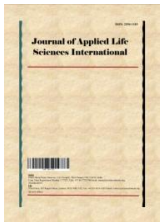

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Acaricidal Activity of *Commiphora merkeri* Bark Exudate against Two Species of *Rhipicephalus* Koch (Acari; Ixodidae) by Larval and Adult Immersion Test

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Authors' contributions

This work was carried out in collaboration among all authors. Authors EI and IAA designed the study, performed fieldwork and the statistical analysis, wrote the protocol and wrote the manuscript. Author SA contributed to study conception, design and revision. All authors read and approved the final manuscript.

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ABSTRACT

Ticks pose a threat in the infestation of both wild and domestic animals, thereby causing an increase in chances for transmission of diseases. Despite of the wide use of *Commiphora* species in tick control, no acaricidal activity of *Commiphora merkeri*. Engl. Exudate have been scientifically assessed. The acaricidal activity of the exudate extract and its Petroleum ether (PE), Dichloromethane (DCM) and Ethyl acetate (ETOAC) fractions were carried out by using the larval immersion test (LIT) and adult immersion test (AIT), against *Rhipicephalus appendiculatus* and *Rhipicephalus averts*. The crude extracts of *C. merkeri* showed 80% and 70% mortality on the LIT bioassay at concentration of 1.0 mg/mL for *R. averts* and *R. appendiculatus,* respectively. There was no statistical difference ($p \geq 0.05$) in activity of petroleum ether and dichloromethane fractions exhibiting 100% mortality at concentration of 1.0 mg/mL for *R. appendiculatus* and *R. averts,* also at 0.8 mg/mL to *R. averts* species. *R. averts* was more susceptible that *R. appendiculatus* showing

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stable incremental mortality in all concentration levels. In the AIT, no statistical significant difference ($p \geq 0.05$) in reduction was observed for crude extract of *C. merkeri* and petroleum ether fractions by having no surviving *R. averts* above 0.025 mg/mL after 24 and 72 h. The same trend was observed for *R. appendiculatus* within 24 h of exposure. However, at lower concentrations the residual effect of treatments on the ticks continued to elicit the effect over time having few or no immediate effect of death after exposure, This was vivid for *R. averts* within 24 and after 72 h. Follow up of survived engorged adults indicated that, the ticks could lay eggs but the eggs were not viable for hatching. This justifies its uses as an alternative agent in an integrated approach in reducing tick infestation among Pastoralist.

Keywords: Acaricidal; Commiphora merkeri exudates; Rhipicephalus appendiculatus; Rhipicephalus averts; larval immersion test; adult immersion test.

ABBREVIATIONS

PE : Petroleum ether fraction
DCM : Dichloromethane fraction
ETOAC : Ethyl acetate fraction
LIT : Larval immersion test
AIT : Adult immersion test

1. INTRODUCTION

Ticks have great negative impact on the economy of pastoral communities. 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 meningo encephalitis, bovine anaplasmosis and East Coast fever [1]. Such diseases in livestock result in reduced meat production, reduced quality and quantity of milk, destruction of hides and death of animals. Control of ticks has for a long time depended much on application of synthetic chemicals, including regular dipping of animals and sprays. Despite of the effectiveness of application methods used the control agents such as pyrethroids, organophosphates, and amitraz experiences resistance to ticks [2]. This situation is pressing for concerted efforts for continue searching for novel innovative application and alternatives effective anti-ticks agents from natural products [3].

Plant species of the genus *Commiphora* grow in the belt of pastoral communities along the Somali-Maasai, acacia-commiphora deciduous bushland and thickets [4]. *Commiphora merkeri* is dioeciously small tree up to 5 m tall; the stem bark is grey with large black lenticels, peeling off around the stems in yellowish papery strips and branchlets spine tipped. The incision of the barks produces oleo-resin copious fluid with fruity smell [5]. The plant species has been reported to relief chest pain, asthma and tuberculosis [6]. In additional, the Maasai of Longido district in Arusha, Tanzania uses it to eliminate ectoparasites, particularly ticks. Thus, the present study was conceived to test different fractions of *C. merkeri* exudate against *R. appendiculatus* and *R. averts* ticks which are of economic importance to Tanzania, so as to establish the effective concentration and potentially promising application techniques for household tick-control.

2. MATERIALS AND METHODS

2.1 Collection of Exudates

The exudate of the bark of *C. merkeri* was collected from Tingatinga village in Longido District by incising the stem bark with a sharp knife and collecting the oozing exudates in a bottle. The plant was identified by Mr. FM. Mbago, a Botanist from the Department of Botany, University of Dar es Salaam. It`s voucher specimen No. EI 34 is deposited in the Herbarium of the University of Dar es Salaam and the Institute of Traditional Medicines of the Muhimbili University of Health and Allied Sciences.

2.2 Preparation of Test Samples

About 50 g of the exudate was dissolved in 500 ml of distilled water and well shaken to obtain enough emulsions. The emulsion was then filtered to remove the un-dissolved particles. Liquid-liquid extraction was done using three organic solvents in the sequence of petroleum ether, dichloromethane and then ethyl acetate. Subsequent shaking of solvents and aqueous exudate fraction for several times was done and then left to settle for 12 h to allow separation of the layers. The three successive fractions were concentrated using rotary evaporator and then dried in a freeze drier to remove water.

Appropriate volume of a stock solution (50 mg/ml) was made and used in preparation of test concentration.

2.3 Development of Tick Colony

Engorged females of *R. appendiculatus* and *R. averts* used in this experiment have been reared at Tropical Pesticide Research Institute, Tanzania. The following strains were used (indicating the original location where was collected from the field animals): *R. appendiculatus* (Kabete strain), *R. appendiculatus* (Lushoto strain), *R. appendiculatus* (Oldonyawas strain) and *R. averts* (Mto wa Mbu strain).

In order to have enough ticks for experiments, a colony was developed according to methods described by FAO [7]. 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 and hatched into larvae. The larvae were allowed to mature for 7 - 10 days. The larvae thus collected were put in a glass vials, stoppered with a muslin cloth, and after two weeks they molted into nymphs. Nymphs of *R. appendiculatus* were fed on rabbit ears, while *R. averts* nymphs fed on sheep scrotum. After 10 days the nymphs became engorged and were collected from the bags,kept on Petri dishes, incubated to develop into adult ticks. The adults were fed for two weeks, then engorged females were collected for the Adult Immersion Test (AIT). After collecting, 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.

2.4 Larval Immersion Test (LIT)

The larvae immersion tests (LIT) were done according to Gazim et al [8] with modifications. Briefly, larvae obtained from engorged females of *R. appendiculatus* and *R. averts,* were rested and unfed for 16 to 25 days after hatchability. Approximately 100 larvae were placed between two rounded filter papers (Whatman no. 1, diameter 120 mm) to form a larvae sandwich, which was placed in a pie plate. A volume of 10 mL of test sample solution at concentrations of 1, 0.8, 0.6 and 0.4 mg/mL was poured over the larvae sandwich to expose them to the treatment. The sequence of pouring started with 3 mL of the test solution being in a petri-dish poured using a syringe, then filter paper of 11 mm was placed on petri dish. Larvae (total n= 100) were transferred with a paintbrush to a paper filter, 4 mL of test sample was added on larvae, and another filter paper was placed above larvae and then 3mL of test sample added on it. The sandwich was left for ten minutes (counted from the start of process) and then removed to open air for drying. After drying for 10 min, larvae were transferred to a squared paper filter that was folded and closed with ‘‘bulldog’’ clips forming a packet. The packets were incubated at 27–28 °C and 80–90% relative humidity then the mortality was counted after 24 h. Only larvae capable of locomotion were considered alive. The same numbers of larvae for control were used during bioassays and experiments were done in duplicate. Live larvae which were still clinging to the filter paper envelope were counted by squashing each larva and recorded. Efficacy of treatments to kill the larvae was determined by calculating corrected mortality [9]. Each run included a positive control (0.05mg/ml alpha Cypermethrin)) and a negative control (distilled water).

2.5 Adult Immersion Test (AIT)

The adult immersion test (AIT) followed Holdsworth et al., [10] with some modifications. Briefly, 0.1 mL of the stock solution was dissolved in 100 mL of distilled water and then serially diluted to obtain concentrations at 50, 25, 12.5, 6.25 and 3.125 μg/mL. Female ticks were randomly picked from the pool, weighed and introduced in the beakers starting with the negative control and then followed the lowest to highest concentration. Ticks were stirred for 30 minutes and then the test sample was poured off after which the ticks were dried gently on a paper towel. For each concentration and controls, 10 ticks were picked, weighed and transferred to petri dishes previously labeled for the appropriate concentration of the treatment 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 at least 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 was observed daily and recorded for 15 days in order to observe whether they will

oviposit and if the laid eggs will hatch into larvae. The criteria used to diagnose dead ticks included the lack of movement of legs and change of cuticle color [11]. Efficacy of extract to kill the adult ticks was determined against negative control which was distilled water by calculating corrected mortalities.

2.6 Data Analysis

Cumulative mean percentage mortality for tick larvae, mean percentage survival for engorged adults and standard errors were calculated using Microsoft excels (2013). Comparison of means was done by one way analysis of variance (ANOVA) at 95% Confidence level.

3. RESULTS

3.1 Results for Larvae Immersion Test (LIT)

Results of the LIT are summarized in Fig. 1. The crude extracts of *C. merkeri* showed 70% and 80% mortality on the LIT bioassay at concentration of 1.0 mg/mL for *R. appendiculatus* and *R. averts,* respectively. However, acaricidal effectiveness below the concentration of 0.8 mg/mL for the *R. averts* and *R. appendiculatus* for the crude extracts was below 30%. There was no statistical difference ($p \geq 0.05$) in activity of petroleum ether and dichloromethane fractions exhibiting 100% mortality at concentration of 1.0 mg/mL for *R. appendiculatus* and *R. averts,* also at 0.8 mg/mL to *R. averts* species. In general, *R. averts* was more susceptible than *R. appendiculatus* with ethyl acetate fraction showing steady incremental mortality in all concentration levels.

3.2 Results for Adult Immersion Test

Results of adult immersion test (AIT) are displayed in Fig. 2 and 3 for the two species of *R. appendiculatus* and *R. averts*. The results indicate a progressive decrease in survival of ticks with duration of exposure and increase in concentration. At lower concentrations there was little or no immediate effect of death after exposure, but the residual amount of treatments on the ticks continue to elicit the effect over time. This was vivid for *R. averts* within 24 h, and after 72 h with no statistical significant difference ($p \geq 0.05$) in reduction observed for crude extract

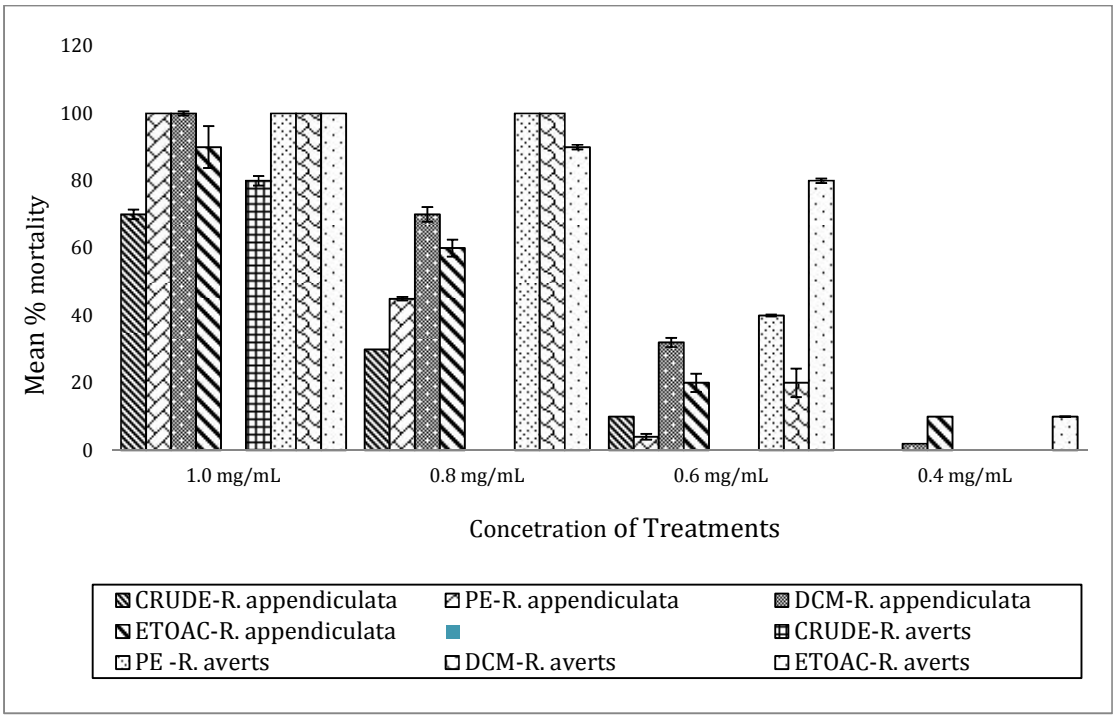

Fig. 1. Mean percentage (±SE) of acaricidal activity by larvae immersion test of extract of *C. merkeri* exudate and its fractions against larvae of *R. appendiculatus* and *R. averts* after 24 hours from exposure

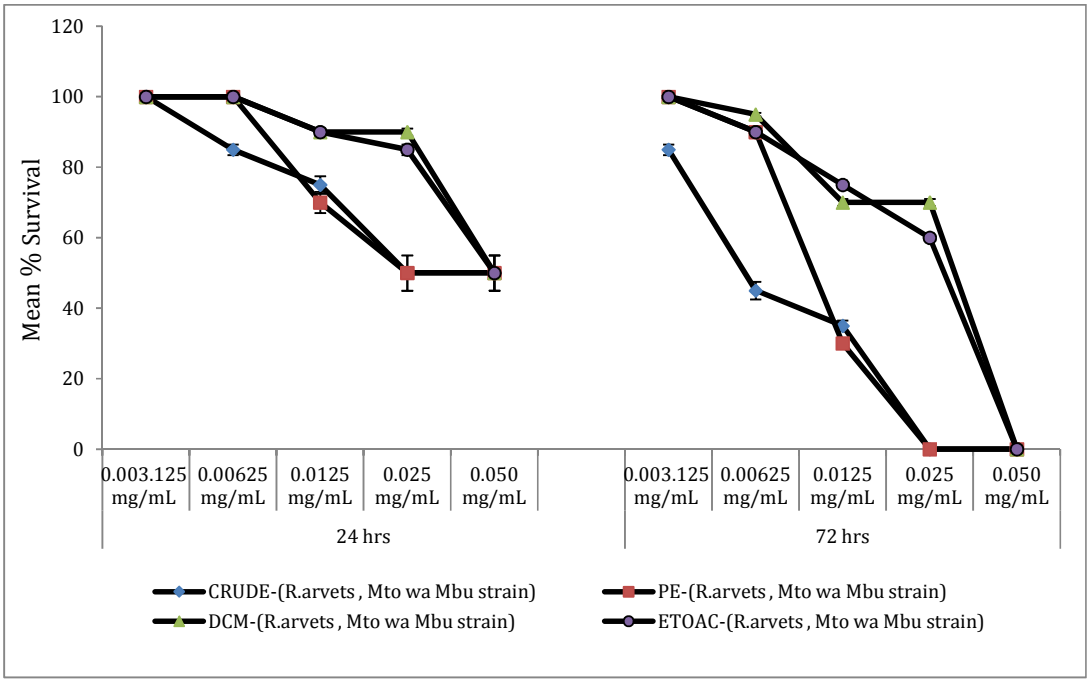

Fig. 2. Cumulative percentage survival (±SE) of engorged *R. averts* ticks to various concentrations of extract of *C. merkeri* exudate and its fractions in water after exposure for 24 h and 72 h

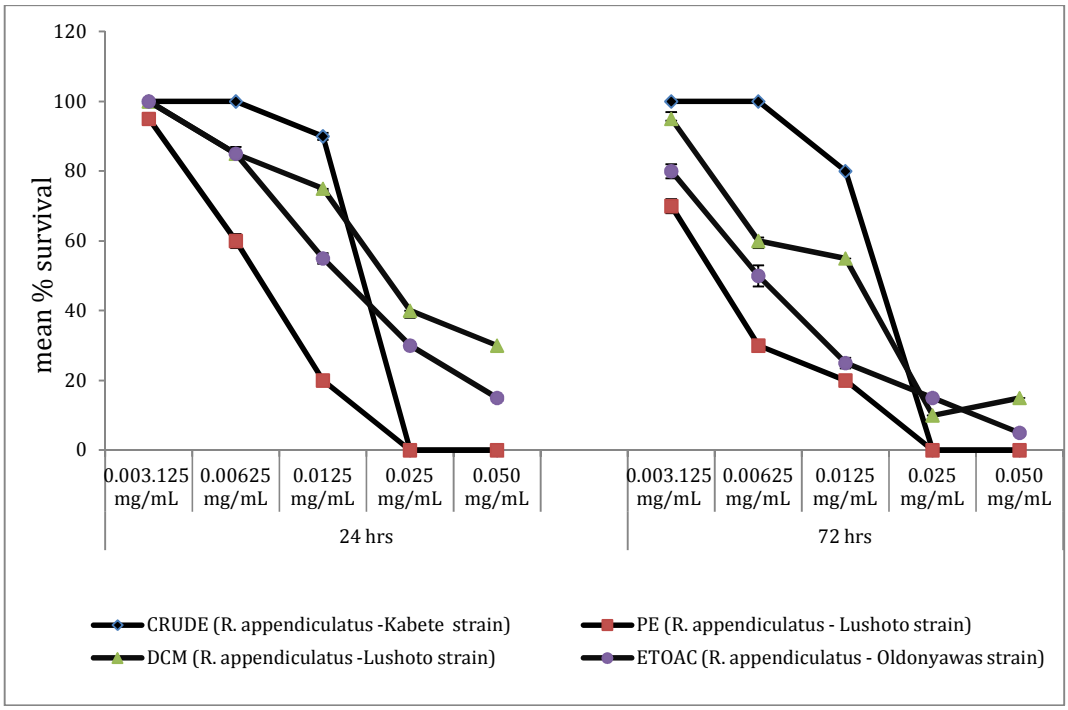

Fig. 3. Cumulative percentage survival (±SE) of engorged *R. appendiculatus* ticks to various concentrations of extract of *C. merkeri* exudate and its fractions in water after exposure for 24 h and 72 h

of *C. merkeri* and petroleum ether fractions by having no surviving tick above 0.025 mg/mL (Fig. 2). The same trend was observed for *R. appendiculatus* where no statistical significant difference ($p \geq 0.05$) and no surviving ticks was observed within 24 h of exposure (Fig. 3). However, a DCM fraction was less active indicating 80% of *R.* averts surviving beyond this concentration even after 72 h after exposure (Fig. 2). Further follow up observations of survived ticks from knockdown effects indicated that, all ticks which survived beyond the test days laid eggs which did not hatch within 15 days of rearing.

4. DISCUSSION

Results for this study shows similarities from previous studies. The trend is similar to that observed for *Azadirachta indica* (Neem) oil which caused mortality of ticks at high concentration and reduces oviposition and egg hatchability in low concentrations [12]. Seeds of *A. indica* show toxicity to *Rhipicephalus micropulus* engorged female ticks with sharp drop in the number of laid eggs and reduced hatching rate [13]. Another study, reported severe reproduction as well as mortalities of *R. microplus* and *R. annulatus* when 50% concentration of *Stemona collinsae* induced 100% nymph and 93.33% adult mortalities at 24 h post treatment [14]. Similarly, all stages of *R, appendiculatus* were repelled and killed by oil extracted from leaves of *Ocimum suave* [15].

Data obtained from the larvae and adult immersion tests for this study show that petroleum ether and DCM fractions of *C. merkeri* have knockdown properties for *R. appendiculatus* at high concentration. The effect of extract and fractions for *R. averts* was steadily slow, however, equally, persistence increased with time of exposure especially at high concentrations. At low concentrations, the treatments were unable to kill the ticks instantly, but affected the reproduction cycle as the hatchability of eggs were not viable for both species tested.

These finding validate the traditional use of *C. merkeri* plant species in control of ticks. However, acaricidal activity in the genus *Commiphora* is not unique to *C. merkeri.* Other plant species whose crude exudate, fractions or characteristic isolated compounds exihibited strong acaricidal activities including dendrolasin derivatives from *C. swynnertonii* [16,17], furanosesquiterpenoids, 2-*O*-acetyl-8,12-epoxygermacra-1(10),4,7,11-tetraene and 2-*O*-methyl-8,12-epoxygermacra-1(10)-4,7,11 tetraene from *C. erythraea* and *C. myrrh* [18,19]. Also, *C. holtziana* extract, and the isolated hydrocarbon fraction, showed strong repellent effects in an olfactometer assay using the red poultry mite, *Dermanyssus gallinae* (Acari: Dermanyssidae) with (+)-germacrene-D, being responsible for repellency [20].

5. CONCLUSION

The findings of this study indicate that, Petroleum ether and dichloromethane fractions gave good larvae mortality while the crude and petroleum ether fraction was more active against engorged adults of *R. appendiculatus* and *R. averts* species. These results provoke further quest for isolation of acaricide compounds from Tanzanian *Commiphora* species for possible development in the control of tick infestation. Also, results for extract at high concentrations justify its uses as an alternative in reducing engorged adult tick infestation in an integrated approach. Moreover, if *Commiphora* plant species have to be used traditionally, then, an effective method of propagation of *Commiphora* should be addressed to pastoralist to avoid extinction of the species due to its several ethno-uses.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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