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IN VITRO EFFICACY OF EXTRACTS FROM PLANTS USED BY SMALL-HOLDER FARMERS IN THE TREATMENT OF DERMATOPHILOSIS IN CATTLE

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Abstract

Background: Bovine dermatophilosis, an important skin disease of cattle caused by *Dermatophilus congolensis*, negatively impacts the livelihoods of small-holder farmers in Zimbabwe. This impact is through, morbidity, loss of draught animal power, costs incurred to manage the disease, losses associated with devalued damaged hides and the resultant culling of some of the affected cattle. Due to the inaccessibility of conventional drugs to manage bovine dermatophilosis, farmers have been reported to use local medicinal plants to manage the disease. The aim of the study was to evaluate the *in vitro* antimicrobial activities of three plants that small-holder farmers in Zimbabwe used to manage bovine dermatophilosis.
Methods: Dried plant materials were ground into powder and extracted individually using, water, 80 % acetone and 80 % methanol. The antimicrobial properties of the plants were evaluated against two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and one Gram-positive (*Staphylococcus aureus*) reference bacterial strains. They were further evaluated against a field isolate of *Dermatophilus congolensis*. The assays used were the disc diffusion, minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC).
Results: Acetone and methanol extracts had superior inhibitory activities than did those of water. *Pterocarpus angolensis* DC extracts had better inhibitory properties with absolute MIC values of 0.156 – 5 mg/ml, *Cissus Quadrangularis* L had MIC values in the range 0.156 – 5 mg/ml while that of *Catunaregam spinosa* Thunb, Terveng was 0.156 – 10 mg/ml. *Dermatophilus congolensis* was more sensitive to *Pterocarpus angolensis* DC average MIC = 0.63 mg/ml than to *Cissus quadrangularis* L average MIC = 1.25 mg/ml and *Catunaregam. spinosa* Thunb, Terveng average MIC = 2.08 mg/ml.
Conclusion: These results suggest the potential antibacterial activities of extracts of the three plants and hence farmers are, in a way, justified in using the plants. Better results (lower MIC) could be obtained by extracting and evaluating pure active compounds of the plants.

Keywords: Zimbabwe, exudative, minimum inhibitory concentration, micro-dilution, ρ- iodonitrotetrazolium, traditional, *Cissus quadrangularis* L.

Introduction

Dermatophilosis is a contagious exudative skin disease of cattle caused by a gram-positive actinomycete, *Dermatophilus congolensis* (Dalis, 2009; Dalis et al. 2010). The disease affects a wide range of mammals including man, cattle, sheep and goats (Woldemeskal and Mersha, 2010). The disease has been reported mainly from cattle in the tropics; where it causes hide damage, prolonged suffering, high morbidity and associated economic losses (Koney, 1996; Chatikobo et al., 2004; Chatikobo et al., 2009; Woldemeskal and Mersha, 2010). In Zimbabwe, dermatophilosis is of importance in small-holder farming communities and has steadily been spreading from its traditional foci to other virgin areas (Chatikobo et al., 2004; Chatikobo et al., 2009).

Conventionally, the treatment for bovine dermatophilosis has been through the use of tetracyclines, penicillin and dihydrostreptomycin, separately or in combination (Arowolo et al., 1987; van Tonder and Horner, 1996; Nath et al., 2010). Gentamycin, given parenterally was more effective than the combination of penicillin and streptomycin (Hamid and Musa, 2010). Topical applications of potassium aluminium hydroxide used with parenteral applications of long acting tetracyclines provided treatment (Nath et al., 2010). Due to the association of bovine dermatophilosis with *Amblyomma variegatum* ticks (Walker, 1996), tick control has been an important adjunct in the control of the disease (Hadrill and Walker, 1996). Non-conventional or ethno-veterinary preparations have been used to manage bovine dermatophilosis. Imam et al. (2008) indicated that the plant, *Mitracarpus hirtus* (L.) DC, had significant inhibitory effects against *Dermatophilus congolensis in vitro*. *Mitracarpus hirtus* has also been used for the treatment of eczema and other skin conditions in man (Imam et al., 2008). Use of alcoholic extracts of *Senna alata* (L) Roxb, *Lantana*

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camara L and *Mitracarpus hirtus* have resulted in the healing of dermatophilosis skin lesions in cattle (Ali-Emmanuel et al., 2003). Dermatophilosis lesions resolved in 8 weeks after using a cream preparation of the plant *Tephrosia vogelii* hook.f (Makoshi and Arowolo, 2011). Non-plant alternative remedies, such as quicklime, sulphur, soap, warm water, oil-mixture of potash and torch battery contents have also been used by farmers to manage dermatophilosis (Stewart, 1972; Arowolo et al., 1987).

In Zimbabwe, farmers reported that they used; *Cissus Quadrangularis* L, *Catunaregam spinosa* Thunb, Terveng and *Pterocarpus angolensis* DC for the management of bovine dermatophilosis (Ndhlovu and Masika 2013). *Cissus quadrangularis* is a fleshy succulent vine occurring in Asia and Africa including Zimbabwe (Mishra et al., 2009; Mullins, 2006). Mishra et al. (2009) reported *in vitro* antibacterial effectiveness against *Staphylococcus aureus* and *Staphylococcus cerivisiae*. Fungicidal, antihelmintic, wound healing and antiinflamatory properties of *C. quadrangularis* have also been reported (Mohanty et al., 2010; Vijay and Vijayvergia, 2010). *Pterocarpus angolensis* is a small to medium sized deciduous tree (Ali et al., 2008), Namibian traditional healers, have used it to manage bleeding, coughs and leg pains (Cheikhyousef et al., 2011) while antibacterial and antiprotozoal activities have also been reported (Samie et al., 2009). *Catunaregam spinosa* has been used for the management of skeletal disorders and was found to be effective against gram positive bacteria (Rajakaruna et al., 2002; Rout et al., 2009). To our knowledge no studies have been conducted to determine the antibacterial activities of the three plants against *D. congolensis*. This study was carried out as a follow up to a field survey (Ndhlovu and Masika 2013) and to investigate antibacterial activities of these plants against *D. congolensis* and selected reference bacterial strains.

Methods

Plant collection

Fresh stems of *C. quadrangularis* were collected from the bush in Chegutu while fresh bark and leaves of *P. angolensis* and *C. spinosa* were collected from Zhombe communal lands. Voucher specimens were authenticated at the National Herbarium and Botanical Gardens, Harare, Zimbabwe and stored at the Faculty of Veterinary Science Toxicology laboratory. The voucher specimens were allocated the following voucher specimen numbers: DNN01-2012, DNN02-2012 and DNN03-2012 representing *Cissus Quadrangularis*, *C. spinosa* and *P. angolensis* respectively.

Plant storage and preparation.

The plant specimens (stems and barks) were air dried at room temperature at the Faculty of Veterinary Science Toxicology laboratory and later ground to fine powder using an electric grinding mill (Fritsch Pulverisette, Germany). The resultant powders were stored in air-tight bottles and kept in a dark cool place until further processing.

Extraction procedure

Powdered plant samples were crudely extracted individually using sterile distilled water, 80 % acetone and 80% methanol. The extractions with acetone and methanol were performed using methods described by Shale et al. (2005; Mathabe et al. (2008); Konate et al. (2011). Twenty grams of the respective powders were mixed with 200 ml of solvent. The extracts were left overnight on a Variomag magnetic stirrer. The extracts were then filtered through Whatman no.1 filter paper and the filtrate centrifuged in a Centaur 2 centrifuge at 3000 rpm for 10 min. The resultant supernatant was concentrated using a rotary evaporator at 40 °C and later the samples were freeze-dried using a HETO FD3 freeze dryer and kept at 4 °C until use. The water extract was prepared as follows; 20 grams of powder were soaked in 200 ml sterile distilled water for 24 hr. The extract was then filtered using Whatman no. 1 filter paper and later, the filtrate was freeze-dried and stored at 4 °C until use. All the extracts were weighed after freeze drying using a Sartorius electronic balance.

Bacterial strains

Three reference bacterial strains, provided by the Zimbabwe National Quality Assurance Programme (ZINQAP) were used: Gram negative *Pseudomonas aeruginosa* (ATCC 25619) and *Escherichia coli* (ATCC 25922), together with Gram-positive *Staphylococcus aureus* (ATCC 25923). These bacteria were chosen due to their availability and because they cause disease in livestock. *Pseudomonas aeruginosa* is an opportunistic human pathogen which also causes chronic mastitis in cattle, fleece rot in sheep and suppurative otitis externa in dogs (Kingsford and Raadma, 1997; Tron et al., 2004). *Escherichia coli* is an economically important inhabitant of the gastrointestinal tract of ruminants (Henton and Hunter, 1996) causing diarrhoea in calves and is associated with abscessation. Hides are the most important source of carcass contamination (Arthur et al., 2007; Arthur et al., 2010). Certain strains of *E. coli*, such as the O157:H7 strain, are of zoonotic importance (Fairbrother and Nadeau, 2006). *Staphylococcus aureus* is a pyogenic pathogen of economic importance in livestock, associated with mastitis in cattle and small ruminants (Lowly, 2000; Fitzgerald, 2012); infected quarters and the skin of the udder and teats are the main reservoirs of infection

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(Larsen et al., 2000). *Staphylococcus aureus* has been reported to be a leading cause of mastitis in cattle (Klein et al., 2012).
Field isolates of *D. congolensis* were prepared from fresh skin scabs collected from naturally infected cattle from Chegutu. The *D. congolensis* bacteria were identified by direct microscopy, culturing on blood agar and biochemical tests as described elsewhere (Awad et al., 2008; Mannan et al., 2009).

All the bacteria were maintained on blood agar plates until use, at which time, three colonies of each were transferred into universal bottles containing Brain Heart infusion broth and grown overnight at 37 °C. Before use of broth cultures, their turbidity was adjusted with sterile saline to match 0.5 MacFaland solution; at this turbidity, bacterial count was estimated at 1 - 2 x 10^8 CFU (Clinical and Laboratory Standards Institute (CLSI), 2006).

Antibacterial assays

The antibacterial activities of the extracts were determined using three methods; agar disc diffusion, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays. Before use, the methanol and acetone extract residues were re-dissolved in 80 % acetone while the water extract residue was re-dissolved in sterile distilled water, each to a final concentration of 10 mg/ml.

Agar disc diffusion assay

The agar disc diffusion method as described elsewhere (Tadeg et al., 2005; Mathabe et al., 2006; Ghalem and Mohamed, 2009 Rout et al., 2009) was used. The inocula of the different bacterial strains were evenly streaked on Mueller-Hinton (M-H) agar using a sterile swab. Sterile Whatman filter paper discs (6 mm diameter); three for each plant extract, were impregnated with reconstituted extracts (10 mg/ml) by soaking them for 15 min. Gentamycin (10 µg) was used as a positive control and discs soaked in distilled water and 80 % acetone, were used as negative controls. Discs were applied on the surface of the dry M-H agar plates and incubated for 18 h at 37 °C. Plates streaked with *D. congolensis* were placed in a candle jar and incubated as for the other bacteria. The plates were observed for zones of inhibition which were measured in millimetres. Absence of a zone of inhibition was interpreted as absence of antibacterial activity. Each extract was tested in triplicate.

Minimum Inhibitory Concentration and Minimum bactericidal concentration

The 96-well micro-plate method for minimum inhibitory concentration (MIC), as described by (Eloff et al., 1998; Masoko et al., 2005; Mathabe et al., 2006; CLSI, 2006) and the minimum bactericidal concentration (MBC) as described by (Moulari et al., 2006) were used. Each reconstituted plant extract; acetone, methanol and water, were two-fold serially diluted starting at a concentration of 10 mg/ml to a final concentration of 0.078 mg/ml. A similar two-fold dilution of gentamycin starting at a concentration of 10 mg/ml was used as a positive control. The negative controls were acetone, methanol and distilled water. An equal volume of 100 µl fresh bacterial cultures were added to the wells. The plates were covered and incubated at 37 °C for 24 h after which, 40 µl of 0.2 mg/ml of ρ-iodonitrotetrazolium violet were added to each well as an indicator and incubated for 30 min. Presence of bacterial growth was indicated by a purple colour whereas non-development of colour indicated inhibition of growth. Each test was replicated three times. Absolute lowest MIC and average MIC values were computed.
For the determination of MBC, a portion of liquid (collected using a sterile loop) from each well that showed no colour change; was plated on blood agar and incubated at 37 °C for 24 h. The lowest concentration that yielded no growth after this sub-culturing was taken as the MBC (Kuete et al., 2008).

Statistical analysis

For the agar disc diffusion assays, the effect of extract and bacteria on zone of inhibition was measured by the General Linear Model (GLM) in the Statistical Package for Social Sciences (SPSS) (SPSS, 2007). Extractant had 3 coded categories; (1-water; 2-acetone, 3-methanol), bacteria had four categories (1- *D. congolensis*, 2-*E. coli*, 3-*P. aeruginosa*, 4-S. *aureus*) and plant had three categories (1- *C. quadrangularis*, 2- *C. spinosa*, 3- *P. angolensis*). Least Significant Differences (LSD) was used as the post-hoc test to measure variances in categories. Values of $P < 0.05$ were considered as significant.

Results

Extraction yields

The quantities of plant materials extracted by the different solvents are as indicated in Table 1. The largest quantity was extracted using methanol from *C. spinosa* (2417 mg) with water extracting the minimum quantity (333 mg) from the same plant. Methanol consistently extracted more material from all the plants while water extracted the least.

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Table 1: Quantities in grams of materials extracted from plants

Plant	Methanol (% of original)[a]	Acetone (% of original)	Water (% of original)
Cissus quadrangularis	1.739 (8.7%)	1.306 (7.5)	0.730 (3)
Catunaregam spinosa	2.417 (12)	2.055 (10.3)	0.333 (1.7)
Pterocarpus angolensis	2.048 (10.4)	1.733 (8.7)	1.084 (5.4)

[a]Calculated using original plant material of 20 gram

Agar disc diffusion assay

Table 2 suggested that extracts from the three plants had some antibacterial activities against mainly Gram-positive bacteria, with diameters of zones of inhibition ranging between 8 and 15 mm. *Dermatophilus congolensis* and *S. aureus* exhibited apparent resistance to the acetone extracts of *C. quadrangularis* and water extracts of *C. spinosa* respectively. Apparent resistance to, mainly water extracts of *C. quadrangularis* L and *C. spinosa*, were seen in the two Gram-negative bacteria, *E. coli* and *P. aeruginosa*. In fact, *E. coli* exhibited resistance to all of the water extracts. Extracts from *P. angolensis* displayed activity against all the bacteria with the exception of *E. coli*. There were significant differences ($P < 0.05$) between the antibacterial activities of the water and methanol extracts of *C. quadrangularis* against *D. congolensis*. Water and acetone extracts of *P. angolensis* exhibited significantly better ($P < 0.05$), activity than methanol extracts against *D. congolensis*. All extracts of *C. spinosa* did not show significant differences ($P > 0.05$), in their activity against *D. congolensis*. There were no significant differences ($P > 0.05$) in activity between acetone and methanol extracts of *C. quadrangularis* against *E. coli* and *P. aeruginosa*. Water and organic extracts of *P. angolensis* did not differ significantly ($P > 0.05$) in their activity against *P aeruginosa*; whilst the activity differed significantly against *S. aureus*. All bacteria in the study were sensitive to gentamycin with *D. congolensis* and *E coli* being more sensitive than *P. aeruginosa* and *S. aureus*.

Table 2: Antibacterial activities of water, acetone and methanol extracts from plants used by small-holder farmers.

Sample	Bacteria[a] tested zone of inhibition (mm)			
	De	*Ec*	*Ps*	*Sa*
Cissus quadrangularis				
Water extract	10.5[a]	R	R	9.5[a]
Acetone	R	8.5[a]	11.5[a]	9.5[a]
Methanol	13.5[b]	11[a]	12[a]	8.5[b]
Catunaregam spinosa				
Water extract	10.5[a]	R	R	R
Acetone	11.5[a]	9.5[a]	R	8.5[a]
Methanol	12.5[a]	7[b]	7[a]	10.5[b]
Pterocarpus angolensis				
Water extract	12.5[a]	R	8[a]	9[a]
Acetone	13[a]	10	9[a]	15[b]
Methanol	11.5[b]	R	7[a]	11[c]
Gentamycin	25.5[g]	24.5[g]	15[g]	14.5[g]

[a] Bacteria: *De, Dermatophilus congolensis; Ec, Escherichia coli; Ps, Pseudomonas aeruginosa*; *Sa, Staphylococcus aureus*; R, resistant

Minimum inhibitory concentration and minimimum bactericidal concentration

Absolute and average MIC values of all the extracts are as shown in Table 3. Organic solvent extracts of *P. angolensis* exhibited the lowest absolute MIC of 0.625 mg/ml, against *D. congolensis* whilst the water extract of *C. quadrangularis* had the highest MIC of 5 mg/ml against the same organism. Acetone and methanol extracts of *C. quadrangularis* and *C. spinosa* had the lowest absolute MIC of 0.156 mg/ml against *S. aureus*. The highest MIC of 10 mg/ml was exhibited by the water extract of *C. spinosa* against *P. aeruginosa*. The lowest average MIC over the triplicates was 0.21 mg/ml exhibited by the acetone extract of *C. quadrangularis* against *S. aureus*, the water extract of *C. spinosa*, had the highest MIC of 10 mg/ml against *P. aeruginosa* (Table 3). Overall, the MIC values, both absolute and average, of all plants indicated that *D. congolensis* and *S. aureus* had higher sensitivity to all the extracts than the gram negative *E. coli* and *P. aeruginosa*. All organisms were sensitive to gentamycin which exhibited the lowest absolute and average MIC of 0.078 mg/ml. There was bacterial growth in the acetone and distilled water negative control while there was none in the methanol control wells.

The MBC assay exhibited a negative response. The response was characterised by regrowth of bacteria after sub-culturing of all the samples, as a result, no MBC values were computed in this study.

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Table 3: Average (and absolute)* Minimum inhibitory Concentrations (mg/ml) of three plant species after 24 hour incubation at 37°C

	Cissus quadrangularis			*Catunaregam spinosa*			*Pterocarpus angolensis*			Gentamycin
	Water	Acetone	Methanol	Water	Acetone	Methanol	Water	Acetone	Methanol	
Dermatophilus congolensis	5.0 (5)*	1.25 (1.25)	2.5 (1.25)	3.33 (2.5)	2.5 (2.5)	2.08 (1.25)	2.08 (1.25)	0.63 (0.625)	1.04 (0.625)	0.078 (0.078)
Escherichia coli	5.0 (5)	2.5 (2.5)	4.17 (2.5)	5 (5)	2.08 (1.25)	3.33 (2.5)	4.17 (2.5)	0.83 (0.625)	0.63 (0.625)	0.078 (0.078)
Pseudomonas aeruginosa	5.0 (5)	1.25 (1.25)	3.33 (2.5)	10 (10)	0.31 (0.313)	0.63 (0.625)	5.0 (5)	1.04 (0.625)	1.67 (1.25)	0.078 (0.078)
Staphylococcus aureus	2.08. (1.25)	0.21 (0.156)	0.42 (0.313)	0.63 (0.625)	0.42 (0.313)	0.26 (0.156)	2.5 (2.5)	0.37 (0.156)	0.52 (0.313)	0.078 (0.078)

*Absolute MICs are in brackets

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Discussion

Of the three extractants used, methanol was the best compared to acetone and water. Results obtained on the extraction yields of the solvents used in this study seem to agree with those of other researchers (Masoko et al., 2005) who reported that methanol extracted more material from *Terminalia* spp. than did acetone, hexane or dichloromethane. Comparable results have been reported, where methanol was shown to extract the most material from seven plants, with the highest yield being from *Loxostylis alata* A. Spreng (Suleiman et al., 2010). The quantities extracted by acetone were greater than those extracted by water from all the plants, this contrasted findings by Abdillahi et al. (2008), who reported that water was a better extractant than acetone. This difference could be as a result of the different extraction procedures used in the two studies. In this study there was no use of sonication and filteration under a vacuum during extraction as was the case with the other study. The other reason for the different results could be that other workers (Abdillahi et al., 2008) studied different plant species which belonged to the *Podocarpus* genus. As water yielded the least amount of material from all the plants in this study, this could have negative implications for farmers, since they frequently use water as an important ingredient when preparing remedies from medicinal plants (Mathabe et al., 2006; Suleiman et al., 2010). Masoko et al. (2005) reported that water was unable to extract non-polar components of plants, as such, for this study, it was possible that the three plants investigated had fewer polar compounds in their structures, hence the reduced yield associated with water extraction. On the other hand, despite methanol having been a better extractant in this study, it is not readily accessible to the small-holder farmers. In a previous study Rios and Recio (2005), it was stressed that it was important, during *in vitro* trials, to utilise extractants which the farmers themselves use when they prepared medicinal plants. The agar disc diffusion assay results generally exhibited a similar trend to those of the MIC although there were certain areas where the results were not in agreement. Extracts that did not exhibit activity in the agar disc diffusion assay were still included in the MIC assay. This was done since there was no knowledge on the diffusion characteristics of the extracts through the M-H agar. It has been reported that zones of inhibition may not be exhibited because discs may lack potency due to storage and preparation (Becton and Company, 2006). Methanol and acetone extracts exhibited better inhibitory activities than water extracts with acetone extracts having lower average MICs than those of methanol, this was in agreement with Masoko et al., (2005) who reported acetone extracts having the lowest average MIC compared to methanol. Hamza et al. (2006); Mulaudzi et al. (2011) described a classification of inhibitory activity of extracts according to MIC as follows: strong inhibitor; < 0.5 mg/ml, moderate inhibitor; 0.5 – 1.5 mg/ml and weak inhibitor: > 1.5 mg/ml. According to this classification, methanol and acetone extracts from all the plants suggested potential inhibitory activity against *S. aureus* while water extracts were weakly inhibitory, this was in agreement with findings by Steenkamp et al. (2004); Mathabe et al. (2006); Suleiman et al., (2010) who reported that *S. aureus* was susceptible to most plant extracts. Moderate inhibitory activity was exhibited by the acetone extracts of *C. quadrangularis* against *C. congolensis* and *P. aeruginosa*. There was variance between the results of the agar disc diffusion and the MIC assays with regards to the activity of the acetone extract of *C. quadrangularis* against *D. congolensis*. *Dermatophilus congolensis* exhibited resistance to extracts, in the agar disc diffusion assay, while in the MIC moderate activity against it was exhibited. The discrepancy between the results of the two assays could be due to failure, by the disc-adsorbed *C. quadrangularis* to diffuse into the agar hence a negative result. Several researchers have reported problems associated with the use of the agar disc diffusion assay. Deviations from the recommended media pH of 7.2 – 7.4 and/or depth of 5 mm, which occur during preparation of the M-H agar media, can negatively affect results of the assay. Murray and Zeitinger (2011) stated that the disc diffusion assay was of limited use in hydrophobic plant extracts since these did not uniformly diffuse through the agar medium. Water soluble constituents of extracts easily diffuse through agar while the more viscous constituents do not (Hood et al., 2003). Despite these disadvantages, currently, several researchers still use the assay (Bharti et al., 2013; Firuzi et al., 2013; Martins et al, 2014). Acetone and methanol extracts of *P. angolensis* performed better (MIC range of 0.63 – 1.04 mg/ml) against *D. congolensis* compared to the other plant extracts. The agar disc diffusion assay results of *P. angolensis* against *D. congolensis* were consistent with those of the MIC, with the micro-organism exhibiting sensitivity. Steenkamp et al. (2004) reported poor inhibitory effects of *P. angolensis* against both Gram-positive and -negative bacteria with an MIC > 4 mg/ml. The difference in activity of *P. angolensis* between the two studies could be due to the different extract preparation methods. Steenkamp et al. (2004) used maceration and heat drying to prepare the extract, which was not the case in the current study. Heat drying might deactivate some antibacterial constituents in the plant as has been reported elsewhere (Lau et al., 2013) where cold and hot extracted preparations of *Lignosus rhinocerotis* (Cooke) Ryvarden had different chemical constituents and bioactivity. The difference could also be due to the fact that the *P. angolensis* plants used were collected from different localities and hence could have different active compounds (Shale et al., 2005). In the current study *P. angolensis* had some inhibitory activities against all organisms with MIC that ranged between 0.37 – 1.67 mg/ml. In general, water extracts from all the plants had weak inhibitory activity (MIC > 1.6) against all bacterial strains. The water extract of *C. spinosa* could be considered to have no inhibitory activity against *P. aeruginosa* since its MIC was 10 mg/ml. Mulaudzi et al. (2011) considered extracts with MIC > 8 mg/ml as having no inhibitory potential. Farmers are therefore faced with two constraints when they use water as an ingredient; (1) poor extraction capacity and (2) weak to very poor inhibitory effects of the resultant extracts.

These two constraints may partly explain the apparent lack of efficacy of plant remedies prepared using water. The lack of efficacy may not be intrinsic to the plant remedy, but attributable to the use of water in the preparation process. To overcome these constraints, farmers steeped the plant material in cold water for longer periods or boiled

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and used the resultant diffusion and decoctions respectively. Alternatively, some farmers prepared and used the plant in the form of a paste as was observed for the management of bovine dermatophilosis in the northern parts of Zimbabwe (Ndhlovu and Masika, 2013).

Gram-positive organisms particularly *S. aureus* were more sensitive to the extracts than were the Gram-negative bacteria. These findings were in agreement with those of Steenkamp et al. (2004); Mathabe et al. (2006); Suleiman et al. (2010) in which *S. aureus* was reported to be highly sensitive to extracts of *Terminalia sericea* Burch. ex DC, *Indigofera daleioides* Benth. ex Harv. var. daleoides and *Khaya anthotheca* (Welw.) C. DC respectively. The Gram-negative *E. coli* was not sensitive to extracts of *C. quadrangularis* and *C. spinosa* whereas *P. aeruginosa* was sensitive to the acetone and methanol extracts of *C. spinosa*. Acetone and methanol extracts of *P. angolensis* had moderate to weak inhibitory effects on both Gram-positive and Gram-negative bacteria with an average MIC < 2 mg/ml. This was in agreement with Shale et al. (2005) who reported that some forms of *Malva parviflora* L were inhibitory against both Gram-negative and Gram-positive bacteria as did *Podocarpus* species (Abdillahi et al., 2008). Luseba et al. (2007) reported that *P. angolensis* was highly effective against the Gram-positive *S. aureus* and the Gram-negative *E. coli*; this was in agreement with the current study. Gram-positive bacteria are reportedly more sensitive to antibiotics due to the lipophilic components of their cell membrane, compared to the Gram-negative bacteria which have hydrophilic components (Abdelkader et al., 2010). Gentamycin had better inhibitory activities than all the plant extracts. Generally, conventional drugs have been reported to exhibit superior antimicrobial properties than traditional medicinal plants (Mabona et al., 2013). Acetone and water negative controls exhibited no inhibitory activities, this was consistent with findings that acetone is non-toxic to microorganisms (Eloff, 1998). The 80 % methanol negative control inhibited growth of all bacteria. In our study, this activity of methanol did not affect the results of the assay since we did not use it for reconstitution. None of the plant extracts, in this study, exhibited MIC values of less than 0.1 mg/ml, it is however, noteworthy, that some authors (Firuzi et al., 2013) regarded plants with MIC ranging from 0.3 – 5 mg/ml as potential candidates for further analysis of their antimicrobial constituents.

The plant extracts had bacteriostatic rather than bactericidal activity as there was re-growth of organisms for all the ranges of MIC when the minimum bactericidal concentration assay was performed. This means that farmers need to repeatedly apply the remedies, daily or every other day until the animal is “cured”.

Conclusion

This study suggests that *C. quadrangularis*, *C. spinosa* and *P. angolensis* could have antibacterial activity against *D. congolensis* and the other tested micro-organisms. The inhibitory effects ranged from moderate to weak with acetone extracts performing better than the other extracts. The extracts would probably perform better if they were extracted in pure form as proposed by Suleiman et al. (2010). Other methods such as pounding the fresh plant material and squeezing out the fluid could lead to the production of a more concentrated test material. Since, in terms of the yield and antibacterial activities, water performed poorly when compared to acetone and methanol, more effective methods for the delivery of plant remedies should be devised and investigated. Such systems should be acceptable and affordable to the farmers. Due to the potential antibacterial activity of the studied plants, it is proposed that further elaborate studies be conducted, to validate these effects. Such studies should include among others, identification and isolation of the active biological compounds that are potentially responsible for the antibacterial activity and toxicity determination assays. The current study could be viewed as a preliminary validation of the ethno-veterinary practises that the farmers used to manage bovine dermatophilosis.

Competing interests: We declare that there are no financial or non-financial competing interests.

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