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Full Length Research Paper

Traditional use, antibacterial activity and antifungal activity of crude extract of *Aloe excelsa*

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The fleshy leaves and roots of most species within the *Aloe* family are used in many traditional treatments (Mabberley, 1987). Traditional healers and indigenous people utilize mainly the leaf sap of this genus widely for the treatment of wounds, burns, rashes, itches, cracked lips and cracked skin (Cera et al., 1980). Antimicrobial activities on the crude extract of *Aloe exclesa* was carried out in attempts to validate the use by traditional healers in the use of there latex and gel exudates for various medicinal ailments.

Key words: *Aloe*aceae, *Aloe excelsa*, antimicrobial, traditional healers.

INTRODUCTION

Within the *Aloe* genus, *Aloe excelsa* is predominantly one of the tree *Aloe*s found in abundance in Zimbabwe, with a few dispersed within the Southern African continent. With the recent advancement of research, in the field of medicinal plants, it has become apparent that many of the species utilized by indigenous people as well as the knowledge of the traditional healers has begun to make its mark on society as a possible avenue for curing to diseases (Anderson 1983; Gjerstad and Riner, 1968; Davis et al., 1986; Crewe 1937; Cera et al., 1980; Heggers et al., 1993; Jain and Filipps, 1991; Kelmanson, et al., 2000; Jager, et al., 1996; Fulton, 1990). Recently, most of the research conducted in the traditional medicines has shown that some remedies obtained from traditional healers are very effective in spite of the fact that there is no scientific justification (Kelmanson et al., 2000; Grierson, 1999; Jager et al., 1996; Fox, 1999). However, most of these species are rich in terms of their biomolecules which can manage health hazard problems.

Currently, the antibacterial activities of many plant species have been reported (Grierson and Afolayan, 1999; Jager et al., 1996; Kelmanson et al., 2000). 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 anti-fungal products (Farnsworth, 1994; Fox, 1999). Furthermore, antibacterial activity has also made a better understanding of the use of traditional medicines as potential drugs in addition to contemporary drugs.

Screening techniques of biologically active medicinal compounds have been conducted on well-known species of plants used in traditional medicines and most plants have shown antibacterial activity (Rabe and van Staden, 1997). Researchers such as Afolayan and Meyer (1997) isolated and identified specific active ingredients such as 3,5,7-trihydroxyflavone (galagin). Furthermore, an isolation of several biological compounds has been achieved and most of these have been registered as pharmaceutical drugs (Afolayan and Meyer, 1995, 1997).

MATERIALS AND METHODS

Traditional healer's method of extraction from the *Aloe* species

Leaves of various *Aloe* species were collected by traditional healers or indigenous people and allowed to dry for a period of time in the sun. Once dried the leaves are boiled to extract the necessary ingredients required to form a decoction for treatment. The amounts of plant material used in this process varies from individual to individual, but in most cases an amount approximate to one kilogram of dried leaves are used. After thirty minutes of boiling in water, the fluid portion was separated from the pulp of the bases. The fluid with the extract is referred to as a "tea". This tea was then

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allowed to cool after which it was administered orally three times a day for a period of three to five days. During this time the patient recovers and is advised to drink a lot of water to compensate for the loss due to dehydration. Further a gel-like lotion is made from the exudates and is applied as moisturizers or skin toners. These lotions are also utilized in treatment of sun burns, rashes, burns, wounds and other skin infections. The exudates are also taken orally as a remedy for blood purification, immune boosters or as a laxative.

Screening for antimicrobial activities

The antibacterial and antifungal susceptibilities tests were carried out 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 agar (BIO-RAD) for all the bacteria. The inoculum was spread on the top of the solidified media and allowed to dry for 10 min. The discs were then applied and the plates were left 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 concentration (MIC) was determined by the dilution method as recommended by the National Committee for Clinical Laboratory Standards (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. Minimum inhibitory concentration (MIC) was defined as the lowest concentration of test samples that resulted in a complete inhibition of visible growth.

Antibacterial Assay

Leaf material from *A. excelsa* was collected from the field and was dried in an oven until sufficiently dried. The dried material was then crushed and then placed in one of three mediums, i.e., water, ethyl acetate and acetone for extraction. 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 properties against five strains of gram-positive (*Bacillus subtilis, Micrococcus kristinae, Bacillus cereus, Staphylococcus aureus, Staphylococcus epidermidis*) and four strains of gram-negative bacteria (*Escherichia coli, Proteus vulgaris, Enterobacter aerogenes,* and *Shigella Sonnei*) for antibacterial activity.

Antifungal assay

A. excelsa leaf latex (4.5 kg after removing the epidermis with the help of a sharp knife) were cut into small pieces were crushed in a homogenizer. The plant materials were soaked in ethanol (95%) and in distilled water in large conical flask for 3 weeks. The extracts (aqueous and ethanol) obtained were evaporated at reduced pressure (45°C) to a syrupy residue. The dried ethanol and aqueous extracts of *A. excelsa* was dark brown in color (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 fungal cultures: *Aspergillus flavus, Aspergillus glaucus, Candida albicans, Candida tropicalis, Trichophyton mentagrophytes,* and *Trichophyton rubrum*. The test organisms were selected on the preference of them being present on patients suffering from superficial mycosis.

RESULTS AND DISCUSSION

A. excelsa leaf material appeared to contain some of the following either one of or a combination of very helpful enzymes, saponins, hormones and amino acids which can be absorbed into the human skin. One of these constituents is acemannan which has been isolated and tested for in *Aloe vera*. Acemannan is a complex carbohydrate with immune stimulating and antiviral properties (Cappasso et al., 1998). Certain lectins, which, are found, for example, in the *Aloe* pith, are assumed to help in stimulation of immune response by increasing the production of lymphocytes that are known to kill bacteria and some tumor cells (Imanishi et al., 1981). These products, in addition, have uronic acids that are natural detoxicants and take part in the healing process by stripping toxic materials of their harmful effects. Modern research has shown that these products are able to restore skin tissue due to their moisturizing effects as well as to relieve pain associated with burns and wounds. The juice of *A. excelsa* have been used in herbal therapies to treat stomach disorders such as ulcers, colitis, constipation and other colon related problems.

The contention of *A. excelsa* application has been supported by the work which was done in India for various stomach ailments as well as a purgative and the leaf pulp has been used for menstrual suppression (Jain and De Fillipps, 1991). In Southern Africa as well as in China and Mexico, the leaf gel or exudates are used in various dermatological remedies, such as minor skin irritations (Grindlay and Reynolds, 1986). Although used as medicinal remedies for centuries, the only two common uses that predominate are the laxative effect as well as external treatment of skin infections or injuries. Table 1 indicates the similarities of treatment from *A. excelsa* in the treatment for various superficial skin treatments and also as a laxative. The other uses of which some claims have been made off has to be more closely studied for effectiveness (Dalton and Cupp, 2000; Davis et al., 1986). Vogler and Ernst (1999) indicated that oral administration of *Aloe* gel might be useful for lowering blood sugar levels. This can be further substantiated by the verbal interviews in which the traditional healers indicate that a mild tea concoction of *A. excelsa* is suitable as blood purifiers. The process of formation of the *A. excelsa* tea concoction is always made fresh and consumed over a short period of time. This concurs with the studies of Grindlay and Reynolds (1986), in which they indicated that the *Aloe* gel unstable and may deteriorate in a short period of time.

The question lies in that, how is it possible for that traditional healers to obtain extractions using water and still

Table 1. Uses of *A. excelsa* by traditional healers.

Plant part	Uses	Preparation used
Leaves	Skin burns	Leaf exudates
Leaves	Rashes	Leaf exudates
Leaves	Sun Burns	Leaf exudates
Leaves	Laxatives	Diluted tea from Leaf Exudates
Leaves	Blood purifiers	Diluted tea from Leaf exudates
Leaves	Skin irritants	Leaf exudates
Leaves	Moisturizers	Paste made from leaf Pulp
Leaves	Immune boosters	Diluted tea from Leaf exudates

provide cures to diseases. It could be that the traditional healers boil their contents would contribute towards possible extractions of active compounds. Without boiling of contents would only extract superficial compounds and not allow for those deep seated compounds to be released. Secondly, traditional healers tend to use larger quantities of plant material to obtain concentrated extracts.

These results are in line with those from previous screenings of medicinal plants for antibacterial activity, where most of the active plants showed activity against Gram positive strains only (Kelmanson et al., 2000; Rabe and van Staden, 1997; Vlietinck et al., 1995). The minimum inhibitory concentration values are relatively high, but active compounds in the extract may be present in low concentrations. This could be justified by investtigations using bioassay-guided fractionation.

The ability of *Aloe* to inhibit growth of microorganisms such as fungi and bacteria has been demonstrated by rapid clearing of infected tissue after induction of *Aloe* therapy. In a study of *Aloe* in treatment of tuberculosis during the early stages, it was found that moderate antibacterial activity was exhibited from the leaf sap (Droscoll et al., 1974; Ghannam et al., 1986). Aloin was the most potent component showing an inhibitory range of 1:50 000 to 1:100 000, and the activity was shown to be greater against Gram positive bacteria and most marked against Mycrobacterium tuberculosis (Droscoll et al., 1974).

When a severe burn occurs, the patient loses the protective epithelial layer of skin, and, as a result, is at increased risk for infection by *C. albicans* and other opportunistic pathogens. *C. albicans* is readily introduced into burn wounds because this yeast is commonly a member of the normal flora found on mucous membranes. Treatment of severe burns is very difficult due to pain and risk of infection. Although *Aloe barbadensis* is not considered a mainstream medicinal agent, anecdotal evidence indicates that when *Aloe* is used on these patients' burns the frequency of fungal infections decreases (Lee et al., 1999). This does not necessarily mean that *Aloe* is an antifungal agent, however, as it could be merely acting as a barrier to contamination or aiding in quicker healing of the wound.

Leaf extracts of *A. excelsa* tends to inhibit gram positive bacterial growth for both acetone and ethyl acetate extracts. Such antibacterial activities were noted on the following gram positive bacteria, *B. subtilis, M. kristinae, B. cereus, S. aereus,* and *S. epidermis.* Relatively lower MIC concentrations were obtained for most of the gram positive bacterial species, *B. subtilis, M. kristinae* and *B. cereus*. *E. coli* and *P. vulgaris* were the only gram negative bacteria to show inhibition for the acetone extracts.

Surprisingly no inhibitory effect has been noted for all water extracts. This could be attributed firstly to the fact that traditional healers boil their plant part to extract the necessary ingredients in water. Secondly, both ethyl-acetate and acetone are more polar than that of water. Hence the more polar substances would extract more of the compounds embedded within the plant cells. More polar solvents are difficult for them to obtain.

The antifungal activity (Table 2 and 3) of the ethanol extracts of *A. excelsa* was found to be quite impressive as compared to aqueous extracts. However, less activity against *C. albicans*. Growth inhibition (zone of inhibition) was recorded as very high (++++), high (+++), medium (++), and low (+), which ultimately indicated zones of inhibition between 41 – 50, 31 – 40, 21 – 30, and 11 – 20 mm, respectively. Some very interesting outcomes were noted in this study. The ethanol extract of all the plant was noted to possess more antimycological effects as compared to the aqueous extracts. The high zones of inhibition noted in the ethanol extract *A. excelsa*, (using a 1:10 concentration) suggest further explanation of the possibility of using this plant against certain skin infections caused by the above fungal organisms. The minimum inhibitions observed are given in Table 4.

Conclusion

The results of the viability assay show promising evidence for the antibacterial and antifungal effect of *A. excelsa* sap. A killing effect was seen even at the 10% dilution, indicating that the candidicidal compound is relatively potent. This evidence has shown *A. excelsa* to hold excellent potential as an antifungal agent.

Table 2. Minimal inhibitory concentration (MIC) of *A. excelsa* antibacterial assay on crude extracts (Controls are chloramphenicol[a] and streptomycin sulfate[b]).

Bacteria	Gram +/-	Medium (MIC) (mg/ml)			Control µg/ml	
		Water	Ethyl acetate	Acetone	Chlor [a]	Strept [b]
Bacillus subtilis	+	Na	3.0	2.0	<2	<2
Micrococcus kristinae	+	Na	4.0	1.0	<0.2	<2
Bacillus cereus	+	Na	2.0	2.0	<2	<2
Staphylococcus aereus	+	Na	Na	1.0	<2	<2
Staphylococcus epidermis	+	Na	Na	1.0	<2	<2
Escherichia coli	-	Na	Na	3.0	<2	<2
Proteus vulgaris	-	Na	Na	2.0	<2	<2
Shigella sonnei	-	Na	Na	Na	<2	<5
Enterobacter aerogenes	-	Na	Na	Na	<2	<2

Na = No activity.

Table 3. Effect of ethanol and aqueous extract obtained from *A. excelsa* on different fungal species.

Fungal Species	Ethanolic extract			Aqueous extract		
	1:10	1:100	1:500	1:10	1:100	1:500
Aspergillus flavus	++++	+++	++	++++	+++	++
Aspergillus glaucus	++++	+++	+++	+++	+++	+++
Candida albicans	++	+	-	++	+	-
Candida tropicalis	+++	++	++	+++	++	+
Tricphyton mentagrophytes	+++	++	++	++	++	+
Trichophyton 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).
N.B. Plates containing potato dextrose agar only served as controls. Control did not show any inhibition of any of the test fungal species.

Table 4. Minimal inhibitory concentration observed in different concen-trations, prepared from stock solution of 100 mg/ml of aqueous and ethanol extracts of *A. excelsa*.

Fungal species	Ethanolic extract	Aqueous extract
Aspergillus flavus	1:500	1:500
Aspergillus glaucus	1:500	1:500
Candida albicans	1:100	1:100
Candida tropicalis	1:500	1:500
Tricphyton mentagrophytes	1:500	1:500
Trichophyton rubrum	1:500	1:500

For work on antifungal activity, the test organisms used are of considerable importance because dermatophyte species *C. albicans, C. tropicalis* and *T. mentagrophytes* and other fungal species were isolated from many patients with superficial mycosis. As the number of organisms increases, the results become more credible. Further, these findings could be used to develop suitable dosage forms such as cream, ointment, and lotion as per the requirement of the treatment.

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