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Full Length Research Paper

In vitro antibacterial activity of seven plants used traditionally to treat wound myiasis in animals in Southern Africa

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In the extreme situation of subsistence farming where insecticides and other veterinary medicines are either unavailable or unaffordable, the use of plants in the treatment of wound myiasis in livestock has been reported worldwide. However, the exact effect of these plants on myiatic wounds has not been established. This study was therefore undertaken to establish the biological activity of seven species of plants which are used traditionally and are claimed to be effective in the treatment of wound myiasis. Plants that have a wide distribution in southern Africa were selected. This paper focuses on the antibacterial activity of these plants on bacteria known to be among the common contaminants of wounds. It has been shown that bacterial action on wounds produce compounds which have an odour that serve as an attractant of myiasis-causing flies. The antibacterial activity of the plants was investigated using a microdilution assay and bioautography methods. All the tested plants had inhibitory activity against the test bacteria. Inhibiting bacterial activity reduces the attractants of myiasis-causing flies to the wound. Thus, inhibiting bacteria action on wounds will interfere with the development of wound myiasis. This could be one of the mechanism through which the plants that are used traditionally in the treatment of wound myiasis work.

Key words: Wound myiasis, ethnoveterinary medicine, antibacterial activity.

INTRODUCTION

Wound myiasis (infestation of wounds by dipterous larvae) in livestock can be devastating due to production losses, veterinary costs and sometimes death (OIE, 2008). The role of bacteria in the attraction of myiasis-causing flies and oviposition has been established in a number of studies (Chaudhury et al., 2010). Bacteria such as *Streptococcus pyogenes, Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli, Proteus mirabilis* and *Klebsiella* spp. found on wounds produce volatile organic, sulphur-containing compounds with an odour that attracts the myiasis-causing flies (Khoga et al., 2002). These compounds can also act as ovipository stimuli to the myiasis-causing flies (Emmens and Murray, 1982). Extracts from unsterile sheep fleeces seeded with *P. aeruginosa, P. mirabilis, Enterobacter cloacae* and *Bacillus subtilis* stimulate oviposition by females of *Lucilia cuprina* (Wied.) (Eisemann and Rice, 1987). Wounds already infested with larvae are also more attractive to the gravid females (Hammack and Holt, 1983). The presence of larvae in wounds by themselves is not enough to attract gravid females, but their activity in

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Abbreviations: INT, *p*-iodonitrotetrazolium violet; **MIC,** minimal inhibitory concentration; **MH,** Müller-Hinton; **TLC,** thin layer chromatography; **EMW,** ethyl acetate/methanol/water; **CEF,** chloroform/ethyl acetate/formic acid; **BEA,** benzene/ethanol/ammonia hydroxide; **LPS,** lipopolysaccharides.

Table 1. Plants used traditionally to treat wound myiasis in South Africa and Zimbabwe.

Scientific name	Family	Plant part used	Distribution	Preparation and administration
Aloe marlothii Berger (Van der Merwe et al., 2001)	Asphodelaceae	leaves	Botswana, Mozambique, South Africa (North-West, Gauteng, Limpopo, Mpumulanga, KwaZulu-Natal north of Durban), Swaziland, Zimbabwe.	The leaves are crushed and the juice is applied onto the wounds
Aloe zebrina Baker (Luseba and Van der Merwe, 2006)	Asphodelaceae	leaves	Angola, Botswana, Malawi, Mozambique, Namibia, South Africa (Gauteng, Mpumalanga, Limpopo), Zambia, Zimbabwe.	Succulent fresh leaves are crushed and applied onto the wound
Calpurnia aurea (Ait.) Benth. (Hutchings et al., 1996)	Fabaceae	leaves	Angola, Mozambique, South Africa, Swaziland, Zimbabwe	Leaf sap is squeezed onto the wound
Psydrax livida (*Canthium huillense*) (Chavunduka, 1976)	Rubiaceae	leaves	Botswana, Malawi, Mozambique, Zambia, Zimbabwe, Angola, Kenya, Namibia, South Africa(North-West, Limpopo, Mpumalanga)	Leaves crushed and packed into the wound
Clausena anisata (Chavunduka, 1976)	Rutaceae	leaves	Angola, Malawi, Mozambique, Zambia, Zimbabwe, South Africa(Limpopo, Mpumalanga, Eastern Cape, KwaZulu-Natal)	Leaves crushed and packed into the wound
Erythrina lysistemon Hutch (Van Wyk et al., 1997)	Fabaceae	leaves	South Africa (North West, Limpopo, Gauteng, Mpumalanga, KwaZulu-Natal, Eastern Cape), Swaziland, Zimbabwe, Botswana, Angola	Leaves crushed and placed on a maggot-infested wound
Spirostachys africana Sond (Hutchings et al., 1996)	Euphorbiaceae		Zimbabwe, Mozambique, Swaziland, South Africa (Mpumalanga, KwaZulu-Natal)	The sap is applied onto the maggot infested wound

media contaminated with bacteria increases attractiveness of the wound (Eisemann and Rice, 1987). As such it is clear that bacterial contamination of wounds is important in the pathogenesis of wound myiasis.

In orthodox veterinary medicine, organophosphate insecticides in conjunction with antibiotics are recommended for the treatment of wound myiasis. The insecticides serve to expel and kill the larvae from the wound (OIE, 2008). The antibiotics deal with the microbial infection on the wound, which promotes wound healing and prevents secondary re-infestation by flies. In the difficult situation of subsistence farming where insecticides and other veterinary medicines are either unavailable or unaffordable, plants have been used in the treatment of wound myiasis in Africa and Asia (Chavunduka, 1976; Van der Merwe et al., 2001; Luseba and Van der Merwe, 2006). However, the exact effect of most of these plants on myiatic wounds has not been established. We therefore, undertook a study to establish the biological activity of 7 species of plants which are used traditionally and are claimed to be effective in the treatment of wound myiasis in South Africa and Zimbabwe (Table 1). The study was conducted in an endeavour to validate the traditional use of the plants and determine those that are highly active. This paper focuses on the antibacterial activity of extracts of these plants on bacteria that are common contaminants of wounds.

MATERIALS AND METHODS

Plant materials

After a study of the literature, seven plant species traditionally used in the treatment of cutaneous myiasis: *Aloe marlothii* A. Berger (Van der Merwe et al., 2001), *Aloe zebrina* Baker (Luseba and Van der Merwe, 2006), *Calpurnia aurea* (Aiton) Benth (Hutchings et al., 1996), *Psydrax livida* (Hiern) Bridson (*Canthium huillense*), *Clausena anisata* (Willd) Hook (Chavunduka, 1976), *Erythrina lysistemon* Hutch (Van Wyk et al., 1997), and *Spirostachys africana* Sond (Hutchings et al., 1996), were selected for further study. More information is provided in

Table 1.

Plant collection and storage

The plant material was collected from the Pretoria National Botanical Garden, South Africa. Voucher specimens and origins of the trees are kept in the garden herbarium. It was dried at room temperature in a well-ventilated room. Collection, drying and storage of plant material guidelines outlined elsewhere were followed (McGaw and Eloff, 2010).

Preparation of plant extracts

Dried leaf material was ground to fine powder using a KIKA-WERKE M20 mill (GMBH and Co., Germany). To obtain the acetone, methanol, dichloromethane and hexane extracts, four separate aliquots of 4 g of the leaf material of each plant were shaken vigorously for 30 min in 40 ml of the respective solvents on an orbital shaker (Labotec®, model 20.2, South Africa). The extracts were allowed to settle, centrifuged at 2000 x g for 10 min and the supernatant filtered through Whatman No. 1 filter paper into pre-weighed glass vials. The extraction process was repeated 3 times for each aliquot of plant material. The extracts were dried in a stream of cold air at room temperature and the mass extracted with each solvent was determined. The dried extracts were reconstituted in acetone to make 10 mg/ml stock extracts which were used for the antibacterial assays. Acetone was used for the reconstitution because of its efficacy in dissolving extracts with a range of polarities (Eloff, 1998a) and its low toxicity to microorganisms (Eloff et al., 2007). Twenty-eight extracts were prepared in total.

Antibacterial assay

A serial microplate dilution method (Eloff, 1998b) was used to screen the plant extracts for antibacterial activity. This method allows for the determination of the minimal inhibitory concentration (MIC) of each plant extract against each bacterial species by measuring the reduction of tetrazolium violet. The test organisms in this study included two Gram-positive bacteria, *S. aureus* (ATCC 29213), and *E. faecalis* (ATCC 29212), and two Gram-negative ones, *P. aeruginosa* (ATCC 27853) and *E. coli* (ATCC 25922). These are some of the most common bacteria known for infecting wounds. The specific strains used are recommended for use in research (NCCLS, 1990). The bacterial cultures were incubated in Müller-Hinton (MH) broth overnight at 37°C and a 1% dilution of each culture in fresh MH broth was prepared prior to use in the microdilution assay. Two fold serial dilutions of plant extracts (100 µL) were prepared in 96-well microtitre plates, and 100 µL of bacterial culture were added to each well. The plates were incubated overnight at 37°C and bacterial growth was detected by adding 40 µL *p*-iodonitrotetrazolium violet (INT) (Sigma) to each well. After incubation at 37°C for 1 h, INT is reduced to a red formazan by biologically active organisms, in this case, the dividing bacteria. The lowest concentration where there was a reduction of the colour intensity was taken to be the MIC. The MIC values were read at 1 h and 24 h after the addition of INT to differentiate between bacteriostatic and bacteriocidal activities. Acetone and the standard antibiotic gentamicin (Sigma) were included in each experiment as controls.

Bactericidal or bacteriostatic?

To confirm the bactericidal activity of the plant extracts the method described by Pankey and Sabbath (2004) was used. Only the acetone plant extracts were used in this assay because in most cases in the antibacterial assay they were more effective and potent. Subcultures of samples from clear dilution wells from the MIC assay were made on MH agar plates by plating 100 µl and subsequently incubating for 24 h at 37°C. The test organisms in this assay were one Gram-negative bacterium, *P. aeruginosa* (ATCC 27853) and one Gram-positive bacterium, *S. aureus* (ATCC 29213). A reduction of at least 99.9% of the colony forming units, compared with the culture of the initial inoculum, was regarded as evidence of bactericidal activity.

Bioautography

Bioautography was carried out to confirm the presence and determine number of antibacterial compounds in the plant extracts (Masoko and Eloff, 2005). Thin layer chromatography (TLC) plates (10 x 10 cm aluminium-baked, Merck, F_{254}) were loaded with 100 µg (10 µl of 10 mg/ml) of the extracts and dried before being eluted in three different solvent systems, that is, ethyl acetate/methanol/water (40:5.4:5): [EMW] (polar/neutral); chloroform/ethyl acetate/formic acid (5:4:1): [CEF] (intermediate polarity/acidic); benzene/ethanol/ammonia hydroxide (90:10:1): [BEA] (non-polar/basic) (Kotze and Eloff, 2002). The test organisms included, *S. aureus* (ATCC 29213), a Gram-positive bacteria and *P. aeruginosa* (ATCC 27853) a Gram-negative bacteria. The bacterial cultures, cultured for 14 h in MH broth were centrifuged at 3500 rpm for 5 min and the pellet re-suspended in minimal volume (20 ml) of MH broth. Developed plates were sprayed until damp with the concentrated bacterial cultures in a Bio safety Class 11 cabinet (Labotec, S.A) and incubated in a humidified chamber (100% relative humidity) overnight at 37°C. The plates were then sprayed with a 2 mg/ml solution of INT and incubated at 37°C for a further 12 h. Clear zone against the purple background indicate inhibition of microbial growth by separated plant constituents on the TLC plate.

To detect the separated compounds, a duplicate set of chromatograms developed in the 3 different solvent systems were sprayed with vanillin-sulphuric acid (0.1 g vanillin (Sigma®): 28 methanol: 1 ml sulphuric acid) and heated at 110°C to optimal colour development.

The mass of extract required to inhibit bacterial growth on an average size animal wound

Whatman No 1 filter papers were cut into circles of 4 cm diameter to mimic an average wound size in an animal. The filter paper circles were weighed and then sprayed with the acetone extracts until they were saturated. The filter paper circles were allowed to dry and re-weighed. The mass of extract required to cover the whole circle was calculated and recorded. Mean separation was done using the PDIFF option of SAS (2006). The volume needed to give determined mass values were determined using the concentration of the extracts which was 10 mg/ml. The quantity of extract in mg required to inhibit bacterial growth on wound of 4 cm diameter was calculated as:

Volume of extract X required to saturate the filter paper circle in ml multiplied by MIC value for a particular bacterium obtained from the antibacterial assay for extract X in mg/ml.

RESULTS

Antibacterial assay

Overall, *E. coli* was the least susceptible bacterium to the plant extracts (Table 2). We considered an MIC of 0.16

Table 2. Antibacterial activity of 7 plant species used to treat wound myiasis in Southern Africa.

Plant species	Extract	Time (h)	Antibacterial activity (MIC in mg ml^{-1})			
			E. coli	*E. feacalis*	*P. aeruginosa*	*S. aureus*
Aloe marlothii	Acetone	1 h	0.313	0.039	0.313	0.313
		24 h	0.313	0.039	0.313	0.078
	Methanol	1 h	1.25	2.5	0.078	0.625
		24 h	0.625	2.5	0.313	0.313
	Dichloromethane	1 h	0.313	0.625	0.313	0.156
		24 h	0.625	0.625	0.625	0.078
	Hexane	1 h	2.5	0.313	0.313	0.625
		24 h	2.5	2.5	2.5	2.5
Aloe zebrina	Acetone	1 h	0.156	0.02	0.156	0.039
		24 h	0.156	0.02	0.156	0.039
	Methanol	1 h	0.313	0.625	0.156	0.078
		24 h	0.156	0.625	0.313	0.156
	Dichloromethane	T1	0.156	0.078	0.156	0.078
		T2	0.156	0.156	0.313	0.039
	Hexane	T1	2.5	0.156	2.5	0.313
		T2	2.5	2.5	2.5	2.5
Calpurnia aurea	Acetone	T1	0.625	0.156	0.156	0.156
		T2	0.625	0.156	0.156	0.156
	Methanol	T1	1.25	1.25	>2.5	>2.5
		T2	1.25	1.25	0.313	
	Dichloromethane	T1	0.625	1.25	0.313	>2.5
		T2	0.625	1.25	0.313	
	Hexane	T1	1.25	2.5	>2.5	>2.5
		T2	1.25	2.5	>2.5	
Clausena anisata	Acetone	T1	0.625	0.625	0.313	0.313
		T2	0.625	0.625	0.156	0.625
	Methanol	T1	0.625	1.25	0.313	0.625
		T2	0.625	1.25	0.313	0.625
	Dichloromethane	T1	0.313	0.313	0.156	0.313
		T2	0.625	0.313	0.156	0.313
	Hexane	T1	0.625	2.5	0.625	>2.5
		T2	1.25	2.5	1.25	>2.5
Erythrina lysistemon	Acetone	T1	0.313	0.156	0.078	0.313
		T2	0.313	0.156	0.078	0.313
	Methanol	T1	0.625	0.625	0.313	0.313
		T2	0.625	0.625	0.156	0.313
	Dichloromethane	T1	0.625	0.156	0.313	0.313

Table 2. Count'd.

		T2	0.625	0.625	0.156	0.313
	Hexane	T1	2.5	1.25	0.625	1.25
		T2	2.5	1.25	0.625	1.25
Psydrax livida	Acetone	T1	0.313	0.078	0.313	0.156
		T2	0.313	0.313	0.156	0.078
	Methanol	T1	0.313	1.25	0.313	1.25
		T2	0.313	1.25	0.625	0.625
	Dichloromethane	T1	0.156	0.156	0.313	0.156
		T2	0.313	0.156	0.313	0.078
	Hexane	T2	2.5	0.313	0.313	1.25
			2.5	0.625	0.313	1.25
Spirostachys africana	Acetone	T1	0.156	0.156	0.156	0.156
		T2	0.156	0.156	0.156	0.156
	Methanol	T1	0.313	0.625	0.313	0.313
		T2	0.313	0.625	1.25	0.313
	Dichloromethane	T1	0.313	0.313	0.313	0.313
		T2	0.313	0.625	0.313	0.313
	Hexane	T1	0.625	2.5	0.313	1.25
		T2	0.625	2.5	0.313	2.5
Gentamycin			1.56×10^{-3}	3.9×10^{-4}	1.56×10^{-3}	7.8×10^{-4}
Acetone			>2.5	>2.5	>2.5	>2.5

mg/ml or less to be significant antibacterial activity based on the guidelines in the Phytomedicine Journal (Instruction to Authors). Only 4 out of 28 extracts had MIC values equal to or less than 0.16 mg/ml against *E. coli*. Nine of the 28 extracts, 11/28 and 8/28 of the plant extracts had MIC values equal to or less than 0.16 mg/ml against *E. faecalis, P. aeruginosa,* and *S. aureus,* respectively. Most of the plant extracts were active against both Gram-negative and Gram-positive bacteria. In 25/28 analyses (89%) hexane extracts had relatively poor activity (1.25 to 2.5 mg/ml) or no antibacterial activity at the highest concentration tested (2.5 mg/ml). In total, 13 extracts (46%) had MIC ≤ 0.16 mg/ml, of which 6 were acetone extracts, 5 were dichloromethane extracts and 2 were methanol extracts. The antibacterial activity of the plant extracts against both the Gram-positive and Gram-negative bacteria varied with the solvent used to extract the plant material (Table 2). As expected, the negative control, acetone, was devoid of any antibacterial activity.

The MICs for each extract type, that is, methanol, acetone, dichloromethane and hexane, were averaged for the four test organisms. The average activity volumes indicating to what volume 1 mg of extract from different extractants can be diluted and it would still kill the bacteria were determined by dividing 1 mg by the average MIC for each extract type. Figure 1 shows the average activity volumes of the different extractants. Acetone is clearly the best extractant, followed by dichloromethane, methanol and finally hexane. These results confirm many observations in our laboratory that the most active antimicrobial compounds have an intermediate polarity. On average 1 mg of the acetone extracts can be diluted in 4.2 ml and still kill bacteria whilst those of hexane can only be diluted in 0.8 ml.

To establish the plant species with the highest activity, the total activity of the different plant species was also determined. Total activity indicates the largest volume to which the biologically active compounds in 1 g of plant material can be diluted and still inhibit the growth of bacteria. It is calculated by dividing the quantity of material extracted from 1 g of dried plant material in milligrams by the minimal inhibitory concentration in mg/ml. It is useful to compare the potency of different

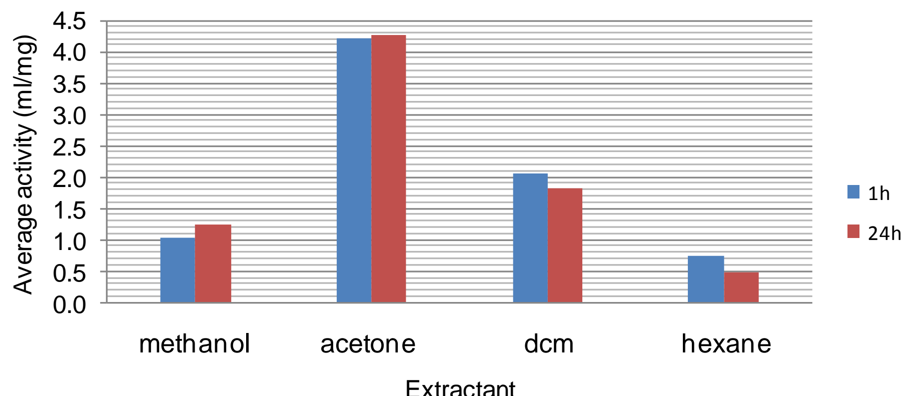

Figure 1. Average activity volumes indicating to what volume 1 mg of extract from different extractants can be diluted and it would still kill the bacteria.

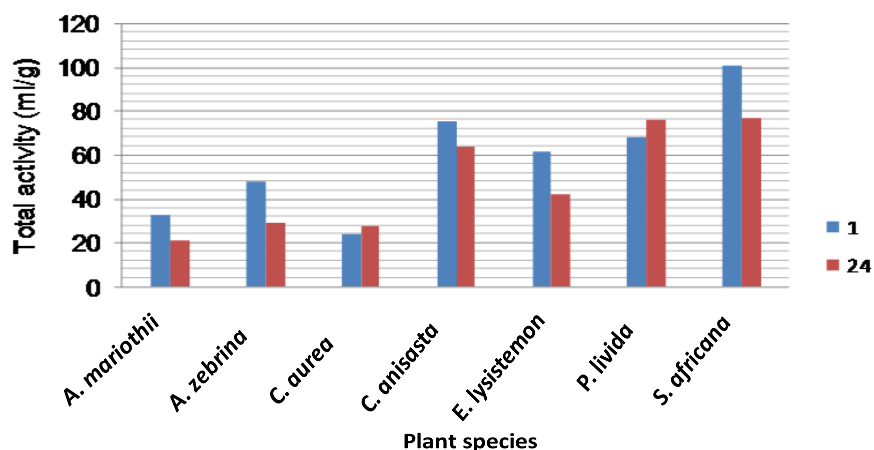

Figure 2. Total activity of the different plant species indicating the volume to which the biologically active compound present in 1 g of the dried plant material can be diluted and still kill the bacteria.

plants and to detect synergism or loss of activity in bioassay guided fractionation. Figure 2 shows the total activity of the different plant species. *Spirostachys africana* had the highest activity followed by *C. anisata, P. livida* and *E. lysistemon*, respectively. Extracts of 5 of the study plants became less potent with time as shown by the reduced activity volumes after 24 h. *Psydrax livida* and *C. aurea* were an exception as the extracts seem to get more potent with time.

Bactericidal or bacteriostatic

All the extracts were bacteriostatic at the determined MICs since growth was observed after plating of the contents of clear wells on MH agar. However they were bactericidal at higher concentrations. All of the tested plant extracts, except *A. zebrina,* were bactericidal against *P. aeruginosa,* at 1.25 mg/ml, with *A. zebrina* being most potent, at 0.625 mg/ml. *S. aureus* was most susceptible to the plant extracts, with all the tested plant extracts being bactericidal at 0.625 mg/ml.

Bioautography

TLC was used to fingerprint the plant extracts. This allowed for visualization of the different compounds in the plant extracts and identification of biologically active bands on the chromatograms. Bioautography, in general, showed more than one active band per plant extract (Figures 3 and 4). Although the hexane extracts had poor antibacterial activity in the microdilution assay bioautography showed that they too contained antibacterial compounds.

The mass of extract required to inhibit bacterial growth on an average size animal wound

Table 3 shows the mass of the acetone extracts of the

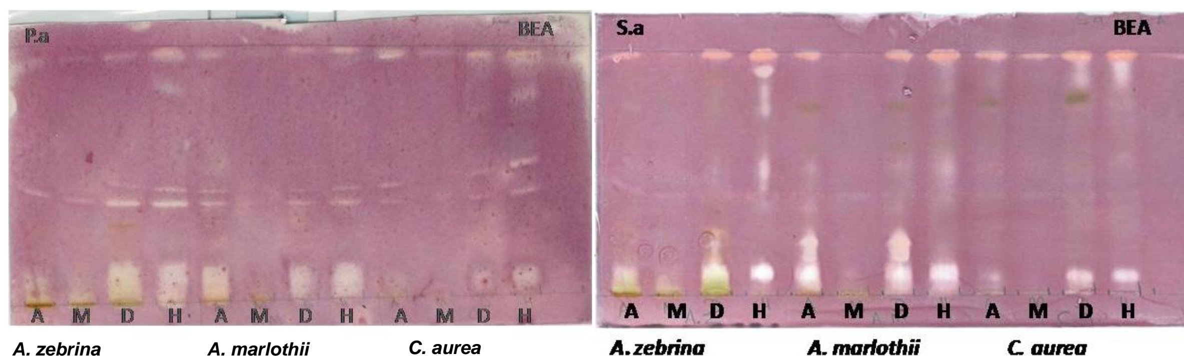

Figure 3. Chromatograms of acetone, methanol, dichloromethane and hexane leaf extracts of *A. zebrina, A. marlothii, C. aurea* eluted with BEA and sprayed with *P. aeruginosa and S. aureus* respectively. White areas indicate the presence of antibacterial compounds.

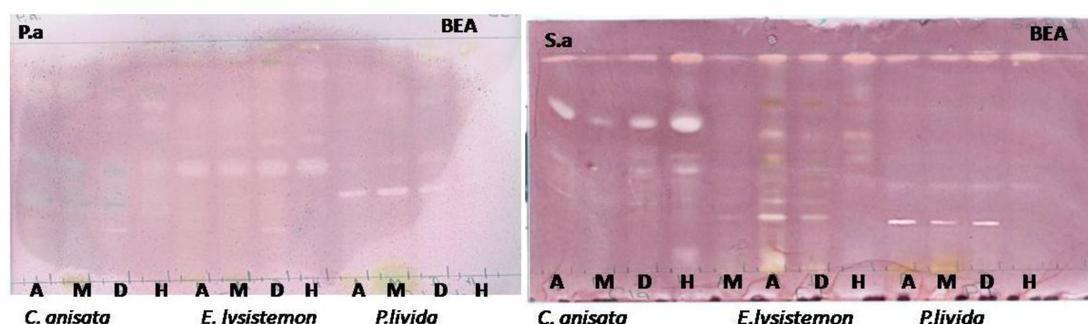

Figure 4. Chromatograms of acetone, methanol, dichloromethane and hexane leaf extracts of *C. anisata, E. lysistemon, P. livida* eluted with BEA and sprayed with *P. aeruginosa* and S. aureus respectively. White areas indicate the presence of antibacterial compounds.

different plant species required to inhibit bacterial growth on a wound of 4 cm diameter. On average the lowest mass of extracts is required when *A. zebrina* is used whilst the highest mass is required when *A. marlothii* is used.

DISCUSSION

Notably, all the plants in this study had antibacterial activity, albeit some at low and others at high minimum inhibitory concentrations. This observed antibacterial property could be one of the mechanisms through which the plants that are used traditionally in the treatment of wound myiasis work. One has to keep in mind that traditional healers mainly use water extracts. The active plant extracts had broad spectrum antibacterial activity, inhibiting both Gram-negative and Gram-positive bacteria, although the MICs were relatively higher for Gram-negative bacteria. It is known that, in general, the Gram-negative bacteria are less susceptible to antibacterials compared to the Gram-positive ones. This is due to the outer membrane composed of lipopolysaccharides (LPS), phospholipids, and lipoproteins that they possess which is absent in the Gram-positive bacteria. The outer membrane serves as a barrier for the bacterium against the destructive effects of various antibacterial compounds (Hodges, 2002).

P. aeruginosa is an opportunistic pathogen and is a common contaminant of wounds. Its action on wounds has been put forward as one of the attractants of myiasis causing flies (Eisemann and Rice, 1987). The fact that the study plants had one or more extracts with activity against *P. aeruginosa* might add validity to their traditional use in the treatment of wound myiasis.

The antibacterial activity of the plant extracts varied with the solvent used for extraction, as expected (Kotze and Eloff, 2002; Eloff et al., 2005). This can be explained in terms of the polarity of the compounds being extracted by each solvent and the amount of that compound, in addition to their intrinsic bioactivity. Notably extracts of the same plant had antimicrobial activity against the same microorganism although at varying MIC values. This means that the compound responsible for the antimicrobial activity was present in each extract, as shown by the bioautography, only at different concentrations. The acetone extracts were more effective and potent and this implies that acetone extracted a higher concentration of the antibacterial compound(s) or less of inactive compounds.

Table 3. Mass of the acetone extract of different plant species required to inhibit bacterial growth on a wound of 4 cm diameter.

Plant species	Average amount of extract sprayed on filter paper (mg)	Volume of extract required to cover the 4 cm filter paper (ml)	Extract required to inhibit bacterial growth on a wound of 4 cm diameter (mg)				Average
			E. c	*E. f*	*S. a*	*P. a*	
A. marlothii	4.60 ± 0.5354[c]	0.460	0.144	0.018	0.144	0.144	0.112
A. zebrina	2.75 ± 07937[b]	0.275	0.043	0.006	0.043	0.011	0.026
C. aurea	1.98 ± 0.1260[a]	0.196	0.123	0.031	0.031	0.031	0.054
C. anisata	2.35 ± 0.3416[a]	0.235	0.147	0.147	0.074	0.074	0.110
E. lysistemon	3.10 ± 0.5715[b]	0.310	0.097	0.048	0.024	0.097	0.066
P. livida	2.25 ± 0.6856[a]	0.225	0.070	0.018	0.070	0.035	0.048
S. Africana	2.70 ± 0.7528[b]	0.270	0.042	0.042	0.042	0.042	0.042

Means with same superscripts are not significantly different ($P < 0.05$).

Most of the plant extracts became less potent with time. This can be explained if the active component were volatile and being lost from the extract with time. This is unlikely seeing that the hexane extract did not have the highest activity. It is more likely that the active antibacterial compounds may have been broken down or the bacteria were able to overcome the initial inhibitory effects of the antibacterial compounds by metabolizing it. *Psydrax livida* extracts were an exception to this trend. This could be attributed to some plant compounds within the extract breaking down with time and releasing compounds that have higher antibacterial activity.

Aloe zebrina had the best antibacterial activity against all the bacteria and had the least quantity of extract required to inhibit bacterial growth on an averaged sized wound. However the quantity of extract from 1 g of plant material was relatively low hence its total activity was low. Generally, the bulk of Aloe leaves are water (Koroch et al., 2009). The leaves of *A. zebrina* are relatively thin compared to those of other aloes such as *A. marlothii*. As a result, the leaves are easy to dry as a whole and this is how they were used in this study. To determine which plants can be used for further testing and isolation, not only the MIC value is important, but also the total activity. This value indicates the volume to which the biologically active compound present in 1 g of the dried plant material can be diluted and still kill the bacteria (Eloff, 1999). Extracts with higher values are considered the best to work with. Among the plants that are used to treat cutaneous myiasis, the best plants in inhibiting bacterial growth are *S. africana*, *C. anisata*, *P. livida* and *E. lysistemon,* respectively, based on total activity.

The antibacterial activity of plants observed in this study concurs with previous findings by other researchers. The acetone extract of *A. marlothii* was reported to be active against *E.coli*, *E. faecalis* and *S. aureus* (Naidoo et al., 2006). *C. aurea* was reported to have antibacterial activity against both the Gram-negative bacteria (*E. coli*, *Salmonella pooni*, *Serratia marcescens*, *P. aeruginosa*, and *Klebsiella pneumoniae*) and the Gram-positive ones (*Bacillus cereus*, *Staphylococcus epidermidis*, *S. aureus*, *Micrococcus kristinae*, and *S. pyogenes*) (Adedapo et al., 2008). Two carbazole alkaloids, clausenol and clausenine, isolated from *C. anisata* are active against both Gram-positive and Gram-negative bacteria with MIC values ranging between 1.3 μg ml^{-1} and 40 μg ml^{-1} (Chakraborty et al., 1995). The volatile oil from the leaves of *C. anisata* also has significant activity against a number of bacteria and fungi (Gundidza et al., 1994). Phytochemically, *E. lysistemon is* rich in flavonoids and alkaloids and over 30 compounds have been isolated from this plant. Three of the isolated compounds have weak activity against the Gram-negative bacteria (*E. coli)* and moderate activity against Gram-positive bacteria (*B. subtilis* and *S. aureus*) (Juma and Majinda, 2005). According to Pillay et al. (2001) the bark of *E. lysistemon* is far more active than the leaves, yielding activity with water, ethanol and ethyl acetate extracts against *S. aureus*, *Micrococcus luteus* and *Bacillus subtlis.* The main anti-bacterial compound in the *E. lysistemon* bark was isolated and was identified as wighteone. Crude extracts from the bark of *S. africana* have antibacterial activity against diarrhoea-causative microorganisms (*Salmonella typhi*, *Shigella sonnei*, *Shigella dysentery*, *Shigella flexneri*, *Shigella boydii* and *E. coli.*) with MIC values ranging between 0.156 and 0.625 mg/ml (Mathabe et al., 2006). Phytochemically, the

Euphorbiaceae family to which S. africana belongs is rich in alkaloids and terpenoids (Webster, 1986). The inhibitory activity of terpenoids on bacteria has been reported (Drewes et al., 2005). One triterpene compound and two diterpenes compounds were isolated from *S. africana* (Mathabe et al., 2008) and are active against some of the diarrhoea-causative microorganisms with MIC values ranging between 50 and 200 μg ml^{-1}.

In some cases the observed results differed from previous findings by other researchers. For example, in this study the hexane extract of *A. marlothii* had some antibacterial activity contrary to McGaw et al. (2000) who reported that crude hexanic, ethanolic and aqueous extracts of *A. marlothii* does not have antibacterial activity. The possible reason for this difference in results could be the difference in plant chemical composition due to different times of plant collection and geographical differences. Unfortunately the TLC fingerprint of the plant from the previous research was not available for us to compare with the results from our study to confirm this postulation.

The antibacterial activities of extracts of *A. zebrina* and *P. livida* are being reported for the first time in this paper. Although the antibacterial activity of the other five study plants against some of the microorganisms have been reported against some of the test organisms in this study, in most of the studies the agar diffusion assay methods were used in determining the antimicrobial activity and high minimal inhibitory concentrations of up to 5 mg/ml were reported. In this study the serial microplate dilution method (Eloff, 1998b) was used. This method allows for the determination of the MICs of each plant extract against each bacterial species by measuring the reduction of tetrazolium violet. It is more sensitive and we were able to show that some of the plant species had antibacterial activity at much lower concentrations than previously determined. For example *C. aurea* was reported to have a MIC of 5 mg/ml against *E. coli, P. aeruginosa, S. aureus* (Adedapo et al., 2008) however, in this study we showed that it could still exhibit antibacterial activity against *P. aeruginosa, S. aureus* at 0.156 mg/ml and *E. coli* at 0.625 mg/ml. In addition, although the antibacterial activity of some of the plant species such as *S. africana* have been previously reported against some of the test organisms in this study, this is a first report of their antibacterial activity against *P. aeruginosa,* an important bacteria in the pathogenesis of wound myiasis. In some cases, the findings of this study add to the information on the antibacterial activity of some plant species. Pillay et al. (2000) reports that the ethyl acetate, ethanol and water extracts of *E. lysistemon* are ineffective against *E. coli* and *P. aeruginosa* however our results show that extracts from other extractants such as acetone, methanol and dichloromethane have reasonable to good antibacterial activity against these bacteria, with MICs ranging from 0.08 to 0.625 mg/ml.

All the plant extracts in this study were bacteriostatic at the determined MICs and bactericidal at higher concentrations. This is in line with the known fact that the MIC is simply the concentration of the drug that inhibits the growth of bacteria and inhibition of bacterial growth does not necessarily mean that the bacteria have been killed (Finberg et al., 2004). The bactericidal activity of an antimicrobial agent against a particular organism tends to be related to its mechanism of action. In general, agents that disrupt the cell wall or cell membrane, or interfere with essential bacterial enzymes, are likely to be bactericidal, whereas those agents that inhibit ribosome function and protein synthesis tend to be bacteriostatic. The tangible benefit of the extracts to be bactericidal comes in its use in the management of topical infection. While the concentration required to kill the tested micro-organisms is high at 0.6 and 1.25 mg/ml their ability to reach this concentration at the wound site in combination with the poor immune response associated with topical wounds make them beneficial in the clinical management of wounds. If a compound or extract only has bacteriostatic activity it does not mean that it will be ineffective as it may allow the natural defence system of the organism to take control. Many commercial antibiotics have bacteriostatic activity. At higher concentrations they may kill the bacteria. The advantage of controlling topical infections is that much higher concentrations can be used.

Generally small quantities, ranging from 0.006 to 0.147 mg, of acetone extracts are required to inhibit bacterial growth on an average sized wound. This may have to be mixed with a grease to apply to the animals. Traditionally the leaves of the plants are crushed and packed onto a wound.

Conclusion

The bacteria used in this study are known pathogens of wounds and their inhibition by the plant extracts in this study might validate the traditional use of plants in the treatment of wound myiasis. It has been shown that bacterial action on wounds produce ammonia and volatile organic, sulphur containing compounds which have an odour that serve as an attractant of myiasis-causing flies. Therefore, inhibiting bacterial activity reduces the attractants of myiasis-causing flies to the wound and the stimuli for oviposition. Thus inhibiting bacteria action on wounds will interfere with the development of wound myiasis. This could be one of the mechanism through which the plants that are used traditionally in the treatment of wound myiasis work. The next step to be addressed is to determine effect of these extracts on larval survival and subsequent development into adult stages.

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