Research Paper

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Bacteriocidal efficacy of *Aloe vera* (Burm L.) against mastitis pathogens *Streptococcus agalactiae, Staphylococcus aureus and Escherichia coli in-vitro*

Science

KEYWORDS : Aloe vera, mastitis, Staphylococcus aureas, Streptoccus agalactiae, Escherichia coli, anti-bacterial, in-vitro

Chikwanda. D.	Faculty of Science and Agriculture, University of Fort Hare, P.Bag X1314, Alice, 5700, South Africa
Nyamushamba. G.B	Faculty of Agriculture, Women's University in Africa, P.O.Box, MP 1222, Mt Pleasant, Harare, Zimbabwe
Matondi. G.H.M.	Faculty of Agriculture, Women's University in Africa, P.O.Box, MP 1222, Mt Pleasant, Harare, Zimbabwe
Marandure.T.	Faculty of Agriculture, Women's University in Africa, P.O.Box, MP 1222, Mt Pleasant, Harare, Zimbabwe
Chikwanda. A.T.	Bindura University of Science Education, Faculty of Agriculture and Natural Resources Management, P.O. Box 1020, Bindura
Imbayarwo-Chikosi V.E.	Departmental of Animal Science, Faculty of Agriculture, University of Zimbabwe, P.O.Box MP 167, Mount Pleasant, Harare, Zimbabwe
Masunda B.	Departmental of Animal Science, Faculty of Agriculture, University of Zimbabwe, P.O.Box MP 167, Mount Pleasant, Harare, Zimbabwe
Masama, E.	Department of Agricultural Management, Faculty of Science and Technology, Zimbabwe Open University, Harare Main Post Office, 3rd Floor, Cnr Julius Nyerere Way/Nelson Mandela, Harare, Zimbabwe.
Chiwara, A.	Faculty of Agriculture, Women's University in Africa, P.O.Box, MP 1222, Mt Pleasant, Harare, Zimbabwe

ABSTRACT

The efficacy of Aloe-vera on mastitis pathogens Staphylococcus aureas, Streptococcus agalactiae and Escherichia coli was determined in-vitro. 1 µml of each of the bacterial organelles were randomly placed in 24 different petri-dishes using a sterile pipette. The petri dishes were randomly allocated to a positive (Gentamycin) and negative (Methanol) control and seven treatments (25%, 50% and 75% w/v concentrations of distilled water-Aloe vera and methanol-Aloe vera leaf extracts) with each treatment replicated three times in a Completely Randomized Design (CRD). Muller Hinton was used as growth media. Reaction time of 30 minutes was allowed for all samples and incubation was done for 72 hours at 37°C. After incubation, bacterial colonies per plate were counted and total bacterial counts calculated. Data was subjected to non-parametric Friedman's 2-way analysis of variance in SAS Version 9.1.3 (SAS, 2004). Total bacterial counts (TBC) were converted to ranks through the PROC FREQ and PROC RANK procedures. Treatment effects were then determined with the general linear model procedure (PROC GLM) of SAS (2004). Means were compared using the lsmeans procedure. There was no significant difference ($P > 0.05$) in TBC ranks among bacteria exposed to Gentamycin and methanol, however, these two had the lowest TBC compared to methanol-Aloe vera and in distilled water-Aloe vera leaf extracts. TBC ranks were significantly lower ($P < 0.05$) methanol-Aloe vera leaf extracts than in distilled water-Aloe vera leaf extracts. Aloe vera exhibits some antibacterial properties but is not as efficient as commercial products.

INTRODUCTION

The major threat to sustained milk production is mastitis. This refers to the inflammation of the mammary gland due to injury and/or infection (Karima *et al*., 2006). According to Oviedo-Boyso *et al*. (2007) the inflammation of the mammary gland is a consequence of the activity of a number of cell and soluble factors that function together to eliminate invading micro-organisms. Mastitis causes substantive economic losses in dairy production as mastitic milk is discarded and not allowed to enter the food chain (Karima *et al*., 2006). Furthermore, expenses are escalated by treatments and preventive/control procedures. Mastitis is also associated (Gueye, 1999) with destruction of milk producing cells within the udder leading to physical, chemical, microbiological and subsequently pathological changes in milk. Depending on stage of disease progression, mastitis can be sub-clinical, clinical, pre-acute or acute mastitis. The most important form of mastitis is sub-clinical as it has no obvious symptoms but can account for more than 40% reduction in milk production. The other forms are relatively easy to manage since they show obvious symptoms and can be controlled promptly.

Methods for mastitis control include the use of long acting synthetic antibiotics such as vancomycin, gentamycin, neomycin, cloxacillin, bacitracin and penicillin among others. One of the major limitations to the use of long acting antibiotics is that antibiotic residues persist into lactation. Treatment and control of mastitis using conventional commercial drugs is not common among resource poor smallholder dairy producers largely because they are expensive and not readily available (Raham *et al*., 2009). Animal rights and welfare activists have, over the years been advocating against synthetic and campaigned for the promotion of ethno-veterinary methods in animal health management systems (Raham *et al*., 2009). This involves the use of medicinal plants and/or plant extracts in the treatment of livestock. Several plants have been shown to possess medicinal properties such as bactericidal activity (Stevenson *et al*, 2012: Kamanula *et al*., 2011)

This research focused on the use of A*loe vera* in the control mastitis. It is a perennial drought resistant succulent plant belonging to the lily family (*liliaceae*) which has been used for a variety of medicinal purposes. It is a bitter herb with anti-inflammatory, astringent, emollient, anti-fungal, anti-bacterial anti-viral properties (Bashir *et al*., 2007). It was proven to be useful in the eradication of a wide range of parasites (Matekaire and Bwakura, 2004). It contains a host of compounds that are biologically active and includes anthraquinones, sacharides and prostaglandins as well as other constituents (Misra and Khumar, 2004).

Aloe vera has been widely used by resource poor farmers to treat and/or manage a wide range of livestock diseases including coccidiosis in poultry (Misra and Khumar, 2004). Despite the widespread use of *Aloe vera* (Mwale *et al*., 2006) a few studies have been carried out in Zimbabwe to verify its effectiveness against mastitic pathogens. This study was therefore, designed to evaluate the efficacy of *Aloe vera* in controlling *Staphylococcus aureas, Streptoccus agalactiae* and *Escherichia coli in vitro* against a positive control of an Amitraz based commercial antibiotic (Gentamycin).

Materials and Methods

Plant collection and identification

Plant specimen were sourced from and identified as Aloe vera at the National Herbarium botanic gardens in Harare, Zimbabwe. The bacteriocidal efficacy study was carried out at the Central Veterinary Laboratories which is located 3 km north east of Harare central business district.

Plant preparation

About 1 kg of fresh *Aloe vera* leaves were crushed using a pestle and mortar and mixed with one litre of warm (40°C ± 2°C) distilled water. Another 1 kg of the same leaves was also crushed and mixed with one litre of methanol. The suspensions were left for about 8 h for extraction of active compounds, with occasional shaking. The contents were then separately filtered first through a clean mutton cloth and then through Whatman filter paper No. 1. The filtrates were concentrated in a rotatory evaporator connected to a vacuum pump. An analytical balance was used to obtain concentrations levels of 25%, 50% and 75% w/v (Table 1). Filtrates were stored at -20°C until used.

Experimental procedure and design

The pour plate method was used. A total of 1 µml of bacterial organelles (*Staphylococcus aureus, Escherichia coli* and *Streptococcus agalactiae*) were randomly placed in 24 petri-dishes using a sterile pipette. The petri-dishes were randomly allocated to six treatments (methanol-*Aloe vera* leaf extracts and distilled water-*Aloe vera* leaf extracts of 25%, 50% and 75%) a positive control group (Gentamycin) and a negative control group (methanol) (Table 1). Each treatment was replicated three times in a completely randomized design (CRD). Muller Hinton was used as growth media. Reaction time of 30 minutes was allowed for all samples before incubation for 72 hours at 37°C ± 1°C.

Plate count on bacterial colonies was done and Total Bacterial Count (TBC) was calculated as shown below:

TBC = Number of colonies observed x Inverse value of dilution factor

Table 1: Experimental treatments

Treatment	Composition	Concentration (%)	Replicates
1	Methanol-Aloe	25	3
2	Methanol-Aloe	50	3
3	Methanol-Aloe	75	3
4	Aloe-distilled water	25	3
5	Aloe-distilled water	50	3
6	Aloe distilled	75	3
7	Gentamycin	100	3
8	Methanol	100	3

Data analysis

Data was analysed using the non-parametric Friedman's two-way analysis of variance in SAS Version 9.1.3 of SAS (2004). Total bacterial counts were converted to ranks through the PROC FREQ and PROC RANK procedures. Treatment effects were then determined with the general linear model procedure (PROC GLM) of SAS (2004). Comparison of means was done using the Predicted Difference statistic of SAS (2004).

RESULTS AND DISCUSSION

Total bacterial counts (TBC) were replaced by ranks for the purpose of analysis. The values with the lowest ranks had the lowest TBC implying highest treatment effects.

Treatment effects

There was no significant difference (P > 0.05) between overall mean TBC ranks across all bacterial types incubated with Gentamycin and Methanol (Table 2). However, these two had the lowest TBC ranks compared to methanol-*Aloe vera* leaf extracts and distilled water-*Aloe vera* leaf extracts. Across all bacterial types, distilled water-*Aloe vera* leaf extracts had significantly higher (P < 0.05) overall mean TBC ranks than organelles incubated in Methanol-*Aloe vera* leaf extract.

Table 2: Overall LS means for TBC ranks for bacterial samples incubated with different antibacterial agents

Treatment	Lsmean TBC ranks		
	E. coli	S. aureus	Str. agalactiae
Methanol-Aloe	7.0[a]	8.0[a]	8.0[a]
Distilled water-Aloe	11.0[b]	11.0[b]	11.0[b]
Gentamycin	4.0[c]	3.0[c]	3.5[c]
Methanol	4.0[c]	3.0[c]	3.5[c]

[abc]Within columns, LS means with similar superscripts do not differ (P > 0.05)

The antibacterial properties exhibited by *Aloe vera* are probably due to the some active antibacterial chemicals present in the extracts. According to Ni *et al.* (2004) *Aloe vera* contains a wealth of substances that are biologically active, including emodin anthrone, dithranol, chrysarobin, carboxypeptidase, magnesium lactate, C-glucosyl chromone, salicylate, and allantoin. Okitoi *et al*. (2007) also confirmed bacteriocidal effects of *Aloe vera* on diarrhoeal infections. Matekaire and Bwakura (2004): Mwale *et al.* (2005) also reported the widespread use of the *Aloe* species in management and treatment of various livestock diseases in Zimbabwe.

Effect of concentration

Distilled water –*Aloe vera* extracts had the same potency at all the three concentration levels (Figure 1). Although there was no previous literature to support this trend, it could be a result of the reaction time allowed for exposure of bacterial organelles to the test treatments. Probably if more reaction time was allowed, some discrepancies could have been noticed. Mwale *et al*. (2005) reported that botanical extracts have been shown to be slow acting compared to synthetics. In some cases (Sarasan *et al*., 2011: Moreki *et al*., 2012) exhibiting their effects after a prolonged period of exposure. There was no significant difference in TBC for organelles incubated in 50% and 75% methanol-*Aloe vera* leaf extracts. However, these concentrations (Figure 1) had significantly higher (P < 0.05) TBCs than organelles in 25% methanol-*Aloe vera* leaf extracts. This could probably have been due to interactive effect between the methanol and the A*loe vera* active constituents which might have led to a reduction in potency at higher concentrations.

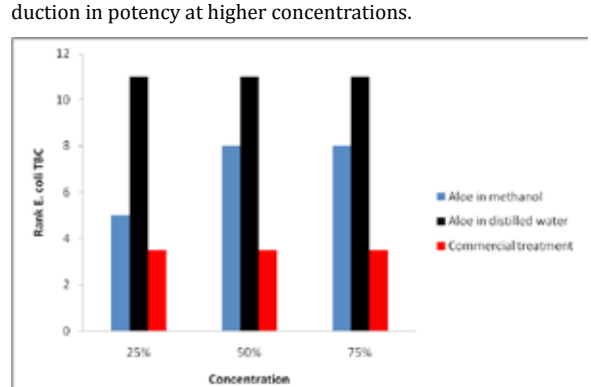

Figure 1: Rank *E. coli* TBC for the three concentration levels.

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CONCLUSIONS

Aloe vera possesses some antibacterial properties that can assist in controlling mastitis pathogens *Staphylococcus aureus, Streptococcus agalactiae* and *Escherichia coli.* However, concentrations tested in this study were not as effective as the commercial product. Methanol- *Aloe vera* leaf extracts at 25% concentration provides the most effective control. Highest levels of inhibition were shown in *E.coli* organelles.

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