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Research Article

The evaluation of *in vitro* repellency and acaricidal efficacy of *Aloe ferox* and *Acokanthera oppositifolia* crude extracts against *Amblyomma hebraeum* ticks

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Abstract

The objective of the study was to examine how solvent extracts derived from *Aloe ferox* and *Acokanthera oppositifolia* leaves influenced the repellency and acaricidal activity against adult engorged female *Ambylomma hebraeum* ticks. The researchers analyzed the acetone, methanol, and ethanol extracts of both plant species. The experiment followed a completely randomized design with a factor arrangement of 2 (plant species), 3 (organic solvent extraction methods: acetone, methanol, ethanol), and 3 (concentration levels: 15%, 30%, 50%). Distilled water and Dazzel dip (15% and 30%) were employed as negative and positive controls, respectively. Repellency was assessed over a six-hour period, while acaricidal activity was measured over seven days. The results of the study demonstrated that the solvent extracts of *Aloe ferox* and *Acokanthera oppositifolia* exhibited a repellent effect ranging from 6% to 89%. Acetone extracts, overall, displayed lower repellency activity compared to methanol and ethanol extracts, with percentages of 58%, 66.5%, and 80.5% for acetone, ethanol, and methanol, respectively. At a concentration of 15% for all solvents, the repellency effect was observed to be 14% for acetone, 9.5% for ethanol, and 11% for methanol. Irrespective of the solvent extraction method used, a higher repellency activity was observed at the 50% concentration level, with statistical significance ($P < 0.05$). The acaricidal activity of *Aloe ferox* and *Acokanthera oppositifolia* significantly increased with the concentration of the extracts. It was concluded that *Aloe ferox* and *Acokanthera oppositifolia* plants possess repellent and acaricidal activities, particularly at a 50% concentration for acetone and methanol extracts, respectively. Additionally, *Acokanthera oppositifolia* demonstrated a higher repellency activity, while *Aloe ferox* exhibited stronger acaricidal activity.

Keywords: Ethno-Veterinary; Medicinal Plants; Herbal Remedies; Repellency; Ticks; Tick Resistance

Introduction

Ticks are highly prevalent external parasites of ruminants in tropical and subtropical regions [1,2]. These blood-feeding arthropods have the ability to transmit various animal and human disease pathogens [2]. One example is Amblyomma hebraeum, a hard tick species responsible for heartwater and African tick-bite fever [1,3,4,5]. Ticks of the Amblyomma and Hyalomma genera are known as hunter ticks, as they remain on the ground until suitable hosts approach their vicinity [6]. They then emerge and crawl

towards the host, exhibiting different hunting strategies depending on their life stage [7]. Amblyomma ticks can either be ambushers (larvae) or hunters (nymphs and adults) and infest a wide range of mammals, birds, and reptiles [6]. These ticks pose significant challenges to animal health.

The current approach to tick control relies heavily on the routine use of synthetic chemicals, which is expensive and often inaccessible to small-scale farmers in sub-Saharan Africa. Moreover, the repeated use and overuse of these chemicals have led to the development of parasite resistance [8]. Therefore, there is an urgent need for cost-effective, environmentally friendly alternatives with different modes of action [2]. Synthetic pyrethroids, in particular, have exhibited high and widespread resistance among ectoparasiticides globally (resistance factor > 100) [2]. *Amblyomma* species have also shown resistance to synthetic pyrethroids and organophosphates [9]. Consequently, there is a demand for the development of management strategies to minimize the development of drug resistance in parasites [10-13].

One potential alternative is the utilization of plant-derived products that possess repellent and acaricidal properties [14-16]. Medicinal plants with acaricidal activities offer significant potential as they are biodegradable and less harmful to the environment [18]. Natural products often contain multiple active compounds, potentially slowing down the development of resistance [2]. Some resource-limited farmers in the Eastern Cape Province of South Africa have already been using *Aloe ferox* and *Acokanthera oppositifolia* for parasite control [19]. *Aloe ferox*, commonly known as bitter aloe, belong to the *Asphodelaceae* family. It is woody and indigenous to Southern Africa, single stemmed with thick-fleshy spined leaves borne on candelabra inflorescences, which bear up to eight very dense, cylindrical and symmetrical racemes. Several *Aloe* species are used to make purgative medication, and also yields a non-bitter gel that can be used in cosmetics. *Acokanthera oppositifolia,* commonly known as bushman poison is an evergreen shrub used as the source of an arrow poison. The shrub contain toxic cardiac glycosides (white latex) strong enough to cause death. It is widespread in Southern and Central Africa. All parts of the plant are toxic (especially seed) except ripe fruit that are edible, however unripe fruit are still poisonous.

While there have been a few studies on *Aloe ferox*, no research has been conducted on *Acokanthera oppositifolia* to date. Additionally, ethanol has been the most commonly used solvent, and *Rhipicephalus (Boophilus) microplus* has been the focus of many tick-related studies [20]. Hence, it remains uncertain whether the findings from experiments conducted on one-host ticks can be extrapolated to other tick species. The detrimental effects and the cost of chemical acaricides have led to the need to investigate alternative eco-friendly methods of tick control such as the use of natural products. Therefore, this study aimed to investigate the acaricidal and repellent effects of solvent extracts from *Aloe ferox* and *Acokanthera oppositifolia* in the control of *Amblyomma hebraeum* ticks.

Materials and Methods

Plant material preparation

Fresh leaves of *Al. ferox* and *Ac. oppositifolia*, were collected in Chris Hani District Municipality (Kwezi village) in Eastern Cape Province, South Africa. Plant materials were identified by a botanist, and the specimens have been deposited in the University of Fort Hare herbarium, *Al. ferox* (MSAN01/2015), *Ac. oppositifolia* (MSAN04/2015). The leaves from *Al. ferox* were cut into small pieces, air-dried for 10 weeks under ambient temperature and then milled into powder using a grinder through a 1mm sieve. The leaves from *Ac. oppositifolia* were oven dried at 60°C over night to a constant weight before grinding. The powder (100g) was soaked for 3 days in three solvents; acetone (99%), methanol (70%) and ethanol (70%) of increasing polarities (15, 30 and 50%). Analytic grade acetone, methanol and ethanol, were obtained from the Botany Department, University of Fort Hare and, supplied by Laboratory Equipment Supplies, Johannesburg, South Africa. Extractions were done according to Nong., *et al*., [22]. Briefly, 75g of plant powder was mixed with 750 ml of the solvent and this was done twice, thoroughly mixed on an orbital shaker for 24 hours and then filtered through Whitman No. 1 filter paper using a Buchner funnel. The material was then transferred into a round-bottomed flask and concentrated by evaporation using a rotary evaporation apparatus(40°C). The condensed extracts were left to dry in a perforated chamber. Finally, they were stored in tightly closed sterile containers and kept in the refrigerator until use. The extraction yields are shown in table 1.

Plant species	Level of extraction	Acetone		Ethanol		Methanol	
		RPM (g)	Yield (g)	RPM	Yield	RPM	Yield
Aloe ferox	1st extraction	75.00	1.15	75.00	1.86	75.00	2.70
	2nd extraction	75.00	2.06	75.00	2.33	75.00	2.31
	Total	150	3.21	150	4.19	150	5.01
Acokanthera oppositifolia	1st extraction	75.00	4.83	75.00	5.82	75.00	8.50
	2nd extraction	75.00	4.92	75.00	6.16	75.00	8.86
	Total	150	9.75	150	11.98	150	17.36

Table 1: Weights of raw plant material and extraction yields of *Aloe ferox* and *Acokanthera oppositifolia* from Chris Hani District, Eastern Cape Province, South Africa.

RPM: Raw Plant Material

Ticks

Fully engorged females of *A. hebraeum* were collected manually from naturally grazing cattle kept at the University of Fort Hare Farm. The repellency method described by Thorsell., *et al.* [23], was used in the bioassay study. Solvent extracts (15, 30 and 50%) from the two plant species were applied at the edge of two filter papers, air-dried for 88 minutes and placed in a petri dish with an inner diameter of 9.5cm. Dazzel dip (Diazinon), at two levels: 15% and 30% was used as a positive control while distilled cold water was used as negative control. Evidence from prior research [14,16,22,24-26]. indicated that solvents do not pose any acaricidal activity hence in this study they were not included as negative control treatments. A total of six adult ticks were introduced in each petri dish and were observed at 30mins, 1, 2, 3, 4, 5 and 6hrs intervals according to Thorsell., *et al.* [23]. If the ticks avoided the treated area, it meant that they had been repelled and if they continued their motion beyond the treated area then they were considered non-repelled. The number of ticks avoiding the area on each occasion was recorded. The average repellency was calculated from the values obtained in the three replicates, using the formula by Thorsell., *et al.* [23].

$$R = p/n \times 100\%$$

where R= repellency; p = number of ticks avoiding the treated area; n= total number of ticks placed on the filter paper

Contact bioassay

The dipping method described by Pirali-Kheirabadi., *et al.* [27], was used. Briefly, 10 ticks were immersed in specific test tubes with treatments for a minute, removed and placed in a 9.5cm petri dish and covered with a lid. The ticks were incubated at 25°C and relative humidity of 85% [21]. The percentage mortality was recorded after every 24 hours for seven days [28]. At the end of 24 hours motionless, and ticks which were incapable of moving, coordinating legs or showing any signs of life were considered dead [17,28]. Live ticks were considered to exhibit normal behaviour when physically moved by a stick. The number of dead ticks was recorded and tick mortality was calculated using the formula by Chungsamarnyart., *et al.* [29]:

$$\text{Corrected mortality (\%)} = (1-{}^{T}/_{C)} \times 100\ \%$$

where

T = Number of ticks alive after being exposed to test material

C = Number of ticks in the control (distilled water).

Statistical analysis

The data were tested for normality and were not transformed. The collected data on repellency bioassay were analyzed using PROC GLM for repeated measures (SAS, 2003). Data on contact bioassay were analyzed using PROC GLM of SAS version 9.1 (2003). Turkey HSD test was used to compare differences between treatment means. A probability value of less than 5% was used to denote a significant difference. The statistical model used for this analysis was as follows:

$$Y_{ijkl} = \mu + T_i + C_j + X_k + (T_i \times C_j) + (T_i \times X_{k)} + (C_j \times X_k) + (T_i \times C_j \times X_k) + E_{ijkl}$$

Y_{ijkl} = response effect due to treatment (mortality and repellency)

μ = overall mean

T_i = effect due to treatment (i; *Al. ferox* and *Ac. oppositifolia)*

$C_{j=}$ effect due to concentration (j; 15, 30, 50%)

Xk = effect due to extract (k; acetone, methanol, ethanol)

(Ti x Cj) = interaction between treatment and concentration

(Ti x Xk) = interaction between treatment and extract

(Cj x Xk) = interaction between concentration and extract

(Pi x Cj x Xk) = interaction between treatment, concentration and extract

E_{ijkl} = random error

Results

In vitro repellency bioassay

Tick repellency increased (P < 0.05) with increasing concentrations of acetone, methanol and ethanol extracts (Table 2) for both plant species. Overall tick repellency decreased (P < 0.05) with time for all treatments and solvents. The highest tick repellency was observed at 50% concentrations in *Al. ferox* acetone extract. Zero to 25% repellency was observed at 15% solvent 139 extraction for both plant species. No repellency was observed for distilled water. Generally, there was a decrease in tick repellency with time in acetone extract for both plant species Diazinon at 15% had the same repellency activity as the 50% acetone extract of *Al. ferox* and 50% ethanol and methanol extracts of *Ac. oppositifolia*. Methanol extracted *Ac. oppositifolia* showed a consistent repellence activity over time with the highest repellence values at 50% concentration. Methanol extraction of *Al. ferox* showed repellency effects on the lower side of the scale (P < 0.05); 50% and below. The repellency effects of the positive control also decreased with time, however at 30% concentration the effects were maintained above average.

						Repellency %			
Treatment (T)	**Extract (X)**	**Concen-tration (C)**			**Time**				
			30mins	**1hr**	**2hr**	**3hr**	**4hr**	**5hr**	**6hr**
Al.ferox	Acetone	15	22.0 ± 1.53^{g}	15.0 ± 1.509^{h}	6.00 ± 1.29^{i}	Nil	Nil	Nil	Nil
		30	67.0 ± 1.49^{d}	63.0 ± 1.513^{c}	46.0 ± 1.29^{e}	61.0 ± 1.34^{d}	39.0 ± 1.501^{f}	46.0 ± 1.66^{e}	50.0 ± 1.23^{e}
		50	85.3 ± 1.91^{b}	81.7 ± 1.81^{b}	63.0 ± 1.92^{c}	65.0 ± 1.88^{c}	65.0 ± 0.97^{c}	63.0 ± 1.75^{c}	61.0 ± 1.84^{d}
	Ethanol	15	13.0 ± 2.93^{h}	6.0 ± 1.49^{i}	Nil	Nil	Nil	Nil	Nil
		30	46.0 ± 1.98^{e}	40.7 ± 1.30^{e}	46.0 ± 1.83^{e}	42.3 ± 1.97^{f}	39.0 ± 0.97^{f}	44.0 ± 1.53^{e}	44.0 ± 1.32^{f}
		50	65.0 ± 1.58^{d}	67.0 ± 1.32^{c}	67.0 ± 1.54^{b}	61.0 ± 1.48^{d}	65.0 ± 1.61^{c}	63.0 ± 1.38^{c}	67.0 ± 1.32^{c}
	Methanol	15	Nil	Nil	Nil	Nil	Nil	Nil	Nil
		30	13.0 ± 1.49^{h}	17.0 ± 1.54^{g}	15.0 ± 1.50^{h}	20.3 ± 1.58^{h}	26. ± 1.320^{g}	24.0 ± 1.66^{f}	31.3 ± 1.29^{g}
		50	31.3 ± 1.57^{f}	28.0 ± 1.30^{f}	37.0 ± 1.66^{f}	35.0 ± 1.58^{g}	42.3 ± 2.13^{f}	48.0 ± 1.91^{e}	50.0 ± 1.25^{e}
Ac.oppositifolia	Acetone	15	Nil	Nil	Nil	Nil	Nil	Nil	Nil

		30	44.0 ± 1.54^{e}	40.7 ± 1.38^{e}	28.0 ± 1.91^{g}	35.3 ± 1.41^{g}	37.0 ± 0.97^{f}	20.7 ± 1.32^{f}	17.0 ± 1.56^{h}
		50	65.0 ± 1.94^{d}	65.0 ± 1.48^{c}	61.0 ± 1.65^{c}	50.0 ± 1.66^{e}	50.0 ± 0.98^{e}	57.3 ± 1.55^{d}	61.0 ± 1.32^{d}
	Ethanol	15	6.0 ± 0.19^{i}	Nil	Nil	Nil	Nil	Nil	Nil
		30	48.0 ± 1.32^{e}	50.0 ± 1.30^{d}	57.3 ± 1.35^{d}	63.0 ± 1.32^{d}	61.0 ± 1.19^{d}	63.0 ± 1.45^{c}	67.0 ± 1.13^{c}
		50	85.0 ± 1.23^{b}	79.7 ± 1.42^{b}	67.0 ± 1.49^{c}	67.0 ± 1.51^{b}	67.0 ± 0.91^{d}	67.0 ± 1.43^{c}	67.0 ± 1.38^{c}
	Methanol	15	11.0 ± 1.11^{i}	11.0 ± 1.23^{i}	Nil	Nil	Nil	Nil	Nil
		30	78.0 ± 1.41^{c}	78.0 ± 1.30^{b}	67.0 ± 1.32^{c}	65.0 ± 1.21^{c}	67.0 ± 0.87^{d}	67.0 ± 1.26^{c}	61.0 ± 1.30^{d}
		50	83.0 ± 1.34^{b}	89.0 ± 1.32^{a}	83.0 ± 1.34^{a}	83.0 ± 1.31^{a}	83.0 ± 1.32^{a}	83.0 ± 0.95^{a}	83.0 ± 0.98^{a}
Dazzel		15	83.0 ± 1.05^{b}	83.0 ± 1.51^{b}	83.0 ± 1.03^{a}	78.0 ± 2.13^{a}	67.0 ± 2.10^{d}	65.0 ± 2.31^{c}	55.7 ± 1.98^{e}
		30	100.00^{a}	83.00^{b}	83.00^{a}	78.00^{a}	78.00^{b}	78.00^{b}	78.00^{b}

Table 2: Least square means ± standard error showing tick repellency (%) of *Am. hebraeum* treated with extracts of *Al. ferox, Ac. oppositifolia* and Dazzel dip at different concentrations.

^{a,b,c,d,e,f,g,h,i} column means with different superscripts are significantly different at,P < 0.05. Nil = zero repellency

Contact bioassay

Acaricidal activity of *Al. ferox* and *Ac. oppositifolia* increased (P < 0.05) with increasing solvent extract concentration (Table 3). Generally, *Al. ferox* showed higher acaricidal activity compared to *Ac. oppositifolia* across all solvent levels. There was an interaction (P < 0.05) between treatments and solvent extraction levels. The highest tick mortality of 100% in *Al. ferox* extract was observed at 30 and 50% acetone extract while 50% ethanol extract of *Ac. oppositifolia* had the highest tick mortality of 83.33%. The tick mortality was similar (P > 0.05) to the positive control (Diaznon) when *Al. forex* was extracted using acetate at 30 and 50% levels. There was no mortality observed in distilled water. *Al. ferox* exhibited better (P < 0.05) contact activity compared to *Ac. oppositifolia* at the same concentration and solvent extract.

Treatments (T) ***Al. ferox***	**Extract (X)**	**Concentration**		
		15	**30**	**50**
	Acetate	83.3 ± 0.413^{bx}	100.0 ± 0.514^{ax}	100.0 ± 0.33^{ax}
	Methanol	63.3 ± 0.271^{by}	73.3 ± 0.299^{ay}	73.3 ± 0.58^{ay}
	Ethanol	83.3 ± 2.98^{ax}	70.0 ± 3.12^{cy}	80.0 ± 1.53^{bxy}
Ac. oppositifolia	Acetate	16.7 ± 0.91^{cz}	31.7 ± 0.69^{bz}	68.3 ± 0.71^{ay}
	Methanol	43.3 ± 0.559^{cy}	66.7 ± 0.337^{ay}	63.3 ± 0.53^{by}
	Ethanol	20.0 ± 0.12^{cz}	53.3 ± 0.14^{by}	73.3 ± 0.13^{ay}
Dazzel dip		100.0 ± 0.46^{ax}	100.0 ± 0.38^{ax}	Nil

Table 3: Least square means ± standard error showing mortality of *Am. hebraeum* ticks exposed to crude extracts of *Al. ferox, Ac. oppositifolia* and Dazzel dip at different concentrations.

^{abc} row means with different superscripts are significantly different at, P < 0.05; ^{xyz} column means with different superscripts are significant at P < 0.05.

Discussion

Repellency activity of plants

Several plant species have shown acaricidal and repellent properties against ticks [30]. Among them, the acetone extract of *Al. ferox* exhibited the highest tick repellency, although the protection it provided was short-lived [48]. This finding is consistent with a study by Wanzala [28], who found that acetone extracts of *Al. ferox* at a 30% concentration showed the highest tick repellency against *Rhipicephalus appendiculatus* ticks compared to methanol and dichloromethane. However, our results contradict the findings of Fourie., *et al*. [31], who observed no tick-killing effect when powdered *Al. ferox* juice was tested on *Rhipicephalus decoloratus* ticks on dogs. Makwarela., *et al.* [32] also reported no tick repellency from *Al. ferox* at 20% and 40% concentrations when water was used as the solvent. The discrepancy in tick repellency could be attributed to the different solvents used, as the choice of solvent and extraction method affects the extraction of active compounds [11,33]. The repellent activities of plants are often attributed to volatile hydrocarbons, particularly sesquiterpenes and monoterpenes [12,34-36]. The short duration of activity observed in acetone extracts may be due to the high volatility of the volatile hydrocarbons [11,15,37], which are effective for only a brief period. Hence, their high volatility limits their effectiveness in providing prolonged protection against ticks. Both *Al. ferox* and *Ac. oppositifolia*, regardless of the extraction method or concentration level, exhibited repellent activity, which could be attributed to their pungent odour that repels ticks. The repellency of *Al. ferox* is likely attributed to its nonpolar compounds, and ticks require a certain degree of volatility to sense their presence. While repellency is often attributed to a specific compound in a plant, it has been reported that compounds can work synergistically, enhancing the bioactivity of the plant. Interestingly, methanol extraction of *Ac. oppositifolia* demonstrated higher repellency activity against ticks over time compared to *Al. ferox*, which was unexpected. This difference may be due to the lower presence of polar compounds like terpenes in *Ac. oppositifolia*. Terpenes, which are natural products found in microorganisms, plants, and animals, are known for their strong biological activities against parasites [38]. *Ac. oppositifolia* contains oxygenated hemiterpenes that produce a strong scent with defence mechanisms against parasites, potentially contributing to its repellency activity [39]. On the other hand, terpinene-4-ol, present in *Al. ferox*, is used as an insect repellent against ticks and mites [40], suggesting that it may play a role in the observed repellency effect. Limited information is available regarding the use of *Ac. oppositifolia* against ticks, but previous studies have reported its use in the treatment of tapeworms and anthrax [41].

Acaricidal activity of plants

The 30% and 50% acetone extracts, as well as the 50% ethanol extract of *Al. ferox*, exhibited the highest mortality rate among ticks [53]. Previous research [42] has identified the presence of terpenes in *Al. ferox*, with oxygenated sesquiterpenes being the most abundant, followed by oxygenated hemiterpenes. These sesquiterpenes have been reported to act as phytoalexins, directly defending against pathogens [33,40]. *Aloe ferox* has been known to contain pharmacologically active substances [10,14]. and has been utilized in treating heartwater, gall sickness, sheep scab, and controlling ticks in cattle [19]. The remarkable tick-killing potential of *Al. ferox* observed in this study is noteworthy. Mawela [43] reported that tick mortality is attributed to the action of anthranoids and anthraglycosides present in *Al. ferox* leaves. Additionally, *Al. ferox* has demonstrated anti-inflammatory effects due to the presence of three malic acid-acylated carbohydrates [44,45]. Therefore, besides its acaricidal properties, it can also be employed to alleviate inflammation and pain in livestock caused by tick infestations (known as tick worry).

Conclusion

Both *Aloe ferox* and *Acokanthera oppositifolia* plants exhibit repellent and acaricidal activities. The 50% methanol extract of *Acokanthera oppositifolia* and the acetone extract of *Aloe ferox* demonstrated the highest repellency effects. *Acokanthera oppositifolia* exhibited greater repellency activity, while *Aloe ferox* showed stronger acaricidal activity. It is recommended to conduct further investigations to assess the safety of these plants before considering their use for commercial purposes. Despite their effectiveness in various cases, plant extracts have not yet been widely applied in industries. Nevertheless, our findings provide valuable insights into the potential application of terpenoids as effective components in acaricidal and repellent formulations.

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