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Evaluating Acaricidal Activity of *Commiphora swynertonii* (Burrt.) bark Exudate against common Ticks in Tanzania

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Abstract

Commiphora swynertonii (CS) exudate tapped from bark is extensively used by Maasai and many other tribes in Tanzania and Kenya for treatment of various ailments in animals and human beings. The exudate is said to have strong wound healing ability and acaricidal activity. The aim of this study was to establish effectiveness of acaricidal activity of the exudate on common ticks *Rhipicephalus appendiculatus* (RA) and *Amblyoma variegatum* (AV). Larval Packet test (LPT) and Adult immersion test (AIT) were used. The exudate was found to have strong acaricidal activity against the ticks with LC₅₀ = 1.72 mg/ml for RA while for AV it was 1.91 mg/ml. The LC₉₉ of the exudate for RA was 3.5 mg/ml and that of AV was 3.7 mg/ml. The exudate also prevented or reduced oviposition and egg hatching capability. Thus CS exudate is recommended for use in protecting livestock and domestic animals against ectoparasites.

Keywords: *Commiphora swynertonii*, Exudate, *Rhipicephalus appendiculatus*, *Amblyoma variegatum*, Acaricidal Activity.

1. Introduction

The genus *Commiphora* (Fam. *Burseraceae*) has been demonstrated to exhibit a wide range of biological activities such as anti-inflammatory and analgesic [1], anti-hyperglycemic and antioxidant [2], hepatoprotective [3], antibacterial [4, 5] and anticancer [6] activities. *Commiphora* species have also been demonstrated to have antiulcer [7], antifungal [8], molluscicidal and antischistosomal activities [9]. *Commiphora swynertonii* (CS) is a small, highly branched and thorny tree or shrub, reaching the height of about 2 to 3 metres, and is used for medicinal purposes in Africa and Asia [10]. It is widely distributed in the northern parts of Tanzania and Southern parts of Kenya. In Tanzania, the plant is used by several tribes including Gogo, Sukuma, Iraqwi and Maasai. The Maasai of Tanzania and Kenya for example use the plant against various ectoparasites afflicting livestock. The plant is also used for treating skin infections, wounds and ulcers, worm infestations, coughs and other chest problems in both humans and livestock [11]. The Maasai and other ethnic groups in Tanzania and Kenya use CS exudate to eliminate ectoparasites particularly ticks. Ticks have great negative impact on economics of livestock keeping. They are vectors of a number of diseases in humans and animals such as African tick bite fever, tularemia, tick-borne relapsing fever, babesiosis, ehrlichiosis, Tick paralysis, tick-borne meningoencephalitis, bovine anaplasmosis and East Coast fever [12]. Disease in livestock result in reduced meat production, reduced quality and quantity of milk, destruction of hides and death of animals. Thus ticks must be eradicated to avoid such problems.

Ticks of economic importance to Tanzania include *Rhipicephalus appendiculatus*, *R. pravorum*, *Amblyomma variegatum*, *A. gemma*, and *A. lepidum* [13], *Boophilus micropulus* and *Rhipicephalus avertis* [14]. The most common method of ticks' elimination is spraying with or dipping in commercial acaricides. However, the cost of available commercial acaricides may be unaffordable to local poor pastoralists. Even instructions to use the available acaricides may not be properly followed by some pastoralists particularly the illiterate ones. Furthermore commercial acaricides are not usually environmental friendly. It is therefore imperative that an alternative is sought to find an affordable and user friendly natural and locally occurring acaricide for use in tick-control. CA bark exudates may provide such an alternative.

Although Maasai and other tribes in Tanzania use the exudates of the bark of CS to repel and kill ticks and other ectoparasites, there are no official publications of the activity.

The aim of this study was to demonstrate the acaricidal activity of the exudates against common ticks afflicting livestock in Tanzania namely *Rhipicephalus appendiculatus* (RA) and *Amblyoma variegatum* (AV) and to determine its LC₅₀ and LC₉₉ in the respective ticks.

2. Materials and Methods

2.1 Bark exudate

The exudate of the bark of *C. swynertonii* was collected by incising the bark with a sharp knife and collecting the oozing exudates in a bottle. The plant was identified by John Elia, a botanist from the National Herbarium in Arusha. Its voucher specimen No. WK01 is deposited in the National herbarium Arusha.

2.2 Preparation of the exudate

The exudate of *C. swynertonii* is practically insoluble in water. Thus it was necessary to prepare an emulsion so as to dilute the emulsion to appropriate concentration as may be required. A 10mg/ml emulsion of the exudates was prepared blending 0.01g of Tween 80 in 90 mls of distilled water and 10 ml of the exudates using Kenwood blender, model SB 054. Tween 80 acted as emulsifying agent.

A milky white emulsion resulted and was stored in a stoppered glass container. Subsequently the following serial dilutions were made: 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml, 0.625 mg/ml, and 0.3175 mg/ml using distilled water and shaking vigorously. Tween 80 solution in distilled water (0.01 mg/ml) was used as negative control and a locally used commercial acaricide, Paranex® containing Alphacypermethrin in the concentration of 0.05 mg/ml was used as positive control.

2.3 Development of a tick colony

Engorged females of RA and AV were collected from naturally infested cattle on farms in Erkesumet, Simanjiro District, Manyara region of Tanzania (GPS Co-ordinates -4° 26' 22.2174", 37° 7' 33.0918" N / E). The animals from which ticks were collected must have not been subjected to any acaricidal treatment for more than 45 days prior to the study [15]. The collected ticks were put in glass vials containing a little sand and small pieces of filter paper, and closed with muslin cloth to allow ventilation while preventing escape. Each vial had a maximum of five ticks as directed [16].

The ticks were immediately transported to Tropical Pesticide Research Institute (TPRI) laboratories. In order to have enough ticks a colony was developed according to methods described by FAO [17]. Briefly the ticks were washed with distilled water, dried using a filter paper and placed in Petri dishes and then incubated at 27–28 °C and 70-80% humidity for one month until oviposition occurred. The eggs laid were placed in glass vials under same conditions of temperature and humidity and hatched into larvae. The larvae were allowed to mature for 7 - 10 days. Some of these larvae were used for the Larval Packet test (LPT). The rest of the larvae were fed on animals. Larvae of RA were dropped in a muslin cloth bag tied on the ear of a rabbit while those of AV were dropped in a bag enclosing the scrotum of a sheep (fig.1). The ticks fed and became engorged. The following day the bags were removed from the ear and scrotum so as to collect the engorged larvae. The larvae thus collected were put in a glass vials, stoppered with a muslin cloth, incubated at 27–28 °C and 70-80% humidity and after two weeks they molted into nymphs. The RA and AV nymphs were again fed on rabbit ears and sheep scrotum respectively. After 10 days the nymphs became engorged and were

collected from the bags and kept on Petri dishes and incubated at 27–28 °C and 70-80% humidity where they developed into adult ticks. The adult ticks were fed on rabbit ears and sheep scrotum again and after two weeks the engorged females were collected ready for the Adult Immersion Test (AIT). After collecting the ticks at each stage (larvae, nymphs and adults), the rabbits and sheep were treated with halofunginone (0.1 mg/kg) for 3 days to prevent any tick-borne infection.



Fig 1: Feeding RA larvae on rabbit ear and AV larvae on Scrotum of sheep. Note the muslin bags tied on the ear and scrotum of rabbit and sheep respectively.

2.4 Ethical Considerations

The protocol for the study was blessed by Muhimbili University of Health and Allied sciences Ethics Committee and the procedures adhered to TPRI Institutional ethical and animal care guidelines as well as Veterinary Laboratory Agency (TVLA) guidelines.

2.5 Experimental procedure

2.5.1 Larval Packet Test. (LPT)

Larval bioassay was carried out according to a method developed by Shaw [18] and modified by FAO's World Acaricide Resistance Reference Centre (WARRC). Filter papers were cut into 9x6 cm pieces. The papers were labeled for each concentration of the test acaricide, positive and negative control. The dilutions were (in mg/ml) 10, 5, 2.5, 1.25, 0.625, and 0.3175. Using a micropipette, the pieces were impregnated with 0.67 ml of each dilution of the test acaricide, positive control and negative control solutions to cover the entire area. Three replicates of each dilution and controls were prepared. The papers were left to dry for 10 minutes and then folded into half along the longer side. The resulting short side was clipped with bulldog clips to form a pocket, where about

100 larvae were introduced and the open end was clipped with another bulldog clip to prevent escape of the larvae.

The pockets were incubated between 27–28 °C and 70-80% humidity and opened after 24 hours to observe dead and live larvae using a X2 lens. The larvae were considered dead if there was no observed movement even after gently blowing them with human breath. The larvae which moved their appendages but did not walk were also considered dead. Both live and dead larvae were counted and recorded.

Equation 1:

$$\text{Percent Mortality} = \frac{\% \text{ Test mortality} - \% \text{ Control mortality}}{100\% - \% \text{ Control Mortality}} \times 100$$

2.5.2 Adult Immersion Test

Adult Immersion Test (AIT) for acaricidal efficacy described by Drummond [19] and modified by FAO [14] was used. 300 Female engorged ticks of RA and AV were rinsed in tap water to remove faeces and other debris. They were then allowed to dry by placing them on paper towel folded on edges in such a way that the ticks were confined and not able to escape.

For each dilution of the exudate (10 mg/ml 5 mg/ml, 2.5 mg/ml 1.25 mg/ml 0.625 mg/ml and 0.3125 mg/ml), 100 ml were prepared and 25 ml were filled in 50-ml beakers in triplicate. The same volume was prepared for both negative and positive controls containing 0.01 mg/ml Tween 80 and 0.05 mg/ml Alpha Cypermethrin respectively.

For each beaker containing the test exudate, 10 ticks were randomly picked from the pool above, weighed and introduced in the beakers starting with the negative control, lowest to highest concentration and finally to the positive control. The ticks were stirred for 30 minutes and then the exudate was poured off after which the ticks were dried gently on a paper towel. For each concentration and controls, 10 ticks were randomly picked, weighed and transferred to Petri dishes previously labeled for the appropriate concentration of the exudate where they were stuck with ventral side up on the bottom surface of the Petri dish using a double-sided sticky tape. The Petri dishes were then incubated between 27–28 °C and 70-80% humidity for five days. During the period of incubation the Petri dishes were not disturbed in order to observe survival of the ticks and the egg laying capacity of each tick. Survival of ticks and their egg laying capacity were observed daily and recorded.

2.5.3 Product effectiveness

After day 5, ticks which died were discarded and those which survived were kept in the incubator for a further 15 days in order to note whether they will ovipose and if the laid eggs will hatch into larvae.

The egg laying capacity was determined by comparing the Weight of eggs of the treated group with the weight of the eggs laid by untreated group (the negative control). The hatching rate was determined by counting the number of larvae and the number of unhatched eggs for each concentration containing survived ticks and calculated as

$$\frac{\text{number of larvae}}{\text{total number of eggs laid}}$$

The total number of eggs laid = Number of larvae + number of unhatched eggs.

The data obtained was used to calculate Percent Hatching Reduction (%HR) and Percent Oviposition reduction (%OR) using equations described by Gonzales [20].

$$\% \text{ Hatching Rate Reduction (\%HR)} = \frac{HC - HT}{HC} \times 100$$

$$\% \text{ Oviposition Reduction (\%OR)} = \frac{WEC - WET}{WEC} \times 100$$

Where HC = Hatching rate in Control group

HT = Hatching rate in Treated groups

WEC = Mean weight of eggs in Control group

WET = Mean weight of eggs in treated groups

Acaricidal efficacy was determined by comparing the Estimated Reproduction (ER) of each group of treated ticks with that of the Control group. ER is an estimate of the number of larvae produced by each female at each concentration of acaricide used in the bioassay and is calculated as follows:

$$ER = \frac{\text{Weight of laid eggs (g)}}{(\text{Number of females})} \times \text{Estimated hatch} \times 20,000$$

(#eggs per g)

The product Effectiveness was then calculated using the equations described by Drummond [19]

Equation 2:

$$\% \text{ Product Effectiveness (\%PE)} = \frac{ERC - ERT}{ERC} \times 100$$

Where ERC is the Estimated Reproduction in Controls and ERT is the Estimated reproduction in Treated groups.

2.5.4 Statistical analysis

The mean and standard error of the dead ticks after exposure to various concentrations of the exudates, positive control and negative control were estimated and then subjected to One Way analysis of Variance (ANOVA) to compare performance of each concentration and to identify differences among sample means. Significance was considered when the probability was below 0.05.

The lethal concentration resulting in 50% mortality LC₅₀, and that resulting in 99% mortality of ticks, LC₉₉, were estimated using the regression of the logarithm of the concentration of the exudate according to the Probits of the cumulative percentage of the corrected mortality rate of ticks using Regression Analysis in SPSS 20 computer program according to the method of Finney (1971). The means of ER and PE at different concentrations were also found by the same statistical package and compared between groups.

3. Results and discussion

Results of the LPT are as summarized in Table 1 and illustrated in Figure 2. At low concentration (below 0.625 mg/ml acaricidal effectiveness of the exudates tended to zero in both species of the ticks, RA and AV, but increased as the concentration increased. A 5 mg/ml concentration of the exudate was found to be as effective as the 0.05 mg/ml concentration of the positive control, Alphacypermethrin. In both cases 100% mortality was realized. Subjecting the results to Regression analysis in SPSS 20 statistical package and subsequent Probit analysis led to establishment of LC₅₀ as 1.72 mg/ml for RA while for AV it was 1.91 mg/ml. The LC₉₉ of RA was 3.5 mg/ml and that of AV was 3.7 mg/ml. The LC₅₀ and LC₉₉ of the exudates are higher for AV than they are for

RA, meaning more concentration of the exudates is required to effect the same mortality in AV than that required for RA. The difference in LC₅₀ and LC₉₉ for AV and RA was small but significant (P<0.05).

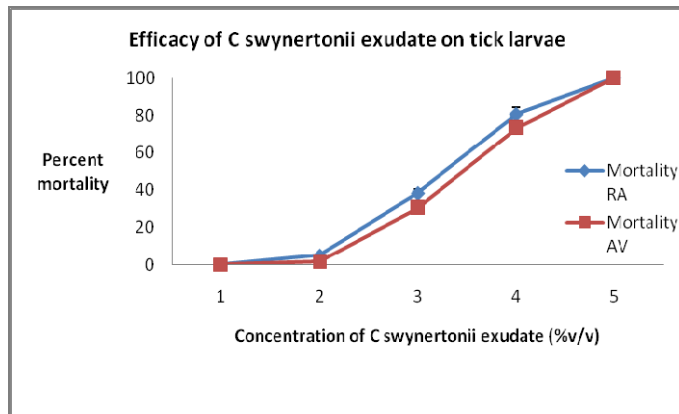


Fig 2: 24-hour Mortality of larvae ticks exposed to various concentration of *C. swynertonii* exudate.

Table 1: Acaricidal activity of *C. swynertonii* exudate against larvae of RA and AV after 24 hour exposure of the ticks to the exudate.

Test materials (mg/ml)	Mean % Mortality	
	$\frac{\% \text{ Test mortality} - \% \text{ Control mortality}}{100\% - \% \text{ Control Mortality}} \times 100$	
	<i>R. appendiculatus</i>	<i>A. Variegatum</i> *
Positive Control (0.05 AlphaCypermethrin)	100	100
5	100	100
2.5	80.6 ± 3.85	73.21.7 ± 2.05
1.25	38.22 ± 3.98	30.38 ± 2.8
0.625	5.21 ± 2.05	2.6 ± 1.7
0.3175	0	0
Negative Control (0.02 Tween 80)	0	0

Table 2: Daily accumulative mortality of engorged RA ticks after exposure to various concentrations of CS exudate

Concentration of the exudate (mg/ml)	Cumulative percent Mortality*				
	Day1	Day2	Day3	Day4	Day5
10	0.67 ± 1.15	84 ± 5.5	100	100	100
5	0	27 ± 4.6	39.33 ± 4.1	83.33 ± 8.02	100
2.5	0	3.7 ± 1	23 ± 5.7	50.33 ± 7.1	85 ± 7.5
1.25	0	0	0	1.7 ± 1.15	17.7 ± 6.7
0.625	0	0	0	0	1 ± 1
Positive Control	65 ± 4.9	100	100	100	100
Negative control	0	0	0	0	0

* Average of three replicates

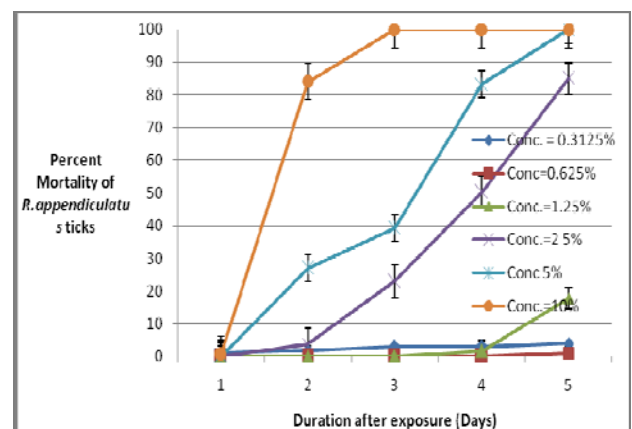
The results of AIT are displayed in Table 2 and Table 3 and graphically illustrated in Figure 3 (i and ii) for AR and AV respectively. The Effectiveness of the acaricidal activity of *C. swynertonii* exudates was shown to be a function of concentration of the exudates as well as the duration of exposure to the exudates. At 10% concentration, more than 85% of ticks died by day 2. A lower concentration of 2.5% did not elicit mortality on day 1 and day two, but progressively the ticks started dying on day 3 and by day 5 more than 85% of ticks had died. At a higher concentration (5% and 10 %) a 100 % mortality was realized on day 2. Thus acaricidal activity of *C. swynertonii* exudate in a function of its concentration and

duration after exposure to the exudate. At a higher concentration the effect is immediate. At a lower concentration there is no immediate action but the residual amounts on the ticks continue to elicit the affect. Fig 5 shows the variation of LC₅₀ between day 1 and day5 after exposure to the exudate. The LC₅₀ is higher on day 1 (6.4 and 15.5 mg/ml for RA and AV respectively) progressively diminishing as duration after exposure increases down to 0.9 and 1.63 mg/ml in RA and AV respectively on day 5. This means even at low concentrations, the exudate will kill the ticks after 3 to 5 days. However for the immediate knockdown effect, a high effective concentration is recommended. Subjecting the ticks to smaller concentration may lead to development of resistance of the ticks to the treatment.

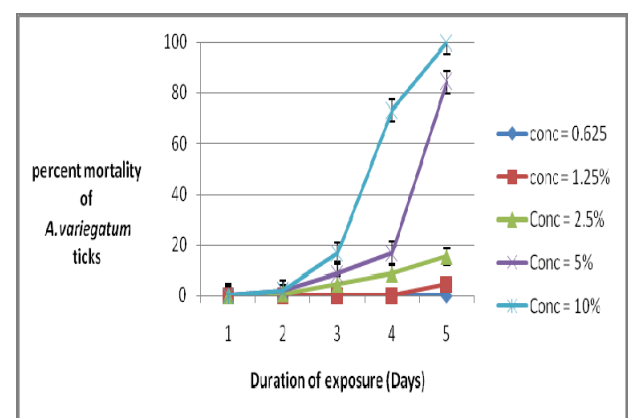
Table 3: Daily accumulative mortality of engorged AV ticks after exposure to various concentrations of CS Exudate

Concentration of the exudate (mg/ml)	Cumulative percent Mortality*				
	Day1	Day2	Day3	Day4	Day5
10	0	4.6 ± 1.15	15.3 ± 5.1	84.3 ± 5.7	100
5	0	0	8.7 ± 0.6	16.7.3 ± 5.8	73.3 ± 11.5
2.5	0	0	4.6 ± 5.7	8.7 ± 0.6	16.7 ± 5.1
1.25	0	0	0.67 ± 1.15	1.7 ± 1.15	1.7 ± 1.15
0.625	0	0	0	0	0
Positive Control	84 ± 6.2	100	100	100	100
Negative control	0	0	0	0	0

* = Average of three replicates ± standard error



(i)



(ii)

Fig 3: Daily cumulative Mortality of engorged RA (i) and AV (ii) ticks after immersion into various concentrations of *C. swynertonii* exudates for five days.

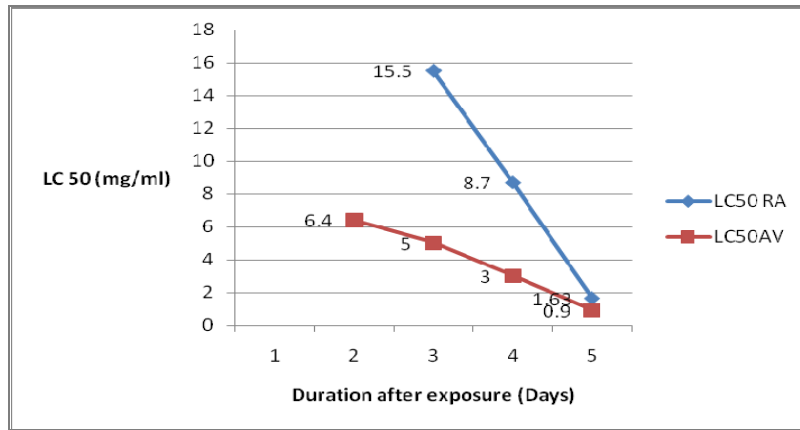


Fig 4: Variation of LC₅₀ as a function of concentration of the exudate and duration after exposure of the ticks to the exudates.

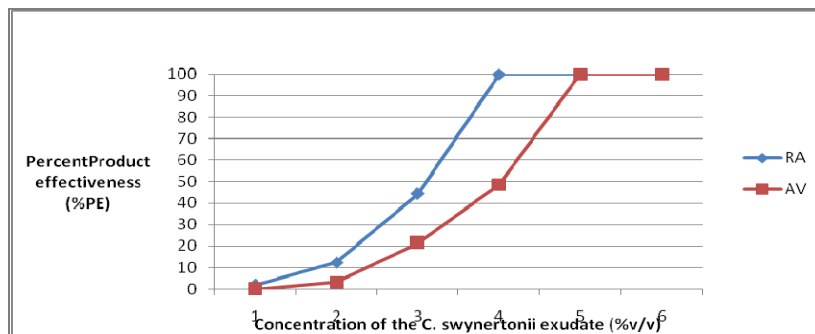


Fig 5: Product effectiveness (%PE) of *C. swynertonii* exudates on adult engorged females ticks.

Table 4: Means and Standard errors of % oviposition reduction (OR) and % Hatching Rate (HR) of adult female of RA and AV exposed to various concentrations of *C. swynertonii* exudates and Product effectiveness of each concentration of the exudates.

Concentration (%v/v)	OR		HR		PE	
	RA	AV	RA	AV	RA	AV
10	All ticks died at day 2	All died at day 3	100	100	100	100
5	All ticks died at day 10	All died at day 15	100	100	100	100
2.5	No egg production for surviving ticks on day 21	24.5±6.2	100	18.6±5.4	100	48.8±8.7
1.25	69.6±12.1	31.4±5.3	54.2±14.3	37.5±9.1	44.3±7.5	21.3±4.4
0.625	49.6±14.1	30.2±8.0	26.2±7.4	18.1±10.2	12.3±3.3	3.2±2.9
0.3215	12.78±10.3	2.7±1.9	1.6±3.1	0.0	1.9±1.7	0

Figure 5 depicts the effectiveness of *C. swynertonii* exudate on adult engorged females as calculated using Equation 2. Not only does the exudate kill the ticks but also it affects oviposition and hatching capability of oviposited eggs. At high concentration (>5 mg/ml) ticks died within three days. At lower concentration, although the ticks did not die, they reduced oviposition capacity. Furthermore most of the eggs that were oviposited did not hatch. The reduction of oviposition and egg hatchability by *C. swynertonii* exudate on ticks is more pronounced in RA than in AV. Thus CS exudate not only kills ticks but it also affects reproduction at low concentrations that are unable to kill the ticks. Similar trends have been observed elsewhere. For instance Abdel-Shafy and Zayed [21] observed that Neem oil effect on *Hyalomma anatolicum axcravatum* ticks by killing the ticks at high concentration and reducing oviposition and egg hatchability in low concentrations. Similarly it has been shown that seeds of *Azadirachta indica* are toxic to *Rhipicephalus micropulus* engorged female ticks through reduction of reproductive parameters, with a sharp

drop in the number of laid eggs and reduced hatching rate [22]. The acaricidal activity of CS exudate parallels other findings. For instance essential oils of CS leaves repels RA ticks and the repellence activity has been associated with presence of sesquiterpenoids [23]. Also bark extracts induces mortality in nymphs and adults of RA [24]. However effective concentration of the bark extract capable of inducing 100% mortality to ticks was 10%. In this study, CS exudate exhibited stronger acaricidal activity by inducing 100% mortality at 5% concentration. Acaricidal activity in the genus commiphora is not unique to CS. There are other species with strong acaricidal activities including *C. erythraea* [25] and *C. myrr* [26]. Additionally CS has also been shown to have strong antibacterial and antifungal activities [27-28] This supports the indigenous use of the plant for treatment of wounds, ulcers, various infections in humans and animals as well as killing ectoparasites afflicting domesticated animals [11]. These findings are encouraging and may be the answer to the quest for an acaricide of biological origin as an alternative or

additional weapon to the currently used commercial chemical acaricides. The latter are said to be expensive, hazardous to environment and some have already exhibited resistance by ectoparasites against which they are produced. Further studies on toxicology and formulation of CS exudate are therefore recommended to warrant vegetative propagation of CS and commercial production and processing of CS exudate. Additionally, active ingredients of the exudate should be isolated and identified so that a synthetic process be designed to obviate use of plants and to conserve environment.

4. Conclusion

CS exudate has strong acaricidal activity. The LC_{50} was 1.72 mg/ml for RA while for AV it was 1.91 mg/ml. The LC_{99} of the exudate for RA was 3.5 mg/ml and that of AV was 3.7 mg/ml. The exudate not only kills the ticks effectively but also affects reproductive processes by reducing or preventing oviposition and egg hatchability. Thus CS exudate is a promising acaricidal agent which is available and affordable to our local communities.

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