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Evaluation of Repellent Properties of Some Plants Used in Venda and Tsonga Ethnoveterinary Medicine

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ABSTRACT Tick infestations are second to worm infestation among ethnoveterinary indications and plants constitute the majority of remedies used in their management in Vhembe District, South Africa. The aim of the study was to investigate the effects of some plants used in the control of tick infestation by farmers in the study area. Leaves of *Euphorbia ingens*, *Leonotis leonorus* and *Lippia javanica* root bark of *Terminalia sericea* and stem bark of *Cissus quadrangularis* were collected under the guidance of farmers who identified them as remedies they use to control ticks. The plants were dried in the laboratory, ground into powder and thereafter extracted with methanol, acetone and dichloromethane. Crude extracts were evaluated for their repellence activities against the tick *Rhipicephalus sanguineus* using the climbing bioassay. Acetone extract of *L. javanica* afforded a sustained activity over time (90%). Similarly, acetone extracts of *L. leonurus* (15 min) afforded limited biological activity as were methanol extract of *C. quadrangularis* and DCM extract of *T. sericea*. At least one extract of each plant except for *E. ingens* had an initial (after 15 minutes) repellent activity. In general, methanol extracts had no noticeable repellent activities as compared to acetone extracts. Aromatic plants are thought to act through their essential oils and irritant plants could be considered as tick toxicants.

INTRODUCTION

Ticks are major vectors of disease agents to humans and domestic animals and the burden of tick-borne disease (TBD) is increasing globally (Dantas-Torres et al. 2012). They are responsible for significant economic and production losses particularly in the cattle breeding industry (Parizi et al. 2012) because they transmit diseases, which cause morbidity and high mortalities. The most important tick-borne diseases of livestock in sub-Saharan Africa are East Coast fever (caused by *Theileria parva*), babesiosis (caused by *Babesia bigemina* and *B. bovis*), anaplasmosis (caused by *Anaplasma marginale*) and heartwater (caused by *Ehrlichia ruminantium*) (Moyo et al. 2009).

The control of ticks is commonly by use of chemicals with acaricidal or repellent activity (for example, organophosphates, synthetic pyrethroids) directly on the affected animals or in the environment (Dantas-Torres et al. 2012). This is increasingly being viewed as problematic because of the emergence of resistance and the ecotoxicity and food safety issues posed by pesticide residues (Parizi et al. 2012). For these reasons alternative means, for example, using anti-tick vaccines or biological control with entomopathogenic fungi are increasingly being sought (Dantas-Torres et al. 2012; Parizi et al. 2012). There is some evidence to suggest that phytotherapy may also be a viable alternative to control ticks in livestock (Gadzirayi et al. 2009; Kalume et al. 2011). In poor resource settings, rotational grazing, manual removal and use of medicinal plants have been documented as ethnoveterinary practices to combat tick infestation (Moyo et al. 2009).

The ethnoveterinary medicine practices among Tsonga and Venda speaking people of South Africa have been previously described (Luseba and Van der Merwe 2006; Luseba and Tshisikawe 2013). From these studies, it was found that tick infestations are second to worm infestation among ethnoveterinary indications and plants constitute the majority of remedies used in the region for their management. As in-

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dicated by Luseba and Van der Merwe (2006), tick infestation, wounds due to tick bites and other skin diseases are treated with similar remedies. The aim of this study was to evaluate the repellent activities of some plants, which have been previously cited by Tsonga and Venda livestock farmers in the Vhembe and Giyani districts of South Africa. The most popularly cited plant species and plant parts were leaves and stem of *Leonotis leonurus* (L.) R. Br. (Lamiaceae), *Lippia javanica* Burm f. Spreng (Lamiaceae), *Cissus quadrangularis* L. (Vitaceae), *Euphorbia ingens* E.Mey. ex Boiss (Euphorbiaceae) and *Terminalia sericea* Burch. Ex DC (Combretaceae).

Lippia javanica is a small, woody annual shrub commonly found in grassland on hillsides and stream banks and on the fringes adjacent to farmland. It is reported to possess analgesic, anti-inflammatory, antipyretic, larvicidal, repellent and antimicrobial properties (Mwangi et al. 1992; Hutchings and van Staden 1994). *Lippia javanica* leaf aqueous extracts consistently demonstrate strong repellent effects (van Wyk 2008; Moreira et al. 2009; Olivier et al. 2010). The bark decoctions and infusions of *Terminalia serecia* are used topically to treat wounds (van Wyk et al. 1997). *Cissus quadrangularis* is a succulent vine native to West Africa and Southeast Asia. Its stem and leaves are used in traditional African and Ayurvedic medicine for pain and inflammation, menstrual disorder, scurvy and as antiflatulence (Panthong et al. 2007). Farmers use it to treat burns, wounds, and as an insecticide (Hutchings et al. 1996; Luseba and van der Merwe 2006).

E. ingens is a multi-branching tree reaching heights of about 10 meters with a sturdy main trunk and large rounded (Gildenhuys 2006). It is the largest of the Euphorbias, which occur in Southern Africa (Van der Linde et al. 2010). *Euphorbia* ingens is used to treat ulcers, cancer and as an insecticide (Luseba and Tshisikawe 2013). *Terminalia sericea* is the dominant species in Southern African savanna bush land (Hutchings et al. 1996). It is used to treat diabetes, diarrhea, and gonorrhea and among the Venda in South Africa it is used to treat menorrhagia (Moshi and Mbwambo 2005).

MATERIAL AND METHODS

Plant Collection, Processing and Extraction

The plant materials for each species were collected from Venda (Vhembe district, 22º 56 S, 30º 28E) and Giyani (23° 19' South, 30° 43' East), air-dried at room temperature in the laboratory and finely ground to powder using the Büchi Grinder B-400 (Labotec). The ground plant materials were extracted with dichloromethane (DCM), acetone and methanol. Three grams of the dry finely ground leaves in 30 mL of solvent was placed in a centrifuge tube and shaken vigorously for 15 minutes. Two mL of the extract was removed from the top and placed in a separate 40 mL glass beaker. This was dried and weighed to determine the concentration of the extract. The remaining 28 mL solution was centrifuged for 5 minutes at 3500 rpm, filtered through Whatman No. 1 filter paper and the supernatant was collected into a 50 mL glass beaker. The process was repeated and the supernatant collected from the second extraction was combined with the first collected supernatant to complete extraction and stored in a fridge at 4ºC to avoid fungal growth (Eloff and McGaw 2006).

Repellency Bioassay

In this study, a modified climbing repellence bioassay described by Mkolo and Magano (2007) was adopted to test for the repellent properties of the plant materials. Ten unsexed *Boophilus (Ripicephalus) appendiculatus* adults were placed on a platform of the treatment apparatus and subsequently the same was done on the control apparatus. Prior to the start of the experiment the ticks were allowed a 15-minute acclimatization period, following which their position on the glass rod was noted at 10 minutes intervals for up to 60 minutes. After each 10-minute interval, the ticks on the glass rods were moved back to the polystyrene platform.

Ticks on the extract filter paper were considered not to be repelled by the extract and ticks found on the neutral filter paper or glass rod below the extract were considered to be repelled. Similarly, ticks found on the negative solvent control were considered not repelled and ticks found on the neutral filter paper or glass rod below the negative solvent control were considered to be repelled by the solvent. Ticks that dropped into surrounding water were dried using Kimberly-Clark paper towel and replaced onto the platform using forceps. The extracts (in the original solvent) at one hundred percent concentration and positive controls (Amitix^R and Bayticol^R dissolved in water) were used. Three replicates were carried out for each extract.

Repellence Data Analysis

The number of ticks that climbed up the glass rod within an hour during the repellence bioassay was counted and their mean number calculated and recorded. The repellence percentage was calculated using the following formula as adopted from Jantan and Mohd (1998). The average percentage repellence was calculated per hour.

$$\% \text{ Repellency} = \frac{[\text{mean number of ticks on test}]}{[\text{mean number of ticks on control}]} \times 100 = 100$$

Statistical Analysis of Repellence Trial

The experiment was designed as a completely random design with 12 replicates each, testing for differences between six plants and three solvents. Analysis of variance (ANOVA) was used to test for plant and solvent effects, as well as the plant by solvent interaction. The angular transformation was applied to percentage repellence to stabilize treatment variances (all negative % repellence values were taken as zero). Treatment means were separated using Fishers' protected t-test least significant difference (LSD) at the five percent level of significance (Snedecor and Cochran 1980). Data was analyzed using the statistical program GenStat (2003).

RESULTS

The results obtained in this study showed that there was no difference in repellent activity between different solvents, and this is important in order to compare the intrinsic effects of the extracts rather than that of the solvent. The activity of the solvents ranged between fifty-one to seventy-nine percent (± 6.1), and therefore only repellent activities equal or above ninety percent (after angular transformation) were considered as promising. Acetone extract of *L. javanica* at thirty percent concentration afforded the highest activity over time (90%) whilst the lower concentration (10%) was only effective at a 15-minute lap (results not shown). Similarly, acetone extracts of *L. leonurus* (15 minutes) afforded ninety percent repellent activity. It is interesting to note that except for *E. ingens*, at least one extract of each plant had an initial (after 15 minutes) good repellent activity. Methanol extract of *C. quandrangularis* and DCM extract of *T. sericea* were also effective. In general, methanol had no noticeable repellent activity.

DISCUSSION

The results reveals that *L. javanica* followed by *L. leonurus* were the most potent plants with regard to repellence activity. It is assumed that these aromatic plants (*L. javanica* and *L. leonurus*) might have direct repellent effects on the tick due to their essential oils, that is, sequiterpene hydrocarbons and β-cubenene are the major constituents of *L. javanica* (Mathabe et al. 2006). Limonene, ocimene, γ-terpinene, β-caryophyllene α-humulene and germacrene D are extracted from *L. leonurus* (Oyedeji and Afolayan 2005). These compounds occurred with several hexuronides of apigenin, luteolin, diosmetin and chrysoeriol as well as the surface flavonoid aglycones luteolin, tricin, apigenin, chrysoeriol, diosmetin, isothymusin, cirsimaritin, eupatorin, 5-desmethylnoboletin, luteolin, 7,4-dimethylether, genkwanin and salvigenin. Xanthine was also detected at very low concentration (Madzimure et al. 2011). Essential oil from *Origanum minutiflorum* (Lamiaceae) was shown to be acaricidal against *Rhipicephalus turanicus* (Cetin et al. 2009). Another member of the Lamiaceae family, *Tetradenia riparia* has also shown activity of its essential oils against *Rhipicephalus microplus* (Gazim et al. 2011). This study showed that the most activity plant extracts were those from Laminiaceae, which also belong to the same taxa.

Madzimure et al. (2011) found that aqueous extracts of *L. javanica* were an effective tick control option even though they were inferior to amitraz, the synthetic positive control. It is possible that organic or essential oil preparation would have shown better activity based on the observation that it is the volatile components which maybe active. The challenge with this is to be able to formulate stable products since vaporization occurs all the time. Similarly, isolongifolenone extracted from turpentine oil, a natural compound effectively repel ticks, more effectively than the widely used synthetic chemical repellent N,N-diethyl-3-methyl benza (DEET) in laboratory bioassays according to Zhang et al. (2009). Hence, many alternatives to DEET are made primarily from plant essential oils. The repellents affect insects by disrupting their natural behavior of blood seeking (Carlson 2000).

Essential oil-based insecticides cause the extrusion of fluid from the epidermis into the cuticle that affects the integument's permeability to respiratory gases and leads subsequently to excessive respiration, dehydration of the central nervous system and histological degeneration (Gerolt 2008). Farooq et al. (2009) have reported that in some parts of Pakistan, pastoralists burn the leaves of aromatic plants "to create a smoke ring around the animals", which serves to repel flies. This possibly releases the essential oils containing the active compounds.

The use of irritant plants for wounds infected with maggots in EVM practice was reported by Luseba et al. (2007). It is also suspected that this is also the case for tick infestation, as *Cissus quandrangularis* and *E. ingens* might have toxicant effects that could not be demonstrated by the methodology used in this study. Moreover, other studies have demonstrated the antibacterial activities of *L. leonurus* (Kamatou et al. 2006) and anti-bacterial and anti-inflammatory activities of *C. quandrangularis* and *T. sericea* (Luseba et al. 2007). Due to these properties, these plant extracts can be considered as good EVM remedies for wound treatment, which is one of the most prominent signs of tick infestation in the study area.

CONCLUSION

Acetone extracts of *L. javanica* and *L. leonorus* were shown to have repellent activities in climbing bioassay. It is thought that aromatic plants act better as repellents due to their essential oils content whilst irritant plants would act better as tick toxicants by direct effects on tick epidermis. Similarly, the use of the same plants for wound treatment suggests that tick infestation is a multi-factorial syndrome. Hence, when validating the use of ethnoveterinary medicines by small-scale farmers, more than one bioassay needs to be conducted in order to have a holistic picture.

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