

An Evaluation of the Antimicrobial Activities of *Aloe barbadensis*, *A. chabaudii* and *A. arborescens* Leaf Extracts Used in Folklore Veterinary Medicine in Zimbabwe

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Abstract: The antimicrobial activities of *Aloe barbadensis* Miller (*Aloe vera*), *A. chabaudii* and *A. arborescens* sap extracts on selected microorganisms were determined. Methanol as well as aqueous extracts of these plants were obtained and then tested for their antimicrobial activities using the disc diffusion assay. The extracts were assayed against gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), gram negative bacteria (*Escherichia coli*, *Salmonella typhimurium*, *S. gallinarum*, *Klebsiella* sp., *Proteus* sp.) and *Candida albicans*. The study showed that the sap extracts of the three Aloes had antimicrobial activity against all the tested microorganisms. The antimicrobial activity of the methanol extracts were significantly higher than those of the aqueous (warm and cold) extracts (one tailed t-test, $p < 0.05$). There was no significant difference in the antimicrobial activities of the aqueous extracts (one tailed t-test, $p < 0.05$). *S. typhimurium* and *S. gallinarum* were the least susceptible to the extracts tested. *E. coli*, *Proteus* sp., *Klebsiella* sp. and *C. albicans* were the most sensitive.

Key words: Ethno veterinary medicine, Aloe sap, antimicrobial activity, extract, microbes, Zimbabwe

INTRODUCTION

Ethno Veterinary Medicine (EMV) also known as folklore medicine (Akinpelu and Onakoya, 2006), encompasses the knowledge, skills, methods, practices and beliefs about animal health care found among the members of a community. The increasing resistance to common antibiotics by microorganisms has prompted the on going search for new antimicrobial agents either by the design or synthesis of new agents or through the search of natural sources for as yet undiscovered antimicrobial agents (Cock, 2008). Much recent attention has focused on extracts and biologically active compounds isolated from plants used in herbal medicine (Essawi and Srour, 2000; Alemda and Agaoglu, 2009).

The Aloe is an entirely African genus, occurring naturally only in Africa and on some islands off the coast (Baker and Linley, 1983). Over 150 *Aloe* species are found in Southern Africa and over 30 species are known to occur in Zimbabwe (West, 1974). The aloe is one of the most commonly used herbs in Zimbabwean folklore medicine where it is used as a basis for medicinal

concoctions for the treatment of both human and livestock diseases (Matekaire and Bwakura, 2004). Examples of *Aloe* species used in Zimbabwe for the treatment of a number of livestock diseases include *Aloe barbadensis* Miller (*A. vera*), *A. chabaudii*, *A. greatheadii*, *A. cameronii*, *A. excelsa*, *A. arborescens* and *A. aculeata*. Aloe preparations are used for a wide range of applications in both human and animal ethno medicine. In fact according to recent studies, the Aloe plant was found to be the most commonly used herb in rural poultry management in Zimbabwe (Mwale *et al.*, 2005). In Zimbabwe, Aloes are used to treat such conditions as diarrhoea, burns and septic wounds. In all cases, fresh leaves are used as a water infusion taken orally in the first case or as a water extract that is used to wash the wounds in the latter two cases (Mwale *et al.*, 2005).

Different *Aloe* sp. are used for different applications and differences on this aspect are minor within communities and wider across communities. According to a study carried out in western Kenya, Aloes were found to be the most commonly used herb in communal poultry

management, mostly in the treatment of a diarrhoeal infection (Okitoi *et al.*, 2007). In Trinidad and Tobago, Aloe sap is used to enhance productivity in poultry and the leaves are used in oestrus induction as a poultice and to treat septic wounds in ruminants (Lans, 2001). *Aloe arborescens* is used extensively in livestock ethno medicine in South Africa. The sap is used to prevent illness in poultry (Jaarsveld, 2002). In the Western world, Aloe preparations are used to treat wounds (including ordinary and x ray burn wounds), flea bites, acne, constipation and to treat herpes lesions (Atherton, 1997).

A decoction of the leaves of *A. chabaudii* Schonland, native to the Democratic Republic of Congo (DRC), Tanzania and Southern Africa is taken orally as a purgative, an infusion of the roots is taken to overcome nausea, a root decoction is drunk to treat blood in the urine and leaf sap may be rubbed into scarifications on the ankle to reduce swelling (Van Wyk and Smith, 1996). In folklore veterinary medicine, *A. chabaudii* is used for poultry which may be dipped in an infusion of the leaves to kill external parasites and the leaf juice may be mixed with drinking water as a cure for poultry with blood in their faeces. Cattle are drenched with the leaf infusion to cure diarrhoea (Van Wyk and Smith, 1996). The aim of this study was to evaluate the antimicrobial activities of *A. barbadensis* Miller, *A. chabaudii* and *A. arborescens* leaf sap extracts against microorganisms of veterinary importance.

MATERIALS AND METHODS

Plant material: Fresh leaves of the 3 *Aloe* specimens positively identified as *Aloe barbadensis* Miller, *A. chabaudii* and *A. arborescens* at the National Herbarium and Botanic Garden, Harare were firstly washed using cold running tap water, rinsed thoroughly with cold distilled water and then allowed to dry.

Preparation of crude extracts: The extraction protocol used was modified from Cowan (1999). Three different solvents were used on all plant specimens; these were cold distilled water, warm distilled water (45°C) and methanol. For each extraction, 100 g of cleaned fresh leaves were weighed using a Mettler PJ3000 analytical balance into 1 L beakers.

The leaves were then lacerated into small pieces using a sterilized blade and then homogenized in a little solvent using an Ultra-turrax T25 basic homogenizer at 13000 rpm to produce a slurry. More solvent was added to the slurry. The total amount of solvent used in each extraction was 150 mL. The homogenate was then filtered using Whatman filter paper number 1. In all cases, the

filtrate was centrifuged once at 5000 rpm for 10 min in a Mistraal 3000i centrifuge. The extracts were sterilized by filtration. Bacteriological membrane filters were used: firstly a 0.8 µm filter was used followed by a 0.22 µm Millex-LCR bacteriological filter. The extracts were then stored at 4°C.

Test microorganisms: All microbial strains except for *Bacillus subtilis* were obtained from the Bacteriology Department at the Central Veterinary Laboratories. *B. subtilis* was obtained from the Department of Biological Sciences, University of Zimbabwe. Stock cultures of *Escherichia coli* ATCC 25922, *Salmonella typhimurium*, *Salmonella gallinarum*, *Klebsiella* sp., *Proteus* sp., *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* were subcultured and maintained in nutrient broth at 4°C. *Candida albicans* was maintained in Sabourauds media at 4°C.

Evaluation of antimicrobial activity: Antimicrobial activity of *A. barbadensis*, *A. chabaudii* and *A. arborescens* sap extracts was determined using the disc diffusion method. A modified Kirby-Bauer (Bauer *et al.*, 1966) disc diffusion method was used. Bacterial strains were cultured in nutrient broth at 37°C for 24 h and reproduced as to contain 10^8 - 10^9 cells mL⁻¹. A total of 100 µL of microbial suspension was then spread onto Mueller Hinton agar (Oxoid). *Candida albicans* was sub-cultured in sabouraud dextrose broth at 30°C for 48 h and reproduced to contain 10^5 - 10^6 cells mL⁻¹. About 100 µL of the prepared culture was then spread onto Sabouraud's dextrose agar. Sterile paper discs, 6 mm in diameter were produced from Whatman filter paper number 1. The sterile paper discs were partitioned into three separate sets. Each set was impregnated with (30 µL⁻¹) either the methanol extract, warm water extract or cold water extract for each of the three *Aloe* sp.

The impregnated discs were allowed to dry then placed onto the inoculated agar plates by pressing them firmly on the agar surface. Plates inoculated with the bacterial strains were incubated at 37°C for 24 h then the diameters of the zones of inhibition were measured in millimeters. *Candida albicans* inoculated plates were incubated at 30°C for 48 h then the zones of inhibition were measured. Each antimicrobial assay was performed in at least duplicate. Standard discs of Furazolidone (50 µg mL⁻¹), Ampicillin (30 µg mL⁻¹) and Sulphamethaxole (25 µg mL⁻¹) (Oxoid) were used as positive controls for antimicrobial activity. Filter discs impregnated with 30 µL of distilled water were used as a negative control for the *Aloe* sp., water extracts. Methanol impregnated discs (30 µL disc⁻¹) were used as a negative control for the *Aloe* sp. methanol extracts.

RESULTS AND DISCUSSION

The antimicrobial activity of *Aloe barbadensis*, *A. chabaudii* and *A. arborescens* leaf (methanol and water) extracts was investigated using the agar disc diffusion method against a panel of bacteria and yeast. For *A. barbadensis* (Fig. 1) methanol extracts, *C. albicans* and *E. coli* gave the largest inhibition zones thus were the most susceptible. Cold water was the second best solvent after methanol, judging by the sizes of the inhibition zones.

The Salmonellae were the least susceptible. For *A. chabaudii* extracts (Fig. 2) the most susceptible organism was *Klebsiella* sp. and the Salmonellae were the least susceptible. The water extracts had lower activity when compared to the methanol extracts against all test organisms. Cold water extracts had lower activity when compared to warm water extracts for all test organisms except for *Klebsiella* sp. and *Proteus* sp. For *A. arborescens* extracts (Fig. 3), the largest zones were observed for *Klebsiella* sp. followed by *E. coli* and *Proteus* sp.

The Salmonellae were the least susceptible and the best activities were recorded by the methanol extracts. Warm and cold water extracts had almost similar effects. Antimicrobial discs used as positive controls consisted of Furazolidone (50 µg mL⁻¹), Ampicillin (30 µg mL⁻¹) and Sulphamethaxole (25 µg mL⁻¹) (Table 1). Antimicrobial activity tended to vary with the *Aloe* species, solvent used in the extraction and the test microbial species. With the disc diffusion assay, methanol was the best extraction solvent as judged by the sizes of the zones of inhibition given by the methanol extracts as compared to the other two solvents. Methanol extracts had the largest zones in all but one case, clearly showing that of the three solvents used methanol is the most suitable for this kind of researcher (Fig. 1-3).

This probably shows that the major antimicrobial components of the Aloes tested and probably of most other *Aloe* species are mainly non polar. In fact, studies have shown that crude alcohol plant extracts have higher antimicrobial activities as compared to aqueous extracts (Cowan, 1999). These preliminary findings are consistent with the results of other studies carried out using either ethanol or methanol and aqueous *A. barbadensis* extracts. In all cases, the alcohol extracts showed the best activity as compared to aqueous extracts (Agarry *et al.*, 2005; Pandey and Mishra, 2009). Though, they are less effective than alcohol extracts, aqueous extracts also showed some positive activity in most cases at times matching or even surpassing the activities of the alcohol extracts or even the standard commercial antibiotics. With the *A. barbadensis* methanol extract (Fig. 1), the most

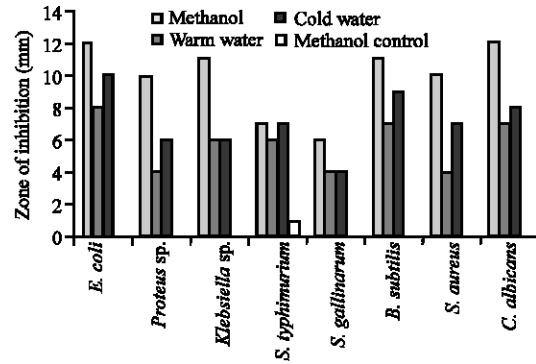


Fig. 1: Antimicrobial activities of *Aloe barbadensis* Miller leaf extracts on all test organisms expressed as zones of inhibition (excluding the diameter of the antimicrobial discs)

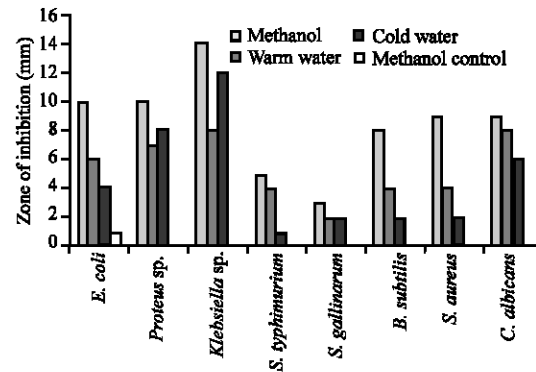


Fig. 2: Antimicrobial activities of *Aloe chabaudii* leaf extracts on all test organisms expressed as zones of inhibition (excluding the diameter of the antimicrobial discs)

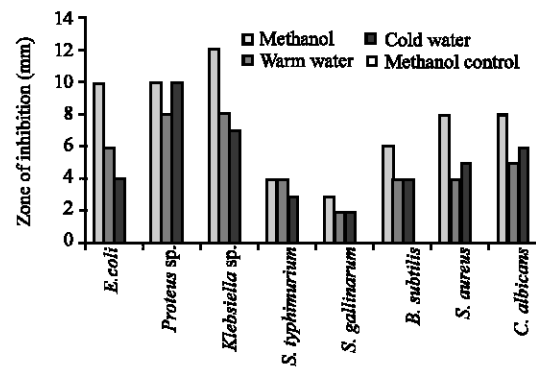


Fig. 3: Antimicrobial activities of *Aloe arborescens* leaf extracts on all test organisms expressed as zones of inhibition (excluding the diameter of the antimicrobial discs)

susceptible organism was *E. coli* followed by *C. albicans*, *B. subtilis*, *S. aureus*, *Proteus* sp. and

Table 1: Sensitivity of test microorganisms to standard antibiotics expressed as diameters of zones of inhibition (excluding the antimicrobial disc diameter)

Microorganisms	Furazolidone (50 µg mL ⁻¹)	Zone of inhibition (mm)	
		Ampicillin (30 µg mL ⁻¹)	Sulphamethaxole (25 µg mL ⁻¹)
<i>E. coli</i>	20	24	-
<i>Proteus</i> sp.	7	-	20
<i>Klebsiella</i> sp.	12	24	-
<i>S. typhimurium</i>	18	20	-
<i>S. gallinarum</i>	16	20	-
<i>B. subtilis</i>	19	-	-
<i>S. aureus</i>	-	-	19
<i>C. albicans</i>	-	24	-

Klebsiella sp. which had zones in the same range. For *A. barbadensis*, cold water was the second best solvent after methanol, judging by the sizes of the zones of inhibition (Fig. 1). The Salmonellae were the least susceptible to the *A. barbadensis* extracts. The zones obtained are consistent with the results of earlier studies. For example in this study, the zones for the methanol *A. barbadensis* extract against *S. aureus* and *C. albicans* were 10 and 12 mm, respectively while zones against the same microbes using the same solvent were found to be 12 mm (*S. aureus*) and 13 mm for the yeast (Agarry *et al.*, 2005). In another study, inhibition zones of 24 and 10 mm were reported for *Klebsiella* sp. and *C. albicans*, respectively (Alemda and Agaoglu, 2009), this concurs with findings in this study (Fig. 1). The other *Aloe* species also showed some antimicrobial activities. In all cases, low susceptibility was recorded with the Salmonellae and comparably higher antimicrobial activities of all the extracts were observed against *E. coli*, *Klebsiella* sp. and *Proteus* sp. (Fig. 2 and 3).

The extracts were also active against the gram positive bacteria *B. subtilis* and *S. aureus* as well as the yeast *C. albicans*. Some of the antimicrobial activities recorded are comparable to the antimicrobial activities given by standard commercial antibiotics (Table 1). For example, *E. coli* posted an inhibition zone of 14 mm with ampicillin (30 µg) against *Aloe* extract assay postings of 12 mm (*A. barbadensis* methanol extract) and 10 mm (*A. chabaudii* and *A. arborescens* methanol extracts). In some cases, *Aloe* extracts showed more antimicrobial activity than standard commercial antibiotics, e.g., Furazolidone (50 µg) had an inhibition zone of 10 mm on *Klebsiella* sp. and 7 mm on *Proteus* sp. In this assay, *Klebsiella* sp. had inhibition zones of 11 mm (*A. barbadensis* methanol extract), 12 and 14 mm (*A. chabaudii* methanol and cold water extracts, respectively) and 12 mm for the *A. arborescens* methanol extract. However despite all this, commercial antibiotics had on average, higher antimicrobial activities than the crude *Aloe* extracts. All these results point to the fact that crude *A. barbadensis*, *A. chabaudii* and *A. arborescens* aqueous and methanol extracts have broad spectrum antimicrobial activity against both the gram positive and gram negative bacteria tested as well as the Yeast

Candida albicans. Activity was consistently high against *E. coli*, *Klebsiella* sp., *Proteus* sp. and *C. albicans*. This is consistent with other studies which found that gram negative bacteria were more susceptible to extracts of *Aloe barbadensis* Miller (Cock, 2008). In another study, *C. albicans* showed marked susceptibility to crude extracts of *A. barbadensis* Miller, although the antibacterial activity of the *Aloe* juice was found mainly against gram positive bacteria (Alemdar and Agaoglu, 2009).

Such activity against some Enterobacteriae e.g., *E. coli* is probably behind the successful use of *Aloe* sap or extract in the treatment of livestock gastrointestinal disorders. It was demonstrated in one study that *Aloe* preparations added to the drinking water of chickens were useful in treating a diarrhoeal condition with bloody droppings, thought to be colibacillosis (Okitoi *et al.*, 2007). Colibacillosis is an important disease of chickens caused by *E. coli* (Barnes *et al.*, 2003).

On the other hand, the gram positive bacteria showed relatively moderate and sometimes high susceptibility to extracts from all the three *Aloe* sp. tested. This probably explains the use of *Aloe* sap to treat septic wounds and abscesses in livestock as well as some skin disorders in humans because *S. aureus* is commonly found on the skin in abscesses and on skin lesions (Cheesebrough, 1998). Also, *Aloe* sap's success in the treatment of septic wounds probably lies in the fact that it is also said to promote wound healing due to the presence of some components like anthraquinones and hormones like gibberellins and auxins (Davis, 1997). Thus, the *in vitro* activity of the *Aloe* extracts against specific microorganisms or groups of microorganisms may be used as justification for the use of these preparations in the treatment of specific livestock diseases.

The antimicrobial activity of *Aloe* sap is a function of a number of many compounds found in the sap. Some of these components are known to have antimicrobial activity against both gram negative and positive bacteria as well as some fungi (Atherton, 1997). This explains why crude *Aloe* extracts are active against a broad range of microorganisms because crude extracts contain all the constituents of *Aloe* sap. However, studies have been carried out involving fractionating *Aloe* sap using

Reverse Phase High Performance Liquid Chromatography (RP-HPLC) and then testing the *in vitro* antimicrobial activities of the various fractions against a range of microorganisms (Cock, 2008). From these tests, the crude extract was active against some gram positive bacteria including *S. aureus* and *B. subtilis* and a number of enterics. This extract also had activity against *Aspergillus niger*. Total 9 RP-HPLC separated fractions were tested and they showed differing activities depending on purity. The first fraction (fraction 1) had broad spectrum activity, however as the extract became more refined the fractions became more selective in their target microorganisms such that fraction 5 and 9 inhibited only *Clostridium freundii* and *E. coli*, respectively (Cock, 2008). This observation shows that Aloe sap contains a number of compounds each with antimicrobial activity against specific microorganisms. These compounds include the anthraquinones, Aloe emodin, aloin, isobarbaloin and a number of some yet to be identified (or named) compounds.

In the face of ever increasing microbial antibiotic resistance, it is becoming more imperative for studies which seek to identify natural antimicrobial compounds and the future development of these compounds not only for use in mainstream medicine but in veterinary medicine to be pursued as they provide a promising avenue for novel antimicrobials. Studying the plants used in folklore medicine promises to yield commendable results as investigating their antibacterial activity has led to a better understanding of the use of traditional medicines as potential drugs in addition to contemporary drugs (Cooposamy and Magwa, 2007).

CONCLUSION

All the three Aloes tested showed antimicrobial activity against all the tested microbes. However, there were no significant differences in the antimicrobial activities of the extracts from the different *Aloe* sp. (one tailed t-test, $p < 0.05$). The results obtained in this study justify the use of Aloes in ethno veterinary medicine in rural communities of Zimbabwe.

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