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Full Length Research Paper

Assessing the potential of *Tetradenia riparia* in treatment of common skin conditions in rural communities of South Africa

R. M. Coopoosamy* and K. K. Naidoo

Department of Nature Conservation, Mangosuthu University of Technology, P. O. Box 12363, Jacobs, 4026, Durban, KwaZulu-Natal, South Africa.

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Tetradenia riparia **has been used by traditional healers for treatment against various ailments including wound healing and skin sores. The scientific validation of the use of *T. riparia* against selected strains of Gram positive and Gram negative bacteria as well as selected fungal strains has substantiated the use by traditional healers. The relatively high inhibitory concentration for both bacterial and fungal strains further indicated the high value of this plant species in the medicinal world and could aid in treatment of secondary infections, such as, mouth sores, pelvic or vaginal sores in individuals who are HIV/AIDS infected.**

Key words: *Tetradenia riparia*, *iboza*, antimicrobial, traditional medicines.

INTRODUCTION

Hospital-acquired and health care related infections are an increasing threat to patient safety and health in the world (Weinstein, 1991, 1998). In the United States, infections encountered in the hospital or a health care facility affect more than 2 million patients, cost $4.5 billion, and contribute to 88,000 deaths annually (Malone and Genuit, 2002; Tasota and Fisher, 1998). Skin infections include impetigo, boils, carbuncles, cellulitis, and complications from burns (Gelfand, 1984; Gold and Eisenstein, 2000). Common pathogens include *Staphylococcus aureus*, group A streptococci, and *Pseudomonas aeruginosa* (Baggett and Hennessy, 2004; Toshkova and Annemuller, 2001; Wysocki, 2002).

Surgical wound infections account for 20 to 30% of cases, but contribute to as many as 57% of extra hospital days and 42% of extra costs. *Staphylococcus epidermidis, S. aureus, Enterococcus faecium, Enterococcus faecalis, Escherichia coli, Enterobacter species,* and *P. aeruginosa* are common pathogens in wound infections (Goldmann and Weinstein, 1996; Weinstein, 1991). Despite being harmless in most individuals, a bacterium such as *S. aureus* is capable of causing various infections of the skin and other organs. These infections are especially common in people with frequent skin injury due to malnutrition. Gastero-intestinal infections exacerbate the problem, especially in young children, due to living in unhygienic conditions.

Tetradenia riparia is found in wooded hillsides and stream banks of coastal KwaZulu-Natal, Mpumalanga and the Northern Province of South Africa; also northern Namibia, Angola, Botswana and east tropical Africa (Figure 1). Its fuzzy green leaves are extremely pungent and have moth-repellent properties. The common indigenous name given to *T. riparia* is “ibosa” which is derived from the Zulu word referring to the aromatic leaves which is routinely prescribed to provide relief from a variety of different ailments. The species has been used as an aqueous infusion or decoction taken internally for colds and flu, bronchitis, stomach upsets, flatulence, mouth ulcers, diarrhoea, and fevers (especially malarial) and used externally, as an inhalation, for headaches.

Plants showing dermatological properties are highly sought after due to their ability to stop bleeding, speed up wound healing and to soothe skin exposed to burns (Lewis and Elvin-Lewis, 1977). Although skin diseases do

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

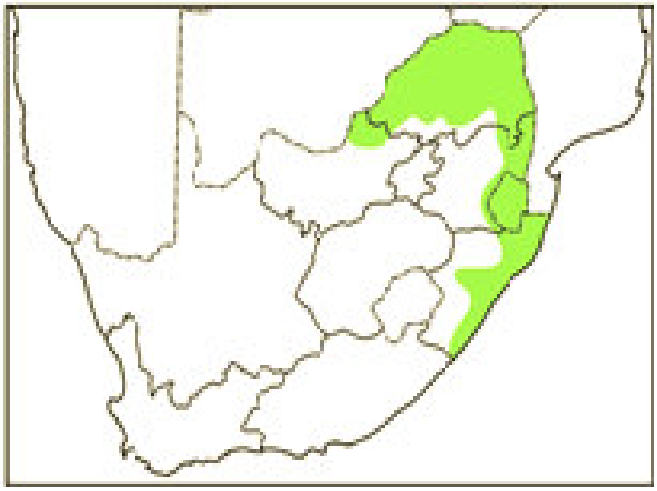

Figure 1. Distribution of *T. riparia* in southern Africa.

not usually threaten life, their unforgiving itching can cause misery and their presence may be a social stigma. In sub- Saharan Africa skin conditions are dominated by bacterial and fungal infection and their clinical expression is often modified by HIV-induced immune-suppression (Naafs, 2004). Skin diseases may therefore constitute a large percentage of all attendees in clinics in rural communities in South Africa.

This investigation makes attempts to validate the use of *T. riparia* to treat common skin conditions, commonly occurring amongst rural indigenous communities, using selected species of bacteria and fungi. Although antibiotics are the mainstay of bacterial treatment, most have lost their effectiveness against common infections because of increasing drug resistance (Barie, 2008; Domin, 1998). Unfortunately, the increased health costs (Amsden, 2004; Apfalter, 2003) and limited accessibility of health care amongst rural dwellers results in an increased reliance on traditionally utilised medicinal plants to treat various ailments.

MATERIALS AND METHODS

Fresh plant material was collected by Mr. G. MacDonald, Department of Nature Conservation, Mangosuthu University of Technology. Additional fresh plant material was obtained from Silverglen Nature Reserve, Chatsworth, Durban, South Africa.

Antibacterial assay

One kilogram of leaf and stem material of *T. riparia* was collected from the field and dried in an oven at 60°C until sufficiently dried (approximately 3 days). The dried material was then weighed and each specimen was then crushed separately before being placed in separate 2 L conical flask containing one of three mediums, that is, hot boiled water, ethyl acetate and methanol, for extraction. The media except the boiled medium were left for 72 h in an orbital shaker at 20 shakes per minute. After 72 h, the ethyl acetate and methanol 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 extracts were then tested for antibacterial properties against three strains of Gram-positive (*Bacillus subtilis* (ATCC 11744), *Staphylococcus aereus* (ATCC 29737), and *Staphylococcus epidermidis* (ATCC 12228)) and three strains of Gram-negative bacteria (*Escherichia coli* (ATCC 13706), *Proteus vulgaris* (ATCC 49132), and *Enterobacter aerogenes* (ATCC 35029)) 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

T. riparia leaves and stems (approximately 1 kg 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 into separate 2 L conical flasks for 3 weeks. The extracts (water and ethanol) obtained were evaporated at reduced pressure (at 45°C) to a residue. Extracts for testing ethanol and aqueous extracts 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,* and *Candida tropicalis.* Plates containing potato dextrose agar were used as controls.

RESULTS AND DISCUSSION

Infections encountered in the hospital or a health care facility have been known to affect more than 2 million patients universally and contribute immensely towards the mortality rate annually (Malone and Genuit, 2002; Tasota and Fisher, 1998). These infections could arise from hospitalization in poor rural communities where sterilization of equipment for surgical procedures cannot be properly carried out, giving rise to bacterial infection on the treated area. Gram positive bacteria are the most prevalent strain that cause human diseases, including infections such as sores (Waihenya et al., 2002). Accordingly, an ability to inhibit these Gram positive bacteria from colonizing a wounded area would provide suitable alternatives to health care practices in rural communities. However, it has also been noted that some strains of Gram negative bacteria are known to occur in wounded areas (Goldmann and Weinstein, 1996; Weinstein, 1991). Hence, any potential agent that could be active against both Gram positive and Gram negative bacterial strains would be an advantageous in reducing infections of wounds and sores.

In the current study, all Gram positive bacteria were inhibited by the boiled water, ethyl acetate, and methanol extracts obtained from the leaves and stems of *T. riparia* (Table 1). This indicates that the crude extracts have an extremely high potential as a cure for wounds and sores

Table 1. Minimal Inhibitory Concentration (MIC) of *T. riparia* antibacterial assay on crude extract (Controls: Chloramphenicol and Streptomycin sulfate) n = 3.

Bacteria	Gram	Medium (MIC) (mg/ml)						Control µg/ml	
		Boiled water		Ethyl acetate		Methanol			
10^6 Bacteria/ml	+/-	Leaf	Stem	Leaf	Stem	Leaf	Stem	Chlor[a]	Strept[b]
Bacillus subtilis	+	6.0	7.0	3.0	5.0	4.0	5.0	<2.0	<2.0
Staphylococcus aereus	+	5.0	7.0	4.0	5.0	5.0	5.0	<2.0	<2.0
Staphylococcus. epidermis	+	5.0	6.0	3.0	5.0	5.0	6.0	<2.0	<2.0
Escherichia coli	-	6.0	7.0	4.0	7.0	5.0	6.0	<2.0	<2.0
Proteus vulgaris	-	Na	Na	4.0	6.0	5.0	6.0	<2.0	<2.0
Enterobacter aerogene	-	Na	Na	5.0	7.0	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 sulfate

Table 2. Effect of ethanol and aqueous extracts obtained from *T. riparia* on different fungal species.

Fungal species	Ethanol extract						Boiled aqueous extract					
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
	1:10	1:10	1:100	1:100	1:500	1:500	1:10	1:10	1:100	1:100	1:500	1:500
Aspergillus flavus	++++	+++	+++	++	+	+	+++	++	++	+	-	-
Aspergillus glaucus	++++	+++	+++	++	+	+	++	+	++	-	-	-
Candida albicans	++++	+++	+++	++	+	+	++	+	+	+	-	-
Candida tropicalis	++++	+++	+++	++	+	+	+++	++	+	-	-	-

- = 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 only served as controls. Control did not show any inhibition of any of the test fungal species.

Table 3. Minimal inhibitory concentration observed in different concentrations prepared from Stock Solution of 100 mg/ml of Aqueous and ethanol extracts of *T. riparia* n = 3.

Fungal species	Ethanol extract		Boiled aqueous extract	
	Stems	Leaves	Stems	Leaves
Aspergillus flavus	1:500	1:500	1:100	1:100
Aspergillus glaucus	1:500	1:500	1:10	1:10
Candida albicans	1:500	1:500	1:100	1:100
Candida tropicalis	1:500	1:500	1:10	1:10

Number of replications = 3.

and could aid in the curative properties of injuries as well as after surgical procedures. The ethyl acetate extract, further, inhibited all gram positive bacterial growth, however at a higher concentration. This would indicate that the ethyl acetate extract would be the most suitable for clinical usage. However, the methanol extracts also inhibited all Gram positive strains except for *Enterobacter aerogene* and can also be a potential extracting medium for crude extracts from *T. riparia*. It is known that traditional healers use boiling of plant material to make decoctions for treatment. The current investigation provided a scientific validation for the use of boiling water as an extraction medium. The boiled extract of the investigation has indicated an inhibition of all Gram positive bacterial strains under investigation as well as one Gram negative strain, that is, *E. coli*. This inhibition by boiled water extracts validates the use of this medicinal plant by rural indigenous people. Traditional healers often prescribe extracts of *T. riparia* for the treatment of sores and wounds that may be caused by unhygenic living conditions in rural communities. Furthermore, as sores and wounds are secondary infections that occur in HIV infected individuals, the use of *T. riparia* boiled water extracts can be used to treat patients, especially where western medicines are scarce or lacking.

The antifungal activity (Tables 2 and 3) of the ethanol extracts of the leaves and stems were found to be more

effective than aqueous extracts. However, the stem extract indicated a greater range of antifungal activity than the leaves against the test organisms. 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 extract of both the leaves and stem indicate a greater antifungal effect inhibiting growth of all fungal test species. As all test species are predominantly found in superficial mycosis, this investigation indicates a potential preventative cure against fungal infections on open wounds and sores. The boiled water of leaf extract provided relatively high inhibition against *A. flavus* and *C. albicans* at 1:100 and 1:10 against *A. glaucus* and *C. tropicalis*. Furthermore, strong inhibition of stem extracts; 1:100 against *C. albicans* and *A. flavus* and 1:10 against *C. tropicalis* and *A. glacus* were noted.

The high zones of inhibition noted in the ethanol extracts (using a 1:500 concentration) suggest further investigation of the possibility of using this plant in alternative uses against sores and wounds caused by the above fungal organisms. Furthermore, further investigations may elucidate greater benefit for the use of this species in the treatment of secondary infection related to HIV/AIDS.

Conclusion

T. riparia has been used extensively by traditional healers for treatment against various ailments. The validation of the use of *T. riparia* in the treatment of superficial sores and wounds indicates a potential of this species as an alternative source of treatment where western medicines are lacking. However, further investigation as a potential household remedy will be attempted as well as validation for the treatment of psoriasis and other skin ailments. The relatively high inhibitory potential of both the boiled extract and ethanol extract could also aid in treatment of secondary infection of HIV/AIDS infected individuals.

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