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ORIGINAL RESEARCH

Acaricidal efficacy against cattle ticks and acute oral toxicity of *Lippia javanica* (Burm F.) Spreng

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Abstract In search for low-cost, safe and environmentally benign plant-based alternatives to commercial pesticides, the efficacy of *Lippia javanica* aqueous leaf extracts in controlling ticks on cattle, acute oral toxicity in mice and phytochemistry were evaluated. *L. javanica* aqueous leaf extracts at 10% and 20% *w*/*v* were effective at controlling cattle ticks but not as good as an amitraz-based acaricide Tickbuster®. However, they can provide an effective tick control option where synthetic products are unavailable or unaffordable, particularly in remote parts of southern Africa. Peripheral blood samples collected showed no haemoparasites in treated cattle implying that animals did not suffer from clinical tick-borne diseases. The leaf aqueous extracts of *L. javanica* were tested for toxicity in BALB/c mice. While anecdotal evidence suggests *L. javanica* has low mammalian toxicity, within 48 h all mice fed with the *L. javanica* leaf aqueous extract at 12.5–37.5% *v*/*v* were lethargic, and overall mortality was 37.5% (n=24). Thus, despite their apparent safety, water extracts of *L. javanica* leaves may have deleterious health implications on humans and animals if consumed at very high doses. Many compounds have been identified from *L. javanica* including an array of phenolic glycosides, flavonoids and essential oils but none of these are known to have acute toxic properties.

Keywords Acaricidal efficacy · Cattle ticks · Leaf aqueous extract · *Lippia javanica* · Mice · Oral toxicity · Phytochemistry

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Introduction

Pesticidal plants have great potential for impact in developing countries (Isman 2008), but a scientific understanding of their activity provides opportunities to optimise their use (Stevenson et al. 2009a). The value of pesticidal

plants comes from the harnessing of plant defence strategies based on the production of toxic chemicals that target herbivores and microorganisms (Futyoyma and Agrawal 2009). These compounds can be repellent or toxic to highly specific pests (Stevenson et al. 2009b) or a wide range of organisms (Aslam et al. 2009; Lukwa et al. 2009; Nzira et al. 2009). The use of pesticidal plants against pests has been reported in tropical regions such as southern Africa (Moyo and Masika 2009; Kamanula et al. 2010; Nyirenda et al. 2010) but largely based on ethno-ecological knowledge, and there are efforts to address optimisation, sustainability and safety issues (Stevenson et al. 2010).

The application of plant extracts to livestock in order to repel or kill parasites is widespread (van Wyk 2008; Moreira et al. 2009; Moyo and Masika 2009) especially in developing countries because synthetic acaricides are not affordable or available and dipping services are unsatisfactory, particularly in areas far from towns. For example, in some parts of Zimbabwe in the 2008/2009 rainfall season, cattle could go for more than 6 months without dipping whereas the general recommended frequency is once a month during winter and once every 2 or 3 weeks during summer (Gunjal et al. 2009).

Many authors have reported botanical products that kill parasites or inhibit oviposition (Chhabra and Saxena 1998; van Wyk 2008; Moreira et al. 2009; Olivier et al. 2010). Acaricidal plants are reportedly widespread (Samie et al. 2010) and there is even the potential for their cultivation (Martin et al. 2001). Their low cost is particularly appealing to farmers, although seasonal availability, harvesting timing *vis a vis* application time, variable efficacy, uncertainty over dosages and standardization (Martin et al. 2001) may be drawbacks.

Lippia javanica (Burm F.) Spreng (Verbenaceae) is reported to be acaricidal by Zimbabwean smallholder farmers (Stevenson et al. 2010). This plant is an erect, small, woody annual shrub that grows to a height of 2 m. The shrub is commonly found in grassland on hillsides and stream banks and as a constituent of the scrub on the fringes of forests that are often adjacent to farmland. The volatile oil of *L. javanica* has been reported to have antimicrobial properties (Samie et al. 2009a, b, 2010; Shikanga et al. 2009). Despite the widespread use of *L. javanica* for medicinal purposes in southern Africa (Manenzhe et al. 2004; Viljoen et al. 2005; Mujovo et al. 2008; van Wyk 2008), not much has been reported in literature about its potential acaricidal properties or its toxicity despite the reported use by farmers for controlling ticks. The objectives of the current study were therefore to test the efficacy of *L. javanica* aqueous leaf extracts and determine the acute toxicity of the aqueous extract when orally administered to BALB/c mice and to elucidate the chemistry of the plant.

Materials and methods

Experiments were conducted to determine the efficacy of *L. javanica* leaf extract in controlling cattle ticks (Experiment I) and to determine the acute oral toxicity of the extract in BALB/c mice (Experiment II). The phytochemistry of the leaf extracts was determined through laboratory analysis.

Experiment I: efficacy of *L. javanica* leaf aqueous extracts in controlling ticks on cattle

Study site

The experiment was conducted at Henderson Research Station (17° 35′ S, 30° 58′ E) located about 32 km north of Harare, Zimbabwe. The vegetation mainly consists of tree savanna or bush clump savanna with tall perennial grasses such as *Hyparrhenia filipendula* on red clay soils. Associated woody species include various *Acacia* species and *Brachystegia spiciformis*. The station is located on the watershed of Zimbabwe at an altitude of 1,200 m. Rainfall is confined to summer (November through to March) and is moderately high (750 to 1,000 mm). The mean annual temperature ranges from 20°C to 30°C.

Preparation of plant extracts

Seven kilogrammes of *L. javanica* were harvested weekly from Henderson Research Station's grassland, crushed and soaked in 28 l of water at room temperature for 24 h followed by filtration through a muslin cloth to produce a 25% (*w*/*v*) stock solution. Serial dilutions of the stock solution were made to provide concentrations of 5%, 10% and 20% (*w*/*v*).

Experimental design and procedure

Prior to the commencement of the study, the strategic policy for tick management at Henderson Research Station was dip-plunging once every week during high rainfall months (December to April) and once monthly for the rest of the year. The conventional dip used was amitraz. All the animals were not dipped for a month before the experiment in order to allow natural tick infestation. The experiment was conducted from April to May 2008 for 7 weeks.

Twenty Mashona steers, normally kept at the research station, were used in a completely randomised design experiment that had five treatments consisting of three application levels of *L. javanica* leaf extract (5%, 10% and 20% (*w*/*v*)), an untreated group (negative control) and a positive control of Tickbuster® spray, a 12.5% EC

amitraz-based compound (Zimphos Private Ltd, Harare, Zimbabwe), applied at the prescribed (label) dilute rate of 0.2% *v/v*. Animals in different treatment groups were kept in separate paddocks which had been managed the same way prior to the study.

Full body counts of the different types of ticks on each animal were done prior to spraying the animals with the treatments. A knapsack sprayer was used to apply 5 l of the aqueous solution/suspension to each animal. The same volume and application method was used for the Tickbuster® treatment group. For the negative control group, 5 l of tap water was applied per animal. The animals were sprayed weekly for 7 weeks from April to May 2008. Ticks were also counted daily in-between spraying. During spraying or sampling, each animal was held in the neck region using a cattle crush to restrain it. All the animals were observed daily for any signs of tick-borne or other diseases.

Blood samples were collected on the first day of the experiment and thereafter, weekly. An ear of each cow was clipped to expose the ear vein, cleaned using alcohol, and then allowed to dry. The ear vein of each animal was pricked with a sharp needle in order to get a small drop of fresh blood. The blood drop was stocked on a clean slide and was rapidly spread into an even thin film using a second clean slide held at a 45° angle and immediately dried. The slide was labelled and kept in an upright position. The blood films were prepared according to the method of Kelly (1979). They were then examined under a light microscope at a magnification of ×1,000 for the presence of haemoparasites. The parasites were identified according to the characters described by Sastry (1983), Levine (1985) and Kreier (1994). Each blood film was examined twice before being considered negative.

Experiment II: single-dose acute oral toxicity of the acaricidal plant *L. javanica* aqueous extracts in BALB/c mice

Study site

The *L. javanica* toxicity study was carried out under ambient conditions in the animal house at the Department of Paraclinical Veterinary Studies, Faculty of Veterinary Science, University of Zimbabwe.

Preparation of plant extracts

Fresh *L. javanica* leaves were pounded in a mortar using a pestle to produce a pulp which was soaked in water at a 1:1 ratio *w/v* for 24 h. The mixture was sieved through a muslin cloth and the filtrate further diluted with water to produce dosages of 0, 12.5%, 25% and 37.5% *v/v*. The dilutions of the treatments were intended to provide the wide range of dosages that farmers normally use.

Animals and experimental design

Acute oral toxicity of the plant was studied using sexually mature BALB/c mice that were kept individually in conventional cages, and given commercial feed and water *ad libitum*. An adaptation period of 2 weeks was allowed before the experiment commenced. Thirty-two mice were used for the experiment in a completely randomised design with four treatments replicated eight times. The experimental unit was one mouse. The treatment groups consisted of the control (placebo), which received only water, and three others that received 12.5%, 25% and 37.5% *v/v* of the *L. javanica* leaf extracts.

Administration of plant extracts

The mice were deprived of feed and water overnight prior to administration of the treatments. A 10-ml plastic syringe with a 16-mm long 22-gauge gavage needle was used to administer the plant extracts suspension per os. The needle was held horizontally, in a position parallel with the head of the mouse, and inserted at the back of the animal's throat. Once the needle was in place, a single dose of 4 ml of the suspension was carefully introduced into the oesophageal opening. The control group received 4 ml of tap water in the same manner. The quantity of 4 ml was based on the minimum water requirement of a mouse for normal basic physiological functions.

Monitoring of animals

Clinical observations were carried out daily for 14 days. Behavioural changes and mortality were recorded. The time of onset and duration of clinical signs was also recorded. Post-mortem examination was carried out on dead mice from all the treatments.

Chemical analysis

Air-dried leaves of *L. javanica* were milled in a coffee grinder. A sample (1 g) was extracted in methanol (10 ml) for 24 h and filtered through Whatman grade 1 filter paper and refiltered through an acrodisc with 45 μm pore size. The sample was diluted ten times before analysis. LC-MS of the extract was carried out using a Thermo LTQ-Orbitrap XL instrument. Calibration was performed using factory solutions containing sodium dodecyl sulfate, sodium taurocholate, L-methionyl-arginylphenylalanyl-alanine acetate.H_2O and Ultramark1621 interfaced to an Accela autosampling LC system. For chromatographic separation, a 150×3.0 mm

i.d., 3 μm, Phenomenex Luna C18 (2) column was used with a linear mobile phase gradient, A=H_2O; B=MeOH; C=1% HCO_2H in MeCN with A=90% and C=10% at t=0 min; B =90% and C=10% at t=20 to 25 min at 400 μL/min flow rate and 30°C. Injection volumes were 2 μL.

Statistical analysis

For Experiment I, the daily tick counts were used to calculate acaricide efficacy ratio per animal using a formula adapted from O'neill (2006):

$$\text{Acaricide efficacy} = 1 - \left(\frac{\text{Treatment tick count}}{\text{Untreated control tick count}}\right)$$

The acaricide efficacy ratio was subjected to arcsine-square root transformations to normalize the data and then analysed by the repeated measures analysis of variance (F test) procedure of SAS (2006). The following model was used:

$$Y_{ijk} = \mu + T_i + W_j + T_i \times W_j + e_{ijk}$$

Where:

Y_{ijk}	the response variable (arcsine-square root transformed tick count efficacy ratio)
μ	the overall population mean
T_i	the fixed effect of the ith treatment (i=treatment 1,...5)
W_j	the fixed effect of time post-treatment application (j=weeks 1,...7)
$T_i \times W_j$	the interaction between time post-treatment application and treatment
e_{ijk}	the residual error

For pairwise comparison of efficacy ratios, the Tukey's studentised range test was used.

For Experiment II, the Chi-square test was conducted using the PROC FREQ procedure of SAS (2006) to determine the association between the treatments and mortality. Data analysis for chemical composition was performed using Xcalibur 2.0.7 software. MS data for individual peaks were compared to the natural products library database at Royal Botanic Gardens in Kew, UK.

Results

Experiment I: acaricidal efficacy

The tick species that were recorded on the cattle were the *Boophilus* species, *Rhipicephalus evertsi evertsi*, *Rhipicephalus appendiculatus* and the *Hyalomma* and *Amblyomma* species. Efficacy ratios on cattle treated with all the *L. javanica* aqueous-extract doses and Tickbuster® spray were higher (P<0.05) than those on cattle in the untreated control (Fig. 1) at the end of the experimental period. There was a significant interaction (P<0.05) between time and treatment effects. In general, efficacy ratios were higher (P<0.05) than untreated control from the fifth week till the end of the 7-week period of weekly treatment application for the 5%, 10% and 20% *L. javanica*. The 5% *L. javanica* treatment only became efficacious from the fifth to the seventh week of treatment. Animals in the amitraz control group had a higher (P< 0.05) mean efficacy ratio against flat ticks than those in all the other treatments (Fig. 1) at the end of the 7-week period.

Efficacy ratios against engorged ticks were higher (P< 0.05) in the treatment groups than in the untreated control (Table 1). There was no interaction between time post-treatment application and the acaricidal efficacy against engorged ticks. The efficacy of *L. javanica* treatments against engorged ticks were not significantly different from those of amitraz (Table 1). No parasites were detected on microscopic examination of the Giemsa-stained thin blood smear.

Experiment II: single-dose acute oral toxicity in mice

In the toxicity experiment, soon after administration of the *L. javanica* treatments all the mice became lethargic. Although treatment had no effect on mortality (P=0.083), some mice in the *L. javanica* treatments died during the first 48 h. The group fed *L. javanica* at 37.5% w/v had higher (P<0.05) mortality than the control and the 25% groups. In all the cases where deaths occurred, the mice were initially depressed and preferred to remain huddled and taking no food from the time of plant extract administration. Post-mortem of the mice that died within 48 h revealed massive congestion around the large intestine, haemorrhages on serosal surfaces of organs and haemorrhagic effusions into the pleural and abdominal cavities. All the mice that survived appeared depressed for about 3 h post-administration of the treatments, after which they recovered and did not display signs of discomfort for the remainder of the observation period. The control group (which received water only) showed no adverse clinical signs. The average mortality for all the mice that were given the plant extracts was 37.5% with the 37.5% *v/v* treatment group recording the highest mortality (Table 2).

Chemical analysis

The major component identified in aqueous extracts of *L. javanica* was verbascoside which occurred as its *E* and

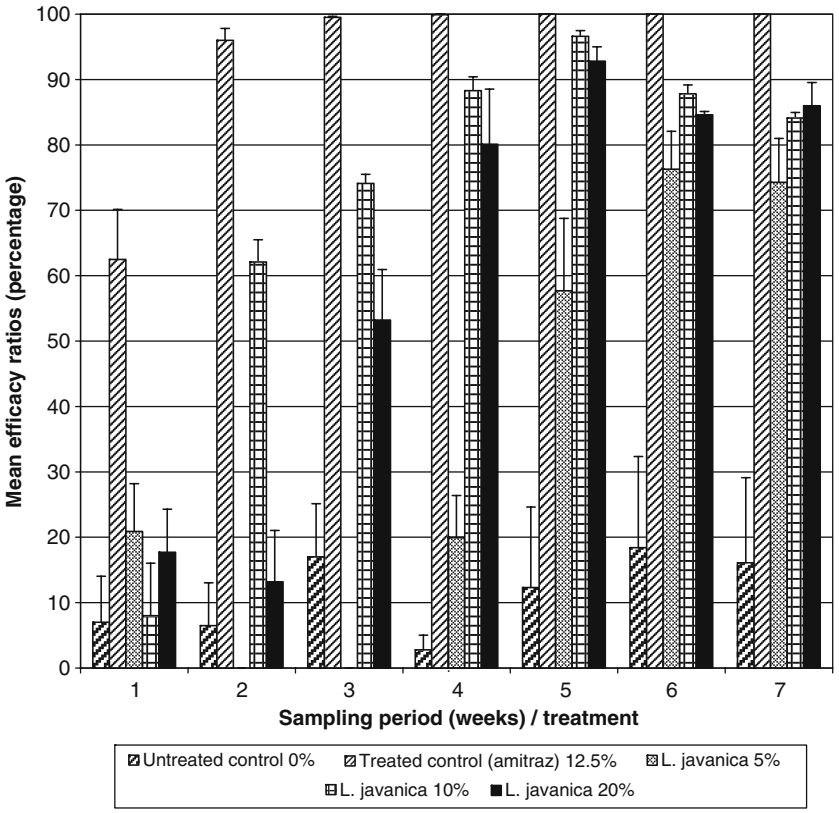

Fig. 1 Comparison of mean efficacy ratios (%) (±SEM) against flat tick counts on cattle over 7 weeks (April–May 2008) after weekly treatment with *Lippia javanica* leaf aqueous extracts and Tickbuster (amitraz) (n=4)

Z regioisomers along with *E* and *Z* regioisomers of crassifolioside. 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 concentrations.

Table 1 Means (%) for treatment efficacy ratios (±SEM) against engorged ticks after seven weekly acaricide applications (April–May 2008) (n=4)

Treatment	Engorged ticks
Amitraz (positive control)	99^{a}
Undipped (negative control)	75^{c}
5% *Lippia javanica*	92^{ba}
10% *L. javanica*	93^{ba}
20% *L. javanica*	89^{ba}
±SEM	5

Within a column, means with a common superscript letter are not different (P>0.05)

Discussion

L. javanica leaf aqueous extracts consistently demonstrated effective acaricidal activity against cattle ticks. The interaction of time and *L. javanica* treatment concentrations (5%, 10% and 20% *w*/*w*) on efficacy demonstrates that apart from the typical dose-dependency that occurs with conventional acaricides (Belmain et al. 2001), the duration of the treatment can influence efficacy. The improved efficacy from the fifth to the seventh week, especially for the 5% *L. javanica* acqueous extract might suggest that there is a residual effect. The effects of 10% and 20% aqueous extracts were not significantly different indicating that a 10% extract provides the maximum activity for this plant and is sufficient to effectively control the cattle ticks. The results compare well with those of Neem, *Azadirachta indica*, in which 5% soapy aqueous seed extract was applied on naturally infested animals every 21 days and controlled the *Boophilus microplus* tick as effectively as an amitraz-based acaricide (Benavides et al. 2001).

Although the efficacy ratios for the acaricidal plant treatments were higher at the end of the experiment, the actual mean tick count implied that the herd would be considered tick infested in Zimbabwe since more than 10%

Table 2 Mortality (%) of BALB/c mice after oral administration of different concentrations of aqueous extracts of *Lippia javanica* leaves

Hours post-treatment	Mortality of mice after exposure to different concentrations of *L. javanica*				Pooled mortality of mice exposed to *L. javanica* (n=24)
	Dose (v/v)				
	0%	12.5%	25%	37.5%	
n	8	8	8	8	
0–12	0	0	0	1	1 (4.2)
13–24	0	0	0	0	0
25–36	0	1	1	2	4 (16.7)
37–48	0	2	0	2	4 (16.7)
49–60	0	0	0	0	0
61–72	0	0	0	0	0
Total mortality (%)	0	3 (37.5)	1 (12.5)	5 (62.5)	9 (37.5)

of the herd had ten or more live ticks per animal (Anonymous 1993). However, this may be of less consequence in smallholder farming areas of Zimbabwe where tick control, which has always been the responsibility of the government veterinary department, had virtually collapsed. Acaricidal plants such as *L. javanica*, therefore, provide a valuable, low-cost option with high efficacy for controlling ticks in cattle herds of resource-poor farmers.

In this study, the ticks that are usually found on cattle at the study site *R. appendiculatus* (brown ear tick), *R. evertsi evertsi* (red-legged tick), *B. decoloratus* (African blue tick) and *Hyalomma* species (bont-legged ticks) were observed, indicating that the normal range of tick species was present during the study period. Findings of the current study supports unpublished survey data produced by Central Veterinary Laboratory of Zimbabwe in 2004, which showed that the genus *Amblyomma* (bont ticks), previously absent around the study site, is now present, though its occurrence is still sporadic. Higher efficacy ratios against engorged ticks on the treated cattle compared to those untreated suggest that ticks had fewer chances of reproduction in the former than in the latter treatment group. Thus the amitraz and *L. javanica* acqueous extracts appeared to be effective in disturbing the tick life cycle. The findings are in agreement with many authors who reported strong repellent effect of *L. javanica* on parasites (van Wyk 2008; Moreira et al. 2009; Olivier et al. 2010). The high efficacy ratio for the untreated group indicates that ticks drop off naturally when they are fully engorged in order to lay their eggs on the ground. This explains the continued increase in tick counts on the undipped treatment group which was grazing on pastures infested with hatched tick larvae.

Seasonal dynamics of tick populations is well documented but information on the effect of cattle density on tick populations is scarce particularly with regards to free-ranging cattle as is often the case for smallholder farmers in many African countries. It is hypothesised that wild animals will play a greater role in influencing tick population size than cattle. However, the use of *L. javanica* could be more beneficial over synthetic acaricides in that it would help increase natural cattle resistance to ticks – a phenomenon which is often compromised when cattle are intensively dipped and become more susceptible to tick attack (Pegram et al. 1993). Thus *L. javanica* treatment will allow tick breeding to continue at low levels, but any effect on tick abundance in the natural habitat is likely to be limited, particularly in the context of smallholder livestock farming systems in Africa where cattle are often grazed on land shared with wild animals.

The absence of tick-borne diseases might confirm the known status of these animals which were well-managed prior to the study. However, the diagnosis of piroplasm infections was based on clinical findings and microscopic examination of Giemsa-stained blood smears. According to Mahmmod et al. (2010), this method is not sensitive enough or sufficiently specific to detect chronic carriers, particularly when mixed infections occur.

The lethargy observed in the mice after administration of *L. javanica* could have been caused by an overdose of xanthine in the extract. Xanthine is a demethylated derivative of caffeine with pharmacological actions such as central nervous system (CNS) stimulation, relaxation of smooth muscle (especially bronchial muscle), myocardial stimulation, peripheral vasoconstriction and diuresis. *Lippia multiflora*, a species found in many parts of sub-Saharan Africa, is also known to contain xanthine (Noamesi et al. 1985a).

Verbascoside was the main component of the leaf extract and this compound has been identified recently in *L. javanica* (Shikanga et al. 2009) and the related species *L. alba* (Quirantes-Pine et al. 2009) but crassifolioside is less common in nature. While it has not been reported previously in *Lippia*, it is known from other genera in the Verbenaceae (Jamzad et al. 2003). None of these com-

pounds has any reported toxicity that might explain the acute toxic effects of *Lippia* water extracts against mice found in the current study, suggesting that other compounds occur in the water extracts that are toxic. While there are some reports of xanthine having mammalian toxicity, this is only when consumed at very high concentrations. It should be noted that xanthine occurs in numerous plant products consumed by humans.

The three preparations used in the toxicity study were presented to the mice at high concentration, thus the presence of xanthine may have over-stimulated the mice and led to a depressant phase without the typical initial stimulation phase with clonic convulsions, which is a rapid alternating contraction and relaxation of muscles. For instance, Noamesi et al. (1985a) demonstrated tranquilizer and analgesic effects of *L. multiflora* in rats, similar to those of diazepam, after doses ranging from 200 to 1,200 mg/kg of *L. multiflora* lyophilisated powder obtained from an infusion of dried leaves dissolved in NaCl administered by either the intraperitoneal or oral route. In that experiment, the tranquilizer and analgesic phase was preceded by a precocious ataxic phase and the quality of the symptoms and signs observed were dose dependant unlike in the present study. In another study, aqueous leaf extracts of the same plant administered intraperitonially produced a profound muscle relaxing action, considered to be primarily responsible for the calming effect which bordered on tranquilization, at doses of 0.25 to 1.0 g/kg and 0.5 to 1.0 mg/ml in amphetamine-medicated mice and unmedicated rats, respectively (Noamesi et al. 1985b). The congestion and haemorrhage observed at post-mortem of some of the mice are consistent with the peripheral vasodilation which occurs with high doses of caffeine (Lewis 1965).

L. javanica leaves have been reported to contain oil whose major constituents are geranial, neral, isopropenyl acetophenone, linalool and some terpenoids (Chowdhury et al. 2003; Manenzhe et al. 2004; Olivier et al. 2010). Previous research showed that *L. javanica* leaf oil is anti-microbial; concentrations of leaf oil above 250 ppm absolutely inhibited pathogens such as *Fusarium equiseti* (Chowdhury et al. 2003). In concurrence, Manenzhe et al. (2004) also reported that hydrodistillation of *L. javanica* leaves, flowers and stems produced oil that was poisonous against *Plasmodium falciparum* when diluted to 1% (*v*/*v*). However, in this experiment, the mice were fed aqueous extracts which are unlikely to contain the non-polar essential oils.

Contrary to the acaricidal effects of *L. javanica* found in the current study, reviews by Pascual et al. (2001) and van Wyk (2008) showed that *Lippia* (Verbenaceae) plant extracts are used in the South and Central America, and tropical Africa, mostly for the treatment of gastrointestinal and respiratory disorders and as seasoning. In addition, some of the *Lippia* plant extracts have antimalarial, spasmolitic, sedative, hypotensive, and anti-inflammatory effects (Lukwa et al. 2009; Nzira et al. 2009). They are frequently consumed in tea and coffee beverages and even in energy-boosting herbal products labelled as "caffeine free" (Viljoen et al. 2005). The plant has a strong smell that may repel ticks. Belmain and Stevenson (2001) argued that repellent plants might indeed be preferable because their human toxicity could be lower than pesticidal plants that by their nature are toxic and therefore may be more hazardous to humans and livestock.

The chemistry of *L. javanica* leaf extracts varies dramatically both within and between natural plant populations owing to edaphic and climatic factors (Viljoen et al. 2005). The knowledge about the plant's phytochemistry would help to identify other plant species with similar active ingredients, thereby reducing pressure exerted by continuously harvesting a single plant species (Bizimenyera et al. 2005). Isolation of some of the compounds identified in the aqueous plant extracts is ongoing with the intention of determining the compounds responsible for the acaricidal effects. This information will help to optimise the process of extraction and application for farmers. Cold water may not be an efficient solvent for the extraction of the acaricidal components but without knowing which compounds are active it will not be possible to optimise this. While organic solvents are more effective for the extraction of plant compounds (Eloff 1998; Bizimenyera et al. 2005), their use is impractical for smallholder farmers in Zimbabwe, especially those located in remote parts of the country. Thus new ways of optimising extraction are required such as using hot water and continuous maceration (Martin et al. 2001) and these can only be developed for new species if we know the active components.

The commercialization of pesticidal plants is often limited by availability in natural stands and problems associated with their propagation or harvesting. *L. javanica*, however, is a widespread and abundant plant, flowers all year and is perennial. Since members of the genus including *L. javanica* are herbal medicines (Van Wyk 2008); there has been interest in their propagation and current research on the closely related *L. multiflora* (Ameyaw 2009) and *L. sidoides* (da Costa et al. 2007) indicates considerable scope for large scale propagation of *Lippia* species.

Conclusions

This study showed that leaf aqueous extracts of *L. javanica* have potent acaricidal effects on ticks on cattle. However, the efficacy of the concentrations is influenced by the duration of application. The 10% and 20% aqueous extract concentrations were efficacious throughout the experiment whereas the lower concentration of 5% only became

efficacious from the fifth week of application. Thus the 10% concentration is sufficient for recommendations to farmers because less plant material is required and it is easier to spray since there is less clogging of spray nozzles. The efficacy of the *L. javanica* aqueous extracts illustrates that they can be used as a mitigatory measure in the absence of synthetic acaricides. Future studies could further explore the enhancement of the efficacy of plant extracts by addition of a surfactant. Further investigations have been initiated to identify the compounds responsible for the effects reported in this study. The results will be used to optimise application procedures for farmers.

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