An aqueous extract of *Maerua edulis* (Gilg & Ben) DeWolf tuber is as effective as a commercial synthetic acaricide in controlling ticks on cattle *in vivo*

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Abstract

Farmers in Zimbabwe claim plant that extracts of *Cissus quadrangularis, Aloe vera* and *Maerua edulis* are effective at controlling cattle ticks. On-station experiments were conducted at Henderson Research Station to determine the *in-vivo* efficacy of crude aqueous extracts of *Cissus quadrangularis* (succulent stems), *Aloe vera* (succulent leaves) and *Maerua edulis* (leaves and tuber) at concentrations of 15%, 15% and 10% *w/v* respectively, against cattle ticks. An amitraz-based acaricide and water were used as positive and negative controls respectively. Thirty Mashona steers were allocated to the six treatments in a completely randomised design experiment where each animal was an experimental unit replicated five times. The animals were each sprayed weekly with 5 L of the test or control solutions using a knapsack sprayer after which full body tick counts were recorded every other day for seven weeks. The experiments were conducted between January and February when conditions are optimal for tick development. The *M. edulis* tuber extract was as effective as the amitraz-based commercial acaricide. The other three plants extracts were however, as ineffective as the negative water control. *Maerua edulis* tuber, soapy water-oil extract is effective against cattle ticks and have potential to be developed into an acaricidal product and thus benefit mostly resource-challenged smallholder farmers who cannot afford commercial synthetic acaricides. *In vivo* studies using acaricidal plants are rare.

Keywords: *In-vivo* acaricidal efficacy; Cattle ticks; *Aloe vera; Cissus quadrangularis; Maerua edulis*; ticks and tick-borne diseases

Highlights

- *Maerua edulis* tuber aqueous extracts were as effective as synthetic acaricide
- *Cissus quadrangularis*, *M. edulis* leaves and *Aloe vera* not effective
- *In-vitro* results alone are not conclusive in determining acaricidal efficacy
- *Maerua edulis* tuber extracts can be developed into a commercial product
- There is scope to further investigate efficacy of *M. edulis* leaves

1. Introduction

For a long time, researchers and farmers have grappled with the negative effects of ticks and tick-borne diseases particularly on the African continent (Estrada-Peña and Salman, 2013) . Ticks are important vectors of various parasites including *Ehrlichia (*Cowdria*) ruminatium, Theileria parva* and *Babesia bigemina* (Bissinger and Roe, 2010; Wanzala et al., 2012; Adenubi et al., 2016; Vudriko et al., 2016). These vectors cause diseases that affect cattle productivity and profitability including *Babesiosis* (Red water), *Theileriosis* (January disease), *Cowdriosis* (Heart water) and *Anaplasmosis* (Gall sickness) if not controlled effectively. Effects of ticks are not limited to diseases alone but they also suck blood and can cause ear and teat damage in cattle. Additionally, they can cause tick "worry" and are generally associated with weight loss and reduced productivity of the animals (Kaaya and Knapp, 2003; Estrada-Peña and Salman, 2013).

Economically, tick control programmes can take up a significant proportion of the national fiscus (Taylor, 2001; Kaaya and Knapp, 2003; Bowman et al., 2004; Rajput et al., 2006; Mapholi et al., 2014). Globally, the cost of controlling ticks is estimated to range between 13.9 - 18.7 billion US dollars (Estrada-Peña and Salman, 2013). There are many examples in Africa where tick and tick-borne disease control programmes have used up millions of US dollars from the fiscus (Moyo and Masika, 2009; Leta et al., 2013; Mapholi et al., 2014). It is estimated that the annual cost in US dollars of importing acaricides in different African countries are: Zimbabwe $9.3 million (Perry et al., 1990), Zambia $10 million (Pegram et al., 1988), Kenya $16 million (Tatchell et al., 1986), Tanzania and Uganda $26 million (Kagaruki, 1997; Okello-Onen and Nsumbuga-Mutaka, 1997), and Nigeria $30 million (Dipeolu, 1991). This is not an African problem alone but other countries also suffer the same fate. Brazil's tick control programmes cost approximately 2 billion US dollars in 2000 (Moyo and Masika, 2013). However, current data on the economic impact of ticks and tick-borne diseases are scarce.

Over the years there have been efforts to look for alternative cost-effective tick control remedies like use of ethnoveterinary plants in response to emerging challenges associated with use of the conventional synthetic acaricides (Isman, 1994; Stevenson et al., 2012; Grzywacz et al., 2013; Khan and Damalas, 2015). These practices have been slowly gaining popularity in many parts of the world particularly in the developing countries (Samie et al., 2010). Traditional practices, especially acaricidal plants, are locally available, affordable and mostly environmentally-benign and therefore can offer a viable alternative or complementary remedy to conventional synthetic-based tick control programmes (Njoroge and Bussmann, 2006; Isman, 2008; Pirali-Kheirabadi et al., 2009; Fouche et al., 2016).

Ethnoveterinary studies have provided databases of plants with claimed acaricidal properties globally (Adenubi et al., 2016). In a limited number of cases the efficacy has been examined in *in vitro* studies. In Zimbabwe, several surveys and literature reviews indicate that many plant species have been used with potential acaricidal activities (Marandure, 2016; Maroyi, 2012; Ndhlovu and Masika, 2012; Nyahangare et al., 2015). Despite the wide availability of these plant species, there are no products available on the market largely because the comprehensive documented scientific evidence of their efficacy is lacking. It is therefore critically important to provide scientific evidence of the efficacy and safety of traditionally acclaimed acaricidal plants for the benefit of the livestock industry.

In most cases where efforts have been made to validate the effectiveness of acaricidal plants, research has been limited to *in-vitro* laboratory bioassays at the expense of *in-vivo* trials (Adenubi et al., 2016). This is because *in-vivo* experiments are expensive and logistically challenging to carry out and many institutions do not have facilities to do these experiments (Moyo et al., 2009; Santillán-Velázquez et al., 2013). However, live animal *in-vivo* data are crucial because they provide evidence of the efficacy of the plant extracts under field conditions on the animal.

The other challenge is the lack of clear guidance on the registration process of these products as the current regulatory framework was designed for registration of synthetic pesticides (Sola et al., 2014). In many countries, it is a legal requirement to have evidence of *in-vivo* activity of a particular product and this explains why there are not many plant-based acaricidal products on the formal markets.

In the current study, the acaricidal efficacies of *Cissus quadrangularis* (L) (Vitaceae), *Maerua edulis* (Gilg & Ben) DeWolf (Capparaceae) and *Aloe vera* (Barbadensis Miller) (L.) Burm.f. (Xanthorrhoeaceae) were tested against cattle ticks *in-vivo*. These plants were selected because they were initially identified by farmers and other stakeholders as acaricidal in a survey conducted in semi-arid cattle producing areas of Zimbabwe. In the survey, the most frequently mentioned plants used against cattle ticks across the surveyed districts in descending order were: *C. quadrangularis* (30.1%), *Lippia javanica* (Burm.f.) Spreng. (Verbenaceae) (19.6%), *Psydrax livida* (Hiern) Bridson (Rubiaceae) (14.9%) and *Aloe* sp. (14.9%) (Nyahangare et al., 2015). It was established from farmers that normally, these plants are prepared by crushing and soaking in water overnight and spraying the extract on the animals. *In vitro* preliminary screening of *C. quadrangularis*, *A. vera* and *M. edulis* showed that water extracts of these plants were indeed acaricidal against tick larvae (Chereni, 2014). While *M. edulis* was not ranked highly in the survey, the few respondents who used it, claimed that it was very effectiveness. Literature search confirmed that in Zambia some preliminary *in-vitro* screening of potentially acaricidal plants showed that *M. edulis* water extract was effective against cattle ticks (Kaposhi, 1992). *Lippia javanica* was not included in the current study because earlier studies confirmed *in-vivo* acaricidal activity of the aqueous extracts (Madzimure et al., 2011). The objective of the current study was therefore to confirm farmer claims of acaricidal efficacy of the selected plants while also validating laboratory efficacy findings (Chereni, 2014), under farm conditions.

2. Materials and methods

2.1 Study site

The study was carried out at Henderson Research Station (17° 35′ S, 30° 58′ E) in Mazowe district about 32 km north east of Harare. The station is in natural farming region II which receives an average annual rainfall of 750 to 1000 mm. Peak tick infestation occurs during the wet summer months between January and March and the trial was conducted in January and February of 2016. The most common tick species found in the area include *Rhipicephalus (Boophilus) microplus* Canestrini (Acari: Ixodidae), *Rhipicephalus evertsi evertsi* Neuman (Acari: Ixodidae), *Rhipicephalus appendiculatus* Neuman (Acari: Ixodidae),, *Hyalomma* spp. and *Amblyomma* spp. (Madzimure et al., 2011).

2.2 Plant collection and preparation of treatments

Cissus quadrangularis stems and *M. edulis* leaves and tubers were collected from Chiredzi district about 430 km south-east of Harare, while *A. vera* succulent leaves were collected at Henderson Research Station located about 30 km north of Harare. The plants were positively identified by a qualified botanist, Mr Christopher Chapano and voucher specimens deposited at the National Herbarium and Botanic Gardens of Zimbabwe. The voucher specimen records are: *C. quadrangularis* (Nyahangare E6), *M. edulis* (Nyahangare E5) and *A. vera* (Nyahangare

E37). The leaves and tubers of *M. edulis* and fleshy stems of *C. quadrangularis* and *A. vera,* were separately crushed and mixed with water containing a 1% w/v detergent (green bar soap) for 24 h to create a 25% g/100 ml stock solution. The green bar soap (Sunlight produced by Unilever Pvt Ltd) is widely available in shops in southern Africa and was added to reduce surface tension of water when applied on the animal bodies. The soap was first pulverized and dissolved in 1 L of the stock solution and then added back to the parent solution. After 24 h, each mixture was filtered through a mutton cloth and sufficient water added to yield 10% extracts v/v of *M. edulis* leaves and tubers and 15 % v/v of *C. quadrangularis* and *A. vera*. Vegetable cooking oil (Olivine brand, Olivine Industries Pvt Ltd, Harare, Zimbabwe) was added to each preparation at 2 % w/v. The vegetable oil was used as a low-cost measure of maintaining and preserving the acaricidal properties of the plant extracts and to aid in penetrating the tick cuticle. Olive oil is a better product but is not affordable to the intended beneficiaries of these technologies. The concentrations (10 % and 15 %) were optimal recommendations from earlier laboratory bioassays (Chereni, 2014). The plant-based treatments were compared to a positive control of Triatix® spray (12.5 % EC amitraz-based compound manufactured by Ecomed Manufacturing, Belmont, Zimbabwe for Coopers Zimbabwe Private Ltd), applied at the prescribed (label) dilution rate of 0.2 % v/v (Table 1). The negative control consisted of the surfactant, the vegetable oil and water.

2.3 Experimental animals and design

Thirty Mashona steers of the same age (approximately two years) and raised under the same environmental conditions at Henderson Research Station were used. The steers were randomly allocated to the six treatments with each steer acting as an experimental unit and replicated 5 times in a complete randomized design experiment. Treatments were applied weekly in accordance with the Government of Zimbabwe regulations for summer dipping as opposed to fortnightly for winter dipping (Ndhlovu et al., 2009; Masuku et al., 2015).

Table 1 Summary of experimental treatments

Treatment	Description
1	10% w/v *Maerua edulis* leaves water extract + 1% w/v surfactant + 2% w/v vegetable cooking oil
2	10% w/v *Maerua edulis* tubers water extract + 1% w/v surfactant + 2% w/v vegetable cooking oil
3	15% w/v *Aloe vera* water extract + 1% w/v surfactant + 2% w/v vegetable cooking oil
4	15% w/v *Cissus quadrangularis* water extract + 1% w/v surfactant +2% w/v vegetable cooking oil
5	Water with 1% w/v surfactant and 2% w/v vegetable cooking oil (Negative control)
6	Triatix® (Positive control; 12.5 % EC amitraz-based compound 0.2 % v/v

The surfactant was a commercial washing solid soap pulverized and dissolved in 1 L of the stock solution and then added back to the parent solution. The vegetable oil was a commercial brand.

2.4 Experimental procedure

Prior to commencement of the experiment, the cattle were exposed to natural tick infestation for 14 days (adaptation period) after which they were subjected to the treatments. During treatments, each animal was restrained in a cattle-crush and a full body tick count for different species made and recorded. After counting, each animal was sprayed with 5 L test extract using a knapsack sprayer. The ticks on the animals were counted every other day thereafter for seven weeks from February to March (duration of the trial). The treatments were applied weekly after counting the ticks. Animals in different treatments were kept in separate paddocks throughout the experiment to avoid cross contamination. All animals were closely monitored throughout the experiment for any signs of tick-borne and other diseases by a veterinary practitioner at Mazowe Veterinary College, located approximately 1 km north of Henderson Research Station, Zimbabwe.

2.5 Data analysis

The efficacy ratio per animal was calculated from the daily tick counts using the formula adapted from O'Neill (2006).

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

The repeated measures analysis of variance the mean efficacy ratios was conducted using the GLM procedures of SAS version 9. 3.1 (SAS, 2006) using the following statistical model:

Yijk = μ + Ti + Wj + Ti × Wj + eijk

Where:

- Yijk is the response variable (tick count efficacy ratio)
- μ is the overall population mean
- Ti is the fixed effect of the i^{th} treatment (i=treatment (1,...5)
- Wj is the fixed effect of time post-treatment application (j=weeks 1,...7)
- Ti × Wj is the interaction between time post-treatment application and treatment
- eijk is the residual error

3. Results

3.1 Acaricidal efficacy ratios

Maerua edulis tuber aqueous extract was the only plant-based treatment with high efficacy ratios against cattle ticks with a mean overall efficacy ratio of 80% over the seven weeks of study (Figure 1). There was no statistically significant difference ($p > 0.05$) in efficacy between the tuber extract and the positive control (Triatix®) throughout the experimental period. In the seven weeks, the tuber extracts had efficacy ratios of greater than 50 % (Fig. 1). *Maerua edulis* leaves, *A. vera* and *C. quadrangularis* treatments were not effective in reducing tick loads and were not significantly different from the negative control (water) ($p > 0.05$).

The weekly overall mean efficacy ratios of treatments between sprayings and after spraying are presented in Table 2. *M. edulis* tuber was the only plant based treatment that was significantly effective against cattle ticks.

3.2 The variation of total tick populations over time

There was both a time and time*treatment effect on total tick population ($p<0.05$). In the first week there was a general increase in the number of ticks for both treatments although the degree of increase differed among treatments. The least increase was on Triatix® (positive control), and the *M. edulis* tuber treatments (Fig 2), while in the other plant-based treatments (*C. quadrangularis*, *A vera* and *M. edulis* leaf) there was a higher record of ticks on the animals. The negative control of water had the highest record of ticks. However, over time, the total tick population across the treatments was reduced significantly.

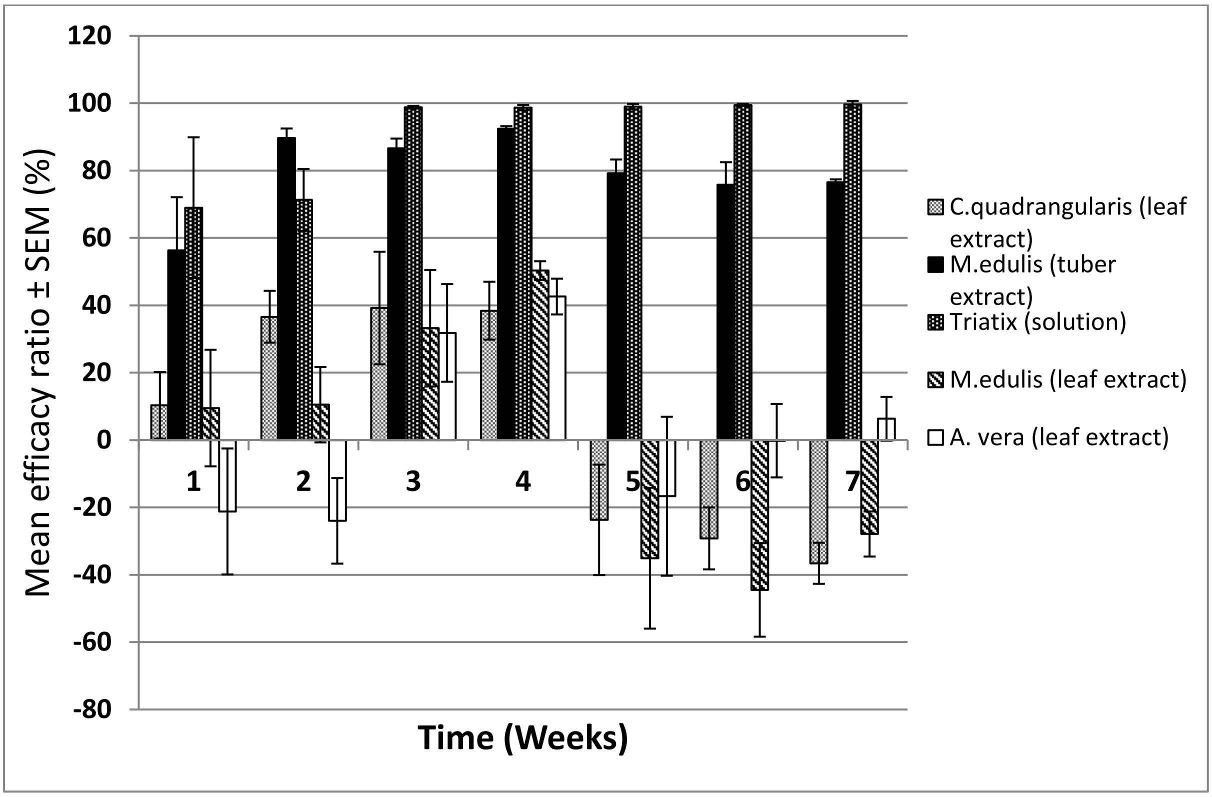

Figure 1: Mean weekly efficacy ratios of treatments against cattle ticks over 7 weeks compared to negative control water (February-March 2016) (*n = 5*)

Table 2: Least square mean tick mortality ratios (%) for treatments against counting time within weeks (*n = 7*)

Treatment	Count 0	Count 1	Count 2	Count 3
M. edulis (leaf)	-14.0[a]	-25.5[a]	-39.0[a]	-15.5[a]
M. edulis (tuber)	71.5[b]	76.7[b]	71.5[b]	69.4[b]
Triatix®	82.5[b]	94.7[b]	89.1[b]	91.2[b]
A. vera	-40.4[a]	-6.4[a]	-36.1[a]	-13.4[a]
C. quadrangularis	-43.9[a]	-27.1[a]	-39.5[a]	-30.1[a]
±SEM	17.7	11.4	14.9	11.6

Within the column means with different superscripts are significantly different ($p < 0.05$).

SEM = standard error of mean

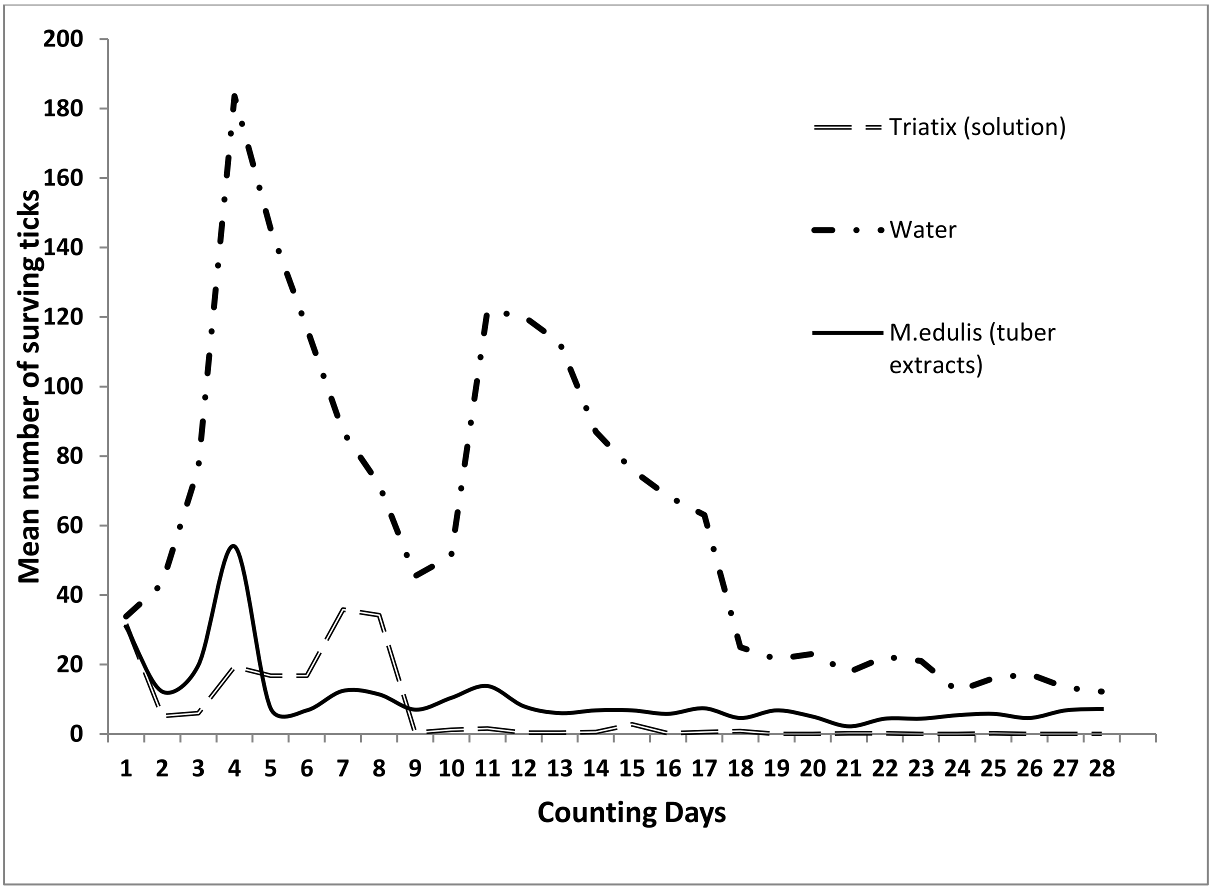

Figure 2: Mean overall ticks surviving in the *Maerua edulis* tuber extract compared to negative and positive treatments over 7 weeks from February to March 2016 (*n = 5*)

3.3 Engorged and tick species identified

In the *M. edulis* leaves, *C. quadrangularis, A. vera* extracts and water treatments, there was a high number of engorged ticks. Only a few engorged ticks were found in the *M. edulis* tuber treatment and the positive control Triatix® (Fig 3). The largest population of ticks for all treatments was of brown ear tick, *R. appendiculatus* followed by blue ticks, *Boophilus decoloratus* (Koch) (Acari: Ixodidae). There were no Bont tick *(Ambylomma* spp.*)* recorded throughout the experiments and very few Red-legged, *R. evertsi* as well as Bont legged (*Hyalomma* spp.) were recorded including a species which was classified under other ticks. There was a slight increase in the numbers of Red-legged ticks in contrast to Brown ear ticks towards the end of the experiments although the numbers were still lower than for Brown ear ticks.

4. Discussion

The *M. edulis* tuber treatments showed high efficacy comparable to the synthetic acaricide (Triatix®). Similar results were reported of the same extract but against tick larvae in the laboratory (Simuunza et al., 2011). The mean tick count for the tuber extract treatment was 9.8 ticks per animal which implies that the animals were not tick infested according to Zimbabwe Veterinary standards which stipulate that when more than 10% of the animals have 10 or more live ticks, they are considered tick infested (Madzimure et al., 2011)

The acaricidal efficacy observed in the *M.* edulis tuber extracts could have been caused by the presence of linear chain unsaturated fatty acids which were found to be the main active ingredients upon preliminary phytochemical analysis (Luo et al., 2011). In the current study, water extract of *M. edulis* leaves was not effective in reducing tick numbers which contradicts

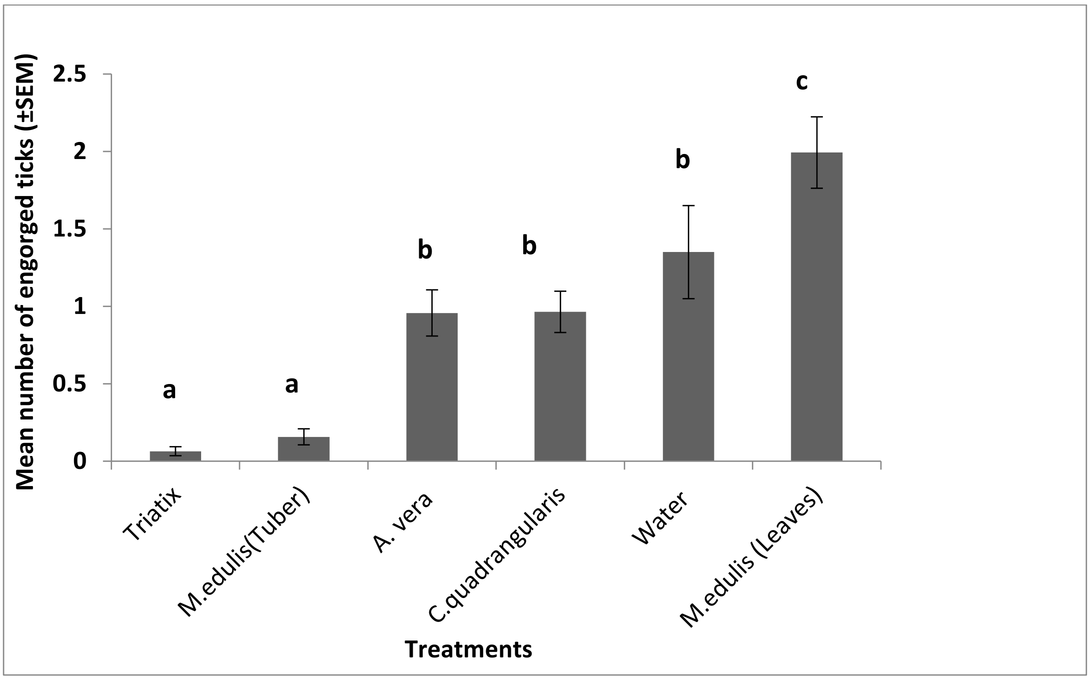

Figure 3: Average number of engorged ticks in the different treatments over 7 weeks (*n = 5*).
Treatment means with different letters (a, b & c) are significantly different

preliminary *in-vitro* bioassays results on tick larvae (Chereni, 2014) and strong perceptions expressed by farmers on the effectiveness of the leaf extracts against pests (Stathers et al., 2002; Nyahangare et al., 2015). However, literature on the chemistry of this plant is scant despite its potential in animal and human health. Some members of the Capparaceae family, including *Marua angolensis* DC. were found to have anthelmintic characteristics against the parasitic nematode *Haemonchus contortus* (Rudolphi) Cobb (Strongylida: Trichostrongylidae) and the activity was ascribed to presence of kaempferol-based and quercetin-based flavonols (Mengistu et al., 2017).

Studies have shown that water is a very poor extractant for antimicrobial and antiparasitic compounds from plants compared to acetone and other intermediate polarity solvents (Eloff, 1988; Kotze and Eloff, 2002; Zorloni et al., 2010). In the current experiments we focused on using an extractant that could be easily available to rural farmers and could deliver active extracts. To increase the solubility of relatively non-polar compounds, soap and oil was added to the water. Perhaps using organic solvents can increase the efficacy of the *M. edulis* leaves because it is known that water has limited extraction properties. Solvents like acetone, which extract a wide array of active compounds and are less toxic to the ticks, may possibly lead to better results (Eloff, 1998; Zorloni et al., 2010). However, acetone cannot possibly be used to treat animals for tick control.

The increase in efficacy in the tuber extracts from week 1 to week 5 could be due to residual effects. It is an indication that apart from the dose-dependency characteristics evident with most conventional acaricides, residual effects may also influence efficacy with increase in application over time. Such a phenomenon is very desirable in the use of acaricidal plants and has been previously reported in another acaricidal plant, *L. javanica.* At the 5% w/v concentration, there was a significant increase in efficacy over time with subsequent applications (Madzimure et al., 2011).

Aloe vera, *M. edulis* (leaf) and *C. quadrangularis* water extracts had no significant acaricidal activity against cattle ticks. This is despite the fact that these plants had been ranked very highly by rural farmers in Zimbabwe in a survey by Nyahangare et al. (2015). In Chiredzi district of Zimbabwe, respondents call *C. quadrangularis "Chiololo"* in their language of Kalanga which loosely translates to "deadly effective" in English. The variance in activity may possibly be explained after a phytochemical analysis to identify the different active components in the water extracts. It has been reported before that *C. quadrangularis* contains a number of bioactive compounds which include alkaloids, resveratrol, piceatannol, pallidol, parthenocissin, quadrangularins, ascorbic acid, carotene, phytosterol substances, calcium, flavonoids, vitamins, enzymes, nicotinic acid, tyrosin and triterpenoids (Joseph and Raj, 2011; Mishra et al., 2010; Rao and Annamalai, 2011). Because of this rich mix of compounds this plant has been reported to have antioxidant (Jainu and Devi, 2005), antibacterial (Murthy et al., 2003), antiosteporosis (Shirwaikar et al., 2003), anti-tumour (Opoku et al., 2000) among other medicinal properties. If reports of acaricidal properties could be confirmed, this will make this plant very special. One of the few reports on activity of water extracts of *C. quadrangularis* against ticks shows that silver nanoparticles made from its stem showed positive results against *R. (B.) microplus* tick larvae and *Hippobosca maculata* L. (Diptera, Hippoboscidae) compared to water extracts (Santhoshkumar et al., 2012). The activity may be caused by the silver nanoparticles and not by a compound from the plant. On the other hand, *A. vera* was shown to be moderately acaricidal against ticks, fleas and mosquitos in *in vitro* experiments in addition to its anti-coccidiosis activity (Urch, 1999; Chereni, 2014). The negative results obtained with *M. edulis leaves, A. vera* and *C. quadrangularis* extracts in this study are contrary to the results from laboratory trials by Chereni (2014). This questions the validity of laboratory results without confirming the activity in animal studies. According to Lim (2011), factors such as temperature, sunlight, as well as air properties could contribute to plant loss of pesticidal efficacy. If the compounds responsible for activity in *in vitro* studies with *A. vera* and *C. quadrangularis* extracts are thermolabile or susceptible to photooxidation and the compounds in *M. edulis* tuber extracts are not sensitive, it could explain the results obtained. Therefore, further research need to be conducted to establish the exact cause for loss of acaricidal properties when the same extracts are tested *in-vivo*.

It is disappointing that the *M. edulis* leaf water extract had no activity against the ticks because the sustainable use of *M. edulis* tuber extracts to protect animals against ticks is questionable. Water is a poor solvent compared to other extractants, especially acetone, in determining antimicrobial and antiparasitic activities (Eloff, 1988; Kotzé et al., 2002). Therefore, there is potential to optimise the extraction from both *M. edulis* leaves and tubers using organic solvents.

The general decline in total tick count over time is not unexpected because tick populations naturally decline as the summer season progresses and rainfall and temperatures decrease because ticks prefer warm and wet conditions (Muchenje et al., 2008). This is why in Zimbabwe dipping is done on a weekly basis in summer and fortnightly in winter in the normal tick management systems. This balance may be changing because of the changing climatic and weather patterns (Collier et al., 2008; Estrada-Peña and Salman, 2013).

There was no change in tick species that are commonly found at the research station with *B. decoloratus* (blue tick), *R. appendiculatus* (brown ear tick), *Hyalomma* species and *R. evertsi evertsi* (red legged tick) found during the trial. All previous trials at the station recorded the same tick species (Madzimure et al., 2011; 2013).

The high number of engorged ticks which were present on *A. vera*, *M. edulis* leaves and *C. quadrangularis* treatments and the negative control, is an indication that ticks were not adversely affected and thus would drop off naturally after being fully engorged so that they would lay eggs (Madzimure et al., 2011). On the contrary, in the positive control and the *M. edulis* tuber treatment, there were fewer engorged ticks recorded on the cattle showing a possible negative effect on the reproductive activities of the ticks.

5. Conclusion

This study showed that *M. edulis* tuber water extracts containing a surfactant and vegetable oil can effectively reduce tick populations on cattle and can be used as an alternative to synthetic acaricides in controlling cattle ticks. *Cissus quadrangularis, A. vera* and *M. edulis* leaf water extracts were not significantly effective against cattle ticks compared to the negative control.

Further investigations are required to compare the *in vivo* efficacy of different concentrations of the tuber extracts. Identification of the anti-tick compounds in the tuber extract could provide useful information and may lead to the identification of a new acaricidal compound. Issues such as optimisation of the extraction, health and safety, propagation and sustainable harvesting should also be investigated. It may be very interesting to investigate why our results differed from the traditional use and from laboratory results. There is also need to determine the possible effects of the harvesting timing on the chemical composition and acaricidal activity of both the *M. edulis* leaves and tuber. It could be one of the reasons causing the differential acaricidal activity that was observed.

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