treatment of many human ailments (Janardhanan and George, 2006).

Antimicrobial activities of many South African medicinal plants have been reported in recent years (Grierson and Afolayan, 1999; McGaw et al., 2000; Afolayan et al., 2002; Mathabe et al., 2006; Lategan et al., 2009). However, very little information is available on *Haworthia limifolia*, despite been extensively used for the treatment of a wide variety of ailments (Table 1). The only study on *H. limifolia* focused on its use in the treatment of gastro-intestinal ailments using test organisms that were directly or in-directly involved in gastro-intestinal disorders (Fawole et al., 2009). However, *H. limifolia* has wider uses ranging from treatment of sores, superficial burns, used as blood purifers and to promote pregnancy in women and cattle (Pers. Comms., 2007). It is also used as a charm to ward off evil and as protection against snakes and to ward off evil. The plant leaves are taken orally; either chewed fresh or brewed in the form of a tea (Pers. Comm., 2007). The plant is in high demand and

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may soon become depleted due to unsustainable harvesting. Approximately 22, 5 tons of the *H. limifolia* is traded in medicinal markets each year, comprising approximately 479 000 individual plants (Mander, 1998). However, *H. limifolia* is unique, in that, unlike other medicinal species where plant parts are collected (bulbs, roots, bark etc.) whole plants are collected thereby increasing harvesting pressure.

Interestingly, *H. limifolia* is mainly used by the Zulu speaking traditional healers and is mostly confined to KwaZulu-Natal. It has been shown that traditional healers from different localities use different medicinal plants for the treatment of common ailments (Mathabe et al., 2006). Ethnopharmacological studies using plant extracts used by traditional healers from different localities and ethnicities have confirmed this (Lin et al., 2002; Ngobeli, 2002).

The need for safe and effective antimicrobial compounds is very important, especially since, pathogens are becoming increasingly resistant to many drugs (Anh et al., 2001). Scientific evidence has brought about the possibility of the utilization of plant extracts in the treatment of fungal and bacterial infections, and the development of antibacterial and antifungal products (Farnsworth, 1994; Fox, 1999).

Furthermore, antibacterial activity has allowed a better understanding of the use of traditional medicines as opposed to modern day drug therapies (Coopoosamy and Magwa, 2007). This investigation attempts to confirm the antimicrobial potential of *H. limifolia* in KwaZulu-Natal, against a selected range of bacterial and fungal microorganisms and further substantiate the use of this plant in traditional practices.

MATERIALS AND METHODS

Traditional uses of *H. limifolia* against various ailments was obtained from Miss M. Mahlangu, technical assistance, Mrs. Dumisile, field ranger and Mr. Genge, Driver, Ezemvelo Kwa-Zulu Natal Wildife, North West Zululand region.

Antibacterial assay

Methods used in extraction of plant extracts by traditional healers were performed according to Coopoosamy et al. (2010). Leaf material of *H. limifolia* was collected from the field and dried in an oven at 60°C until sufficiently dried. One kilogram of dried material was then crushed and placed in a 2 L conical flask containing one of three mediums, that is, water, ethyl acetate and acetone, for extraction. A further extraction was attempted in hot water (80°C). The media, except the boiled medium, were left for 72 h in an orbital shaker at 20 shakes per minute. After 72 h the extracts were filtered. The boiled medium was placed on a hot plate at constant 80°C for a period of 4 h after which it was filtered. The extracts were then used for further tests.

The plant crude extract was then tested for antibacterial properties against five strains of Gram-positive (*Bacillus subtilis, Micrococcus kristinae, Bacillus cereus, Staphylococcus aereus, Staphylococcus epidermidis*) and four strains of Gram-negative bacteria (*Escherichia coli, Proteus vulgaris, Enterobacter aerogenes,* and *Shigella sonnei*) for antibacterial activity. 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}.

Antifungal assay

H. limifolia leaves (approximately 1 kg) were cut into small pieces and crushed in a homogenizer. The plant material was soaked in ethanol (95% v/v) and in distilled water in 2 L conical flasks for 3 weeks. A further extract was performed in boiled distilled water over 4 h (each having separate portions of plant material). The extracts (water, boiled water and ethanol) obtained were evapo-rated at reduced pressure (45°C) to a residue. Extracts for testing ethanol, aqueous extract and boiled 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, 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 were used as controls.

RESULTS AND DISCUSSION

Many of the uses of *H. limifolia* in traditional treatments can be credited to the presence of Gram-positive bacteria. Extensive investigations have shown that these Gram-positive bacteria cause various human diseases such as colds, wounds and sores (Coopoosamy and Magwa, 2007, 2010; Waihenya et al., 2002). Personal communications with various individuals have indicted the traditional use of *H. limifolia* to be used both in spiritual as well as traditional medicinal practices (Table 1). The traditional use has been linked to sores, burns and sun-burns. During the process of sore development or other superficial mycosis, bacteria and fungi accumulate on the surface of wounds and burns All Gram-positive bacteria were inhibited by the ethyl acetate extracts of leaves of *H. limifolia* (Table 2).

The aqueous extract did not inhibit activities of both acetone and ethyl acetate extract. Coopoosamy et al. (2010) indicated that boiling could assist in the extractions being used by traditional healers. It was observed that boiled extraction inhibited most Gram positive bacteria under investigation with the exception of *Micrococcus kristina* (Table 2). *E. coli* was the only Gram-positive bacteria inhibited by the ethyl acetate, acetone and boiled water extract. This investigation aids in the understanding of how traditional healers may be able to unlock the active ingredients of various traditionally used medicinal plants to their benefit. Previously, water extracts have proven to be ineffective in inhibiting either Gram-positive or negative bacteria. However, this investigation has successfully substantiated the use of *H. limifolia* by traditional healers as a source of treatment

Table 1. Traditional uses of *H. Limifolia.*

Plant part	Uses	References
Whole plant (growing)	Removal of evil	Pers comm. (2007). Miss. M. Mahlangu, Mrs. Dumisile and Mr. Mgenge
Leaves	Tea of boiled leaves for blood purifiers	Pers comm. (2007). M. Mahlangu, Mrs. Dumisile
Leaves	Tea of boiled leaves to promote pregnancy	Pers comm. M. Mahlangu
Leaves	Treatment of sores	Pers comm. (2007). M. Mahlangu, Mrs. Dumisile
Leaves	Treatment of superficial burns	Pers comm. (2007. M. Mahlangu, Mrs. Dumisile
Leaves	Treatment of sun burns	Pers comm. (2007). M. Mahlangu, Mrs. Dumisile
Leaves	Cleansing of digestive system	Pers comm. (2007). M. Mahlangu and Mr. Mgenge

Table 2. Minimal Inhibitory Concentration (MIC) of *H. limifolia* antibacterial assay on crude extract (Controls: Chloramphenicol and Streptomycin sulfate) n = 3.

Bacteria 10^6 Bacteria/ml	Gram +/-	Medium (MIC) (mg/ml)				Control µg/ml	
		Cold water	Boiled water (80°C)	Ethyl acetate	Acetone	Chlor[a]	Strept[b]
Bacillus subtilis	+	Na	7.0	3.0	3.0	<2.0	<2.0
Micrococcus kristinae	+	Na	Na	4.0	5.0	<2.0	<2.0
Bacillus cereus	+	Na	8.5	4.0	4.0	<2.0	<2.0
Staphylococcus aereus	+	Na	7.0	4.0	5.0	<2.0	<2.0
Staphylococcus epidermis	+	Na	7.5	5.0	5.0	<2.0	<2.0
Escherichia coli	-	Na	8.0	5.0	5.0	<2.0	<2.0
Proteus vulgaris	-	Na	Na	Na	Na	<2.0	<2.0
Shigella sonnei	-	Na	Na	Na	Na	<2.0	<5.0
Enterobacter aerogene	-	Na	Na	Na	Na	<2.0	<2.0

Na = No Activity.All tests were done in triplicates and the averages are indicated. Chlor[a] = Chloramphenicol, Strept [b]= Streptomycin sulphate.

towards various ailments.

The results of the present investigation on the effect of antimicrobial activity are in line with those from previous screenings of medicinal plants for antibacterial and antifungal activity, where most of the extracts of various plant species showed activity against Gram-positive strains (Coopoosamy and Magwa, 2007; Grierson and Afolayan, 1999; Kelmanson et al., 2000; Rabe and van Staden, 1997; Shamim et al., 2004; Vlietinck et al., 1995). The minimum inhibitory concentration values obtained in the current study are relatively high. High Minimum Inhibitory Concentrations (MIC) were obtained for the Gram-negative *E. coli* for the acetone, ethyl acetate and boiled water extract when compared to the control. Ethyl acetate, acetone and boiled water extracts also exhibited high MIC values for the Gram-positive bacteria; *B. subtilis*, *M. kristinae* and *B cereus* (Table 2). This could be due to the active compounds in the extracts being present in relatively low concentrations.

The antifungal activity (Tables 3 and 4) of the ethanol and boiled water extracts were found to be more effective than aqueous extracts. 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 (Coopoosamy et al, 2010). The ethanol and boiled water extracts were noted to possess more antimicrobial effects as compared to the aqueous extracts.

Conclusion

It is evident that traditional healers do not have effective extraction media to maximize the effects of the plant material. However, this investigation assists in the understanding of the methods employed by traditional healers to obtain maximum benefit from various medicinal plants. Comparatively, it has been shown that the boiled water extract can produced just as effective results as that of the more polar media. Similar methodology should be employed when investigating the antimicrobial activities of other traditionally important plants.

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Table 3. Effect of Ethanol, aqueous extracts and boiled aqueous extracts obtained from *H. limifolia* on different fungal species.

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
Aspergillus flavus	+++	++	+	+	+	-	++	++	+
Aspergillus glaucus	+++	+	+	+	+	-	++	+	+
Candida albicans	+++	++	+	+	-	-	++	++	+
Candida tropicalis	+++	++	+	+	+	-	++	+	+
Trichophytonm entagrophytes	++	+	+	+	-	-	++	+	-
Trichophyton rubrum	++	+	+	-	-	-	+	-	-

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

Table 4. Minimal Inhibitory Concentration (MIC) observed in different concentrations prepared from Stock solution of 100 mg/ml of aqueous, boiled aqueous and ethanol extracts of *H. limifolia* n = 3.

Fungal species	Ethanol extract	Aqueous extract	Boiled aqueous extract
Aspergillus flavus	1:500	1:100	1:500
Aspergillus glaucus	1:500	1:100	1:500
Candida albicans	1:500	1:10	1:500
Candida tropicalis	1:500	1:100	1:500
Trichophyton mentagrophytes	1:500	1:10	1:100
Trichophyton rubrum	1:500	1:10	1:10

Number of replications = 3.

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