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Repellency activity of *Commiphora swynnertonii* exudates against *Rhipicephalus appendiculatus* larvae

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Abstract

Evaluating plant extracts for anti-tick properties is essential towards development of alternative method for controlling tick infestation and other harmful insects. This study aimed to evaluate repellency activity of *Commiphora swynnertonii* hexane and chloroform extracts against *Rhipicephullus appendiculatus* larvae using climbing bioassay method. Concentrations of 110mg/ml, 100 mg/ml, 90mg/ml, 80mg/ml, 70mg/ml, 60mg/ml and 50mg/ml of hexane and chloroform extracts were used. *C. swynnertonii* chloroform and hexane extract had repellency activity in all concentrations at all-time intervals. The repellency activity was appeared to be concentration and time dependent. The results from this study strengthen the view that *C. swynnertonii* is the potential source of anti-tick agents.

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Introduction

The use of synthetic acaricides is the most common method used for decades by livestock keepers to control both hard and soft ticks (Opdebeeck *et al*., 1988). Synthetic acaricides that has been employed for the management of ticks are organophosphates (OP), phyrethroid (SP), amitraz, macrocyclic lactone (ML), Arsenic, organochlorine, and benzenehexachloride (BHC), polychloroterpine, dieldrin and aldrin, cyclodiene compounds and toxaphene just to mention a few. Despite the fact that synthetic acaricides has been effective in the management of ticks, their use has become less reliable, acceptable and sustainable due to several reasons, such as high cost of acaricides, tick resistance to synthetic acaricides and contamination of the environment or food with toxic residues (Mkolo *et al*., 2007; Bissinger *et al*., 2010; Kalala *et al*., 2014;). These challenges promote the necessity to seek for alternative tick control methods which is affordable, effective, ecofriendly and less toxic to the livestock and livestock keepers.

The use of natural substances such as plant-based products to kill or repel parasitic arthropods on livestock have been widely used by many communities as an alternative to synthetic acaricides (Opiro *et al*., 2013). However scientific studies have been conducted by scientific researchers to validate the ethno veterinary use of plant based products which are used by indigenous people in the communities. For example, a study conducted by Silva Lima *et al*., (2016) on repellency activity of *Lippie alba* essential oils growing in Brazil found to possess repellency activity against *Rhipicephalus appendiculatus*. On another study, Wanzala *et al*., (2014) observed repellency activity of *Tagetes minuta* and *Tithonia diversifolia* growing in Kenya against *R. appendiculatus*.

Commiphora swynnertonii (Burseraceae) is a small highly branched and thorny tree with a height of about 3 meters tall. The plant is widely distributed in Africa and Asia and is among the plant species commonly used in Tanzania and Kenya for the management of ticks (Kalala *et al*., 2014).

Although several studies have been carried out to assess and validate the medicinal value of *Commiphora* species (Aliyu *et al*., 2002), only little information on *C. swynnertonii* is available in the literature. This paper therefore reports the repellency activity of *C. swynnertonii* extracts against *R. appendiculatus* larvae.

Materials and methods

Exudates and ticks collection

Exudates of *C. swynnertonii* were collected from Mererani, Simanjiro district in Arusha region, Tanzania while ticks were obtained from Tanzania Pesticides Research Institute (TPRI). The plant material was identified in the field by Mr. Innocent Mboya, a botanist from TPRI and voucher specimen coded CS 001 is deposited at the Nelson Mandela African Institution of Science and Technology.

Extraction of plant materials

Exudates weighed 78.8g were mixed with 150ml of distilled water followed by 150ml of hexane. The solution was thoroughly shaken and allowed to settle in order to form two layers. The hexane layer was separated from aqueous layer by decantation.

Thereafter, 150ml of chloroform was poured into the separating funnel containing aqueous layer. The solution were shaken and left for 6 hours and chloroform layer was separated from the aqueous layer. The two extracts obtained were concentrated through vacuum rotary evaporator, and the extracts obtained were kept in a beaker covered with aluminium foil and stored at 4°C for further use.

Climbing bioassay

The climbing bioassay was performed as described by Magano and Mkolo, (2011). The bioassay is based on climbing behavior of ticks. Except for the genus *Amblyomma* ticks naturally climb up vegetation to quest for a host (Norval *et al*., 1987).

A beaker of 50ml filled with paraffin was firmly inserted in the center of a 250ml beaker which was filled with water to completely surround the small beaker in order to discourage tick from crawling away from paraffin platform (Carrol, 1998).

The paraffin provided support to the vertically inserted wood rod (length 11cm) and also served as platform on which ticks were placed. Concentrations of chloroform and hexane extracts were prepared by dissolving 110mg 100mg, 90mg, 80mg, 70mg, 60mg, 50mg in 10% Dimethyl sulphoxide (DMSO).

Two filter papers (12cm^2 each) were prepared on which one filter paper was impregnated with tested extract while the other filter paper was not impregnated with any extract or solvent.

The impregnated filter paper was pasted on the top of the wood rod followed by non-impregnated filter paper which was pasted below it. The same procedures were followed for the positive and negative control. Positive control used was jungle formula commercial insects' repellant and negative control was DMSO.

Twenty five *R. appendiculatus* larvae were randomly placed on a paraffin platform of the treated apparatus while the same numbers of larvae were placed on controls platform. The position of the larvae on the wood rod was recorded at 10 minutes intervals for 60 minutes. Ticks (larvae) on the impregnated filter paper and on negative control were considered not repelled while those found on non- impregnated filter paper and on wood rod were considered repelled. Three replications were done for each concentration of the extracts and standard insect repellant (Jungle formula).

Statistical analysis

Data obtained were analyzed using GenStat computer software version 4 (Gen Stat 4). Percentage repellency was calculated using the formula below as demonstrated by Silva Lima *et al.*, (2016) while effective concentration which repel 50% of the ticks (EC_{50}) was obtained using linear regression equation.

PR= NTF / (NTF +TF) X 100

Where:

PR is percentage repellent

NTF is number of ticks on non-impregnated filter paper

TF is number of ticks on impregnated filter paper.

Results

The repellency activity of *C. swynnertonii* hexane and chloroform extracts were evaluated for repellency activity against *R. appendiculatus* larvae and results obtained are summarized in Tables 1 and 2. The findings from this study indicated that the repellency activity of *C. swynnertonii* hexane and chloroform extracts was concentration and time dependent.

The repellency declined as time elapsed (Table 1 and 2). The standard repellant (Jungle formula) used displayed the same activity patterns. It was revealed that there was very high significant difference ($P<0.001$) in repellency effects between control and tested extracts in the first 40 minutes.

Hexane and chloroform extracts had comparable repellency activity as indicated in Table 1. It was evident statistically that there was nonsignificant difference ($P>0.001$) among extracts after 50 and 60 minutes exposure time (Table 1). Furthermore results revealed that, there was very high significant difference ($P<0.001$) in repellency concentrations of control and tested extracts from 10 to 60 minutes.

The concentrations of chloroform and hexane extracts with higher percentage repellency activity comparable to the control were 110mg/ml and 100 mg/ml (Table 1). Additionally, findings from this study indicated that there was very high significant interaction effect ($P<0.001$) in both control and treated extracts against concentrations in the first 20 minutes while no significant interaction effect ($P>0.001$) was observed from 30 to 60 minutes.

The concentrations that can repel 50% of the larvae EC_{50} was calculated and results are shown in Table 2. The EC_{50} was generally increasing with an increase in exposure time for both extracts and standard. The EC_{50} could not be calculated at the 10th minutes because all the tested concentrations of the standard repellant had 100% repellency while at the 20th minutes the Y-intercept was greater than 50.

Table 1. Mean percentage repellency of chloroform and hexane *C. swynnertonii* extract against *R. appendiculatus* larvae.

Extract	Concentration (mg/ml)	PR (10 min)	PR (20 min)	PR (30min)	PR (40min)	PR (50 min)	PR (60 min)
Chloroform	50	53.63de	44.84hi	24.04ij	10.89j	8.00i	6.72h
	60	58.00bcde	56.06fgh	41.44fghij	32.36ghij	22.27efghi	22.02efgh
	70	61.36bcde	61.94fg	54.04defg	41.58efgh	26.26efghi	23.18efgh
	80	71.45bc	67.13ef	50.40efgh	43.21defh	41.36cdef	27.11efg
	90	73.06b	75.88de	62.36cdeg	55.81cdefg	47.63bcde	36.69def
	100	89.00a	90.60abc	84.07abc	65.36bcde	63.41bcd	54.04bcd
	110	95.41a	92.81ab	87.10ab	74.87bc	71.17ab	57.79bc
Hexane	50	26.34f	21.21j	19.06j	14.65ij	11.06hi	7.32h
	60	47.72e	35.47i	28.26hij	24.51hij	13.71ghi	13.51gh
	70	53.03de	42.93i	39.37ghij	25.23hij	19.93fghi	17.53fgh
	80	55.97cde	46.43hi	41.49fghij	37.48fghi	27.45defgi	20.83efgh
	90	68.61bcd	54.43gh	45.13fghi	40.58fgh	37.96defg	35.04ef
	100	87.85a	77.60de	73.83bce	66.52bcd	65.72abc	56.64bc
	110	94.97a	92.26ab	75.79bc	65.55bcde	70.88ab	62.32b
Control	50	100.00a	76.77de	63.70cdef	25.13hij	23.94defgi	21.89efgh
	60	100.00a	79.66cd	60.49cdeg	42.69defh	30.45defgi	26.39efgh
	70	100.00a	80.42cd	60.65cdeg	56.66cdefg	30.35defgi	32.79efg
	80	100.00a	87.08bcd	76.63bcd	57.24cdef	36.64defh	27.80efg
	90	100.00a	100.00a	89.81a	69.12bc	46.62bcde	29.84efg
	100	100.00a	100.00a	100.00a	88.44ab	49.09bcd	40.54cde
	110	100.00a	100.00a	100.00a	100.00a	88.19a	80.41a
P-value (extracts)		***	***	***	***	ns	ns
P-value (concentration)		***	***	***	***	***	***
P-value (interaction)		***	***	ns	ns	ns	ns

***Significant difference, ns=no significant difference, values followed by different letters denote statistical significance (P<0.001).

Table 2. Effective concentrations (EC_{50}) of chloroform, hexane and control against *R. appendiculatus* larvae at different time intervals.

Extracts		Time (Minutes)					
		10	20	30	40	50	60
chloroform	EC_{50}	49.21	55.45	72.44	83.81	89.54	101.22
	R^2	0.9545	0.9785	0.9376	0.9621	0.988	0.9455
Hexane	EC_{50}	68.8	77.37	84.05	91.97	93.71	100.36
	R^2	0.9588	0.9438	0.9206	0.9227	0.9146	0.92
Control	EC_{50}	-	-	42.92	69.13	87.26	97.98
	R^2	-	0.8909	0.8777	0.9704	0.7707	0.6629

R^2 - Regression correlation coefficient; EC_{50} – Concentrations (mg/ml) at which 50% of the *R. appendiculatus* larvae were repelled.

Discussion

Repellency is caused by the release of volatile secondary metabolites which are known to cause disorders in the movement of the target species away from the odor source (Jaenson *et al.*, 2005). Plants with insecticidal repellency effects were the main insecticidal agents in Africa for management of insect vectors before the introduction of synthetic insecticidal agents. Validation of such plants has been viewed as the best option of developing bio-pesticides that are readily available in developing countries.

The *Commiphora swynnertonii* exudate is widely used by pastoralists for the management of ticks. It has therefore ethnomedical and ethnoveterinary significance in Tanzania which necessitated scientific validation of the exudate. Results emanated from this study demonstrated that hexane and chloroform extracts of *C. swynnertonii* exudate has repellency activity against *R. appendiculatus* larvae. Both *C. swynnertonii* chloroform and hexane extracts exhibited repellency activity in all concentrations (50mg/ml, 60mg/ml, 70mg/ml, 80mg/ml, 90mg/ml, 100mg/ml and 110mg/ml) which indicated that the

activity was caused by release of volatile compounds that cause the targeted organism to move against the odor source. This study confirms that the *Commiphora* species contain volatile substances that are responsible for the repellency effect that can be extracted with different organic solvents.

Findings from this paper were comparable to the results obtained from standard insects' repellant (Jungle formula). The observation was supported by Lwande *et al*., (1999) findings who reported that the essential oil of *Gynandropsis gynadra* was compared to the commercially synthetic arthropod repellant in repelling *Rhipicephalus appendiculatus* ticks. These findings clearly suggest that some plant species may contain anti-tick agents that may be equally effective against ticks as some of the commercially synthetic arthropod repellants.

The effectiveness of the extract against *R. appendiculatus* larvae was dose dependent as evidenced by an increase in percentage repellency as the concentration increased from 50mg/ml to 110mg/ml. The lower concentrations appeared to be less effective since the amount of volatile compound which enhances the repellency activity was lower than in higher concentration.

However the effectiveness of the extracts against *R. appendiculatus* was decreased as time declined from 10 to 60 minutes. EC_{50} appeared to be increasing as the time increases for both tested extracts and the standard insects' repellant. The lowest EC_{50} was appeared to be the best concentration in repelling the ticks since it induces high repellency effect within a short period of time and also minimize the utilization of plant products and hence conservation of *C. swynnertonii* diversity.

Findings from the current study on the repellency properties exhibited by *C. swynnertonii* exudates contribute to the body of knowledge on the insecticidal properties of the genus *Commiphora*. Furthermore findings from this study validate the ethnoveterinary information on the use of *C. swynnertonii* exudate on the management of tick infestation on livestock among Maasai community in Tanzania.

Conclusion

The use of plant-based products, particularly those from indigenous plants, may minimize the costs incurred by the countries to import synthetic acaricides. The results from this study strengthen the view that *C. swynnertonii* is the potential source of anti-tick agents and this validates the ethno veterinary use of the plant for management of tick infestation on the animals. The results of this study, further strengthens the widely held view that plant products can be used as an alternative to synthetic tick-repellent.

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