

## Review

# Crude sample preparation, extraction and *in vitro* screening for antimicrobial activity of selected wound healing medicinal plants in KwaZulu-Natal, South Africa: A review

S. Ghuman<sup>1</sup> and R. M. Coopoosamy<sup>2\*</sup>

<sup>1</sup>Department of Environmental Health, Mangosuthu University of Technology, P. O. Box 12363, Jacobs, KZN 4026, South Africa.

<sup>2</sup>Department of Nature Conservation, Mangosuthu University of Technology, P. O. Box 12363, Jacobs, KZN 4026, South Africa.

Accepted 17 May, 2011

Plant materials (leaves, stems, bulbs and roots) of many species of medicinal plants are used in the treatment of skin conditions and wound healing. Indigenous people are known to widely use the crude extracts of many plants. A large number of plants have been identified for their antimicrobial activities for various medicinal ailments particularly for skin conditions and wound healing. Plant extracts of *Aloe arborescens*, *Aloe aristata*, *Bulbine natalensis*, *Bulbine frutescens* and *Scilla natalensis* were tested for antibacterial activity against 5 strains of Gram positive (*Bacillus subtilis*, *Micrococcus kristinae*, *Bacillus cereus*, *Staphylococcus aureus* and *Staphylococcus epidermidis*) and 4 strains of Gram negative (*Escherichia coli*, *Proteus vulgaris*, *Enterobacter aerogenes* and *Shigella sonnei*) bacteria and antifungal activity for 6 fungal cultures: *Candida albicans*, *Candida tropicalis*, *Aspergillus flavus*, *Aspergillus glaucus*, *Trichophyton mentagrophytes* and *Trichophyton rubrum*.

**Key words:** Sample preparation, extraction, antimicrobial screening, wound healing, medicinal plants.

## INTRODUCTION

South Africa's rich plant diversity makes it one of the countries where the demand for medicinal plant use is high. It is estimated that 70% of its black population consult traditional healers for health related conditions thus utilizing natural medicines. Currently, over 27 million consumers of traditional medicine, most of which are indigenous plant derivatives rely on traditional healers for their cures (Mander, 1998, 1997; Jager et al., 1996).

Medicinal plants maybe obtained from the traditional healers, herb markets or gathered in the wild. The problem is that many of these are protected species as the wild populations are declining due to excessive harvesting for the purpose of medicinal use, typical of developing countries (Farnsworth, 1994; Srivastava et al., 1996). Traditional and developed practices have leaned towards the development of technologically advancement in the identification of safe medicinal compounds for the treatment of a wide range of conditions, especially as many pathogens are already and becoming resistant to allopathic medication (Ahn et al., 2001).

Many well known plant species have been identified for

\*Corresponding author. E-mail: [rogercoopoosamy@gmail.com](mailto:rogercoopoosamy@gmail.com).  
Tel: +27 82 200 3342. Fax: +27 31 907 7665.

treating burns, rashes, boils, mouth blisters, insect bites, cold sores and cracked skin (van Wyk et al., 1997; Raina et al., 2008; Nagori and Solanki, 2011). Extraction and screening techniques of biologically active medicinal compounds have been conducted on many well known species of plants used in traditional medicines and most plants have shown antimicrobial (antibacterial and antifungal) activities (Rabe and van Staden, 1997; Grieson and Afolayan, 1999; McGraw et al., 2000; Afolayan et al., 2002; Mathabe et al., 2006; Coopoosamy and Magwa, 2007; Lategan et al., 2009; De Wet et al., 2010). Scientific evidence for the development of antibacterial and antifungal products from plants has made advances for further studies and some have been patented successfully (Farnsworth, 1994; Fox, 1999; Ofodile et al., 2010).

## MATERIALS AND METHODS

Fresh whole plant material (leaves, stems, bulbs and roots) of *Aloe arborescens*, *Aloe aristata*, *Bulbine natalensis*, *Bulbine frutescens* and *Scilla natalensis* were collected from the Silverglen Nursery. SG2011/01; SG 2011/02; SG2011/03; SG 2011/04; SG2011/05; SG2011/06 are the voucher specimens housed at Mangosuthu University of Technology in the Medicinal Plant Research Laboratory. Fresh samples were used for immediate extraction, using both traditional and laboratory based methods outlined according to Coopoosamy et al. (2010).

### Traditional healer's method of extraction from plants species

Plant materials (leaves, roots, stem and corm) were allowed to sufficiently dry for approximately 3 days. Once dried the material was boiled to extract the necessary ingredients required to form a decoction for antimicrobial screening. The amount of plant material used in this process varies; however, approximately a kilogram of each was used. After 30 min of boiling in water, the fluid portion was separated from the pulp. This fluid with the extract was referred to as a "tea". The tea was then allowed to cool and refrigerated for testing.

### Laboratory method of extraction of compounds from plant species

One kilogram of fresh and dried material of each (leaf, stem, bulb and root) was used. Fresh material was cut into small pieces before crushing and both fresh (using mortar and pestle) and dry material was crushed using a mortar and pestle. The leaf latex of the *Aloe* spp. and *Bulbine* spp. was weighed after removing the epidermis with the help of a sharp blade. The crushed material was then placed respectively in 2 L conical flasks containing one of each of the 5 extractant mediums namely ethanol, ethyl acetate, methanol, acetone and distilled water for extraction based on the varying polarity of the solvents. The media was left for 72 h in an orbital

shaker at 20 shakes per min. After 72 h, the extracts were filtered, refrigerated and used for testing.

The methanol, acetone, ethanol, ethyl acetate and sterile distilled water were used as extraction solvents for extracting the compounds from the dried plant material. 5 g of crushed fresh plant material and 5 ml of each solvent were placed in a screw cap tube, shaken vigorously for 5 min. Centrifugation of the extracts for 5 min separated the supernatant from the plant material for each extract. Supernatant of each plant extract was transferred into pre-weighed beakers. The procedure was done in triplicate for each plant extract. The pre-weighed beakers containing the plant extract supernatants were allowed to dry completely to obtain a solvent free dried extract residue. Methanol and acetone plant extract supernatants were subjected to overnight drying under airflow in a fume cupboard. Dried beakers were re-weighed and calculated extract residues resuspended in 0.5 ml DMSO (Merck). Plant extract concentrations varied between the different plants and solvents used for extraction. Prior to assaying, a 1.25% extract solution was made using the stock DMSO extracts and sterile distilled water.

### Screening for antimicrobial activity

The antibacterial and antifungal susceptibilities were tested using the agar diffusion method (Janssen et al., 1987) followed by the dilution method for products which presented a bioactivity. Petri plates were prepared by pouring 20 ml of Mueller Hinton (MH) agar (BIO-RAD) for all the bacteria. The inoculum was spread on top of the solidified media and allowed to dry for 10 min. The discs were then applied and the plates were left for 30 min at room temperature, to allow the diffusion of the oil before their incubation for 24 h at 37°C in air for all bacteria (Collins et al., 1989). The inhibition zones formed around the discs were evaluated in millimeters. Each test was carried out in triplicate.

Minimum inhibitory concentrations (MIC) were determined by the dilution method as recommended by the National Committee for Clinical Laboratory Services (NCCLS, 1997). Experiments were carried out in triplicate. Inhibition of microbial growth in the plates containing tested solutions was judged by comparison with growth in blank control plates. Solvent at 10% had no inhibition effect (Coopoosamy and Magwa, 2007). MIC was defined as the lowest concentration of test samples that resulted in a complete inhibition of visible growth.

### Antibacterial assay

Traditional healers tend to use water (being most polar) for extractions. Less polar mediums such as ethyl acetate and acetone will selectively isolate the less polar compounds and provide a form of separation. The plant extracts were then tested for antibacterial activity against 5 strains of Gram positive (*Bacillus subtilis*, *Micrococcus kristinae*, *Bacillus cereus*, *Staphylococcus aureus* and *Staphylococcus epidermidis*) and 4 strains of Gram negative (*Escherichia coli*, *Proteus vulgaris*, *Enterobacter aerogenes* and *Shigella sonnei*) bacteria. 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 per ml. Cultures were done in triplicate.

**Table 1.** Minimal inhibitory concentration (MIC) of *H. limifolia* antibacterial assay on crude extract (controls: chloramphenicol and streptomycin sulfate) n=3.

10 <sup>6</sup> bacteria (ml)	Gram +/-	Medium (MIC) (mg/ml)			Control (µg/ml)		
		Cold water	Boiled water (80 °C)	Ethyl acetate	Acetone	Chlor <sup>a</sup>	Strept <sup>b</sup>
<i>Bacillus subtilus</i>	+	Na	7.0	3.0	3.0	<2.0	<2.0
<i>Micrococcus kraitsinae</i>	+	Na	Na	4.0	5.0	<2.0	<2.0
<i>Bacillus cereus</i>	+	Na	8.5	4.0	4.0	<2.0	<2.0
<i>Staphylococcus aureus</i>	+	Na	7.0	4.0	5.0	<2.0	<2.0
<i>Escherichia epidermis</i>	+	Na	7.5	5.0	5.0	<2.0	<2.0
<i>Escherichia coli</i>	+	Na	8.0	5.0	5.0	<2.0	<2.0
<i>Proteus vulgaris</i>	-	Na	Na	Na	Na	<2.0	<2.0
<i>Shigella sonnei</i>	-	Na	Na	Na	Na	<2.0	<5.0
<i>Enterobacter aerogene</i>	-	Na	Na	Na	Na	<2.0	<2.0

Na = no activity. All tests were done in triplicates and the averages are indicated. Chlor<sup>a</sup> = chlchloramphenicol, Strept<sup>b</sup> = Streptomycin sulphate.

### Antifungal assay

Plant material will be soaked in ethanol (95% v/v) and in distilled water in 2 L conical flasks for 3 weeks. The extracts obtained will be evaporated at reduced pressure (45 °C) to syrupy residue (Ahmad, 1992). Preparation of extracts of 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 to obtain a concentration of 100 mg/ml dilutions (1:10, 1:100, 1:500) of these stock solutions were 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 6 fungal cultures: *Candida albicans*, *Candida tropicalis*, *Aspergillus flavus*, *Aspergillus glaucus*, *Trichophyton mentagrophytes* and *Trichophyton rubrum*. The test organisms were selected on the preference of them being present on patients suffering with superficial skin and wound conditions. Plates containing potato dextrose agar served as controls.

### DISCUSSION

A study using *Aloe excelsa* leaf extract reported impressive antibacterial and antifungal activity against similar microorganisms, suggesting further information against skin infections (Coopoosamy and Magwa, 2007; Kelmanson et al., 2000; Rabe and van Staden, 1997; Vlietinck et al., 1995). Compounds like acemannan (a complex carbohydrate) and lecithins found in *Aloe* spp. is

known to have immune stimulating, antiviral and antitumor cellular properties (Cappasso et al., 1998; Imanishi et al., 1981). Uronic acids have also been found to be active in the healing process. Studies in India, China, Mexico and Southern Africa report leaf gel exudates as effective dermatological remedies (Grindlay and Reynolds, 1986; Jain and De Fillipps, 1991; Dalton and Cupp, 2000; Davis et al., 1986). Although MIC's were relatively high active compounds were thought to be low and it is recommended that further investigations using bioassay-guided fractionation be conducted (Coopoosamy and Magwa, 2007). *Bulbine* spp. similarly like *Aloe* spp. are reported to be successfully used for skin conditions and wound healing amongst many other treatments (Coopoosamy and Magwa, 2007; Jager et al., 1996).

While it is known that wound healing is a complex and dynamic process (McNees, 2006), a study by Jia et al. (2008) concludes the successful capacity for *A. arborescens* Miller and *Aloe ferox* Miller to accelerate progressive wound healing. Studies on *S. natalensis* extracts were found to have good anthelmintic activity against the parasite *Schistosoma heamatobium* (Sparg et al., 2002) and antibacterial activity against a wide range of skin infections (Matthe, 1989) and is used as remedies for livestock and poultry (Matchett, 2002). In South

**Table 2.** 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
<i>Aspergillus flavus</i>	+++	++	+	+	+	-	++	++	+
<i>Aspergillus glaucus</i>	+++	+	+	+	+	-	++	+	+
<i>Candida albicans</i>	+++	++	+	+	-	-	++	++	+
<i>Candida tropicalis</i>	+++	++	+	+	+	-	++	+	+
<i>Trichophyton mentagrophytes</i>	++	+	+	+	-	-	++	+	-
<i>Trichophyton rubrum</i>	++	+	+	-	-	-	+	-	-

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

Africa, according to Van Staden (2010), bulbs and leaves form part of the diversified flora that the country is endowed with and seasonal phytochemical dynamics of the plant extracts revealed a fairly comparable *in vitro* antibacterial and antifungal activity (*B. subtilis*, *S. aureus*, *Klebsiella pneumoniae*, *C. albicans* between the bulb and leaf extracts in all the investigated plant species (*Tulbagia violacea*, *Hypoxis hermerocallidea*, *Drimys robusta* and *Merwillia plumbea*). MIC's for antibacterial activity (Table 1) were reported by Coopoosamy and Naidoo (2011).

The MIC's were relatively high in this study, especially for Gram negative *E. coli* for acetone, ethyl acetate and boiled water extracts also exhibited high MIC values for Gram positive *B. subtilis*; *M. kristinae* and *B. cereus* and possibly accounted for by active compounds in the extracts presented in relatively low concentrations (Coopoosamy and Naidoo, 2011). Antifungal activity presented in Table 2, reported that ethanol and boiled water extracts were more effective than aqueous extracts. Growth inhibition zones recorded in similar studies (Coopoosamy et al., 2010a,b; Stafford et al., 2005; Fennell et al., 2004) indicated inhibition between 41 to 50 for very high; 31 to 40 for high; 21 to 30 for medium and 11 to 20 mm for low in the fungal species mentioned in Table 2. Similar results were found by Coopoosamy and Magwa (2007) in *A. excelsa* and four species of *Bulbine* (*B. narcissifolia*, *B. frutescens*, *B. longifolia* and *B. latifolia*) (Coopoosamy, 2011).

## Conclusion

The results of the assays are promising evidence for

further comparisons with pure extractions and identification of antibacterial and antifungal compounds in future *in-vivo* and *in-vitro* wound healing research. These findings could be used to develop suitable dosage forms as per requirement for treatment of skin conditions and wound healing. The results are further an indication of validation of use of these plants by traditional healers in traditional medicines for various ailments.

## REFERENCES

- Afolayan AJ, Grieson DS, Kambizi L, Madomombe I and Masika PJ (2002). *In vitro* antifungal activity of some South African medicinal plants. S. Afr. J. Bot., 68: 72-76.
- Ahn NT, Cam PD and Dalsgaard A (2001). Antimicrobial resistance of *Shigella* spp. Isolated from diarrheal patients between 1989 and 1998 in Vietnam. South Asian J. Trop. Med. Public Health, 32: 856-862.
- Afolayan AJ, Meyer JJM (1997). The antibacterial activity of 3,5,7-trihydroxyflavone isolated from the shoots of *Helichrysum aureonitens*. J. Ethnopharmacology, 57: 177-181.
- Afolayan AJ, Meyer JJM (1995). Morphology and ultrastructure of secreting and nonsecreting foliar trichomes of *Helichrysum aureonitens* (Asteraceae). Int. J. Plant Sci., 156: 481-487.
- Ahmad SW (1992). PhD Thesis, Department of Pharmacognosy, University of Karachi, p. 79.
- Cappasso F, Borrelli F, Cappasso R, Di Carlo G, Izzo AA, Pinto L, Mascolo N, Castaldo S, Longo R (1998). *Aloe* and its therapeutic use. Phytother. Res., 12: 124-127.
- Champion RH, Burton JL and Ebling FJG (1992). Textbook of Dermatology, 5<sup>th</sup> ed., 3: 1130-1175. London, Blackwell Scientific Publications.
- Collins CH, Lyne PM, Grange JM (1989). Microbiological Methods (6<sup>th</sup> ed.), Butterworths, London, p. 410.
- Coopoosamy RM (2011). Traditional information and antibacterial activity of four *Bulbine* species (Wolf). Afr. J. Biotechnol., 10(2): 220-224.
- Coopoosamy RM, Naidoo KK, Buwa L, Mayekiso B (2010). Screening of *Siphonochilus aetopicus* (Schwein.) B.L. burtt for antibacterial and antifungal properties. J. Med. Plant Res., 4(12): 1228-1231.
- Coopoosamy RM, Magwa ML (2007). Traditional use, antibacterial

- activity and antifungal activity of crude extract of *Aloe excelsa*. Afr. J. Biotechnol., 6(20): 2406-2410.
- Dalton T, Cupp MJ (2000). *Aloe*. In: Toxicology and Clinical Pharmacology of Herbal Products, Ed. Cupp, MJ Totowa, New Jersey: Humana Press Inc.
- Davis RH, Kabbani JM, Maro NP (1986). Wound healing and antiinflammatory activity of *Aloe vera*. Proc. Pennsylvania Acad. Sci., 60: 79.
- De Wet H, Nkwananya MN, van Vuuren SF (2010). Medicinal plants used for the treatment of diarrhoea in northern Maputaland, KwaZulu-Natal Province, South Afr. J. Ethnopharmacol., 130(2): 284-289.
- Farnsworth NR (1994). The role of medicinal plants in drug development. In: Krosgaard-Larsen S, Brogger-Christensen S, Kofod H. (Eds), Natural Products and Drug Development, Munksgaard, Copenhagen.
- Fennell CW, Lindsey KI, McGraw LJ, Sparg Gi, Stafford EE, Elgorashi OM, Van Staden J (2004). Assessing African medicinal plants for efficacy and safety: pharmacological screening and toxicology. J. Ethnopharmacol., 94: 205-217.
- Fox TR (1999). *Aloe vera* revered, Mystery Healer. Health Foods Business, pp. 45-46.
- Grieson DS, Afolayan AJ (1999). An ethnobotanical study of plants used in the treatment of wounds in the Eastern Cape, South Afr. J. Ethnopharmacol., 67: 327-332.
- Grindlay D, Reynolds T (1986). The *Aloe vera* phenomenon: A review of the properties and modern uses of the leaf parenchyma gel. J. Ethnopharmacol., 16: 117-151.
- Imanishi K, Ishiguro T, Saito H, Suzuki I (1981). Pharmacological studies on Plant Lecithin, Aloctin A.I. growth inhibition of mouse methylcholanthrene-induced fibrosarcoma (Meth A). In ascites form by Aloctin A. Experientia, 37: 1186-1187.
- Jain SK, De Fillipps A (1991). Medicinal plants of India. 1, Algonac, Michigan: USA Reference Publication.
- Jager AK, Huchings A, van Staden J (1996). Screening of Zulu Medicinal plants for prostaglandin-synthesis inhibitors. J. Ethnopharmacol., 52: 95-100.
- Janssen AM, Scheffer JJC, Baerheim Svendsen A (1987). Antimicrobial activity of essential oils: A 1976-1986 literature review Aspects of the test methods. Plant. Med., pp. 395-398.
- Jia Y, Zhao G, Jia J (2008). Preliminary evaluation: The effects of *Aloe ferox* Miller and *Aloe arborescens* Miller on wound healing. J. Ethnopharmacol., 120: 181-189.
- Kelmanson JE, Jager AK, van Staden J (2000). Zulu medicinal plants with antibacterial activity. J. Ethnopharmacol., 69: 241-246.
- Lategan CA, Campbell WE, Seaman T and Smith PJ (2009). The bioactivity of novel furanoterpenoids isolated from *Siphonochilus aethiopicus*. J. Ethnopharmacol., 121: 92-97.
- Mander M (1998). The marketing of indigenous medicinal plants in South Africa: A case study in KwaZulu-Natal. Food and Agriculture Organization of the United Nations. Rome. 151.
- Mander M (1997). The marketing of indigenous medicinal plants in South Africa: A case study in KwaZulu-Natal. Inst. Nat. Res. Pietermaritzburg.
- Matchett JR (2002). A Review of the Use of Plants as Ethno-Veterinary Medical Replacements for Antibiotic Growth Promoters.
- Mathabe MC, Niiolova RV, Lall N, Nyazema NZ (2006). Antibacterial activities of medicinal plants used for the treatment of diarrhoea in Limpopo Province, South Afri. J. Ethnopharmacol., 105: 286-293.
- Matthe DS (1989). Letter to the editor. Phytother. Res., 3: xii.
- Matthe DS (1988). Letter to the editor. Phytother. Res., 2: 107.
- McGraw LJ, Jager AK and van Staden J (2000). Antibacterial, anthelmintic and antiamoebic activity in South African medicinal plants. J. Ethnopharmacol., 72: 247-263.
- McNees P (2006). Skin and wound assessment and care in oncology, Seminars in Oncology Nursing, 22: 130-143.
- Nagori BP, Solanki R (2011). Role of plants in wound healing. Res. J. Med. Plant, 5: 392-405.
- National Committee for Clinical Laboratory Standards (1997). Methods for dilution antimicrobial susceptibility tests of bacteria that grow aerobically. Approved Standard M7-A4, National Committee for Clinical Laboratory Standards, Wayne, PA.
- Ofofiele LN, Kanife UC and Arojoye BJ (2010). Antifungal activity of a Nigerian plant *Chrysanthellum americanum*. Acta SATECH, 3(2): 60-63.
- Rabe T, van Staden J (1997). Antibacterial activity of Southern African plants used for medicinal purposes. J. Ethnopharmacol., 56: 81-87.
- Raina R, Prawez S, Verma PK, Pankaj NK (2008). Medicinal plants and their role in wound healing. Vet Scan, 3(1): 1-7.
- Sparg SG, van Staden J, Jager AK (2002). Pharmacological and phytochemical screening of two Hycinthaceae species: *Scilla natalensis* and *Ledebouria ovatifolia*. J. Ethnopharmacol., 80: 95-101.
- Srivastava J, Lambert J, Vietmeyer M (1996). Medicinal plants: An expanding role in development. The World Bank, Washington DC, p. 18.
- Stafford GI, Jager AK, van Staden J (2005). Effect of storage on the chemical composition and biological activity of several popular South African medicinal plants. J. Ethnopharmacol., 97: 107-115.
- van Staden J (2010). Annual Report. Research Centre Plant Physiology, Molecular Biology and Ethnobotany. UKZN.
- Van Wyk BE, van Oudtshoorn and Gericke N (1997). Medicinal Plants of South Africa. Briza Publications, p. 292.
- Vlietinck AJ, van Hoof L, Totte J, Lasure A, Candan Berghe D, Rwangabo PC and Mvukiyumwami J (1995). Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties. J. Ethnopharmacol., 46: 31-47.