Acaricidal Activity of*Commiphora Swynnertonii* (Burtt) Stem Bark Extracts against adult *Rhipicephalus Appendiculatus* Newman and *Amblyomma Variegatum*

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ABSTRACT

The *Commiphora swynnertonii* (Burtt) stem bark petroleum ether, ethyl acetate and methanolic extracts were evaluated for acaricidal activity against adult *Rhipicephalus appendiculatus* and *Amblyomma variegatum* using the contact method. The extracts were tested at concentrations of 60, 70, 80, 90 and 100 mg/mL. All extracts exhibited acaricidal activities which were concentration and time dependent. The *Commiphora swynnertonii* (Burtt) stem bark petroleum ether extract exhibited relatively high acaricidal activity with 50% lethal concentration value (LC_{50}) of 72.31 and 71.67 mg/mL resulting mortality of 100% against *Amblyomma variegatum* and 87% against *Rhipicephalus appendiculatus* respectively after 156hours of exposure to treatments. These findings validate traditional use of *Commiphora swynnertonii* for control of tick.

Keywords: acaricidal activity, *Amblyomma variegatum*, *Rhipicephalus appendiculatus*, plant extracts, concentration.

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1. INTRODUCTION

The bottleneck challenge to livestock industries in pastoralists' communities in Sub-Sahara Africa is tick infestation and tick borne diseases (Young et al., 1988; Olwoch et al.,2008).Tick borne diseases that are of greater economic importance in Sub-Saharan Africa are East Coast Fever (ECF), babesiosis, anaplasmosis, dermatophilosis, and cowdriosis. The effects associated with these diseases includes low quality hides and skins, low meat and milk production, high veterinary services and loss of labour animals (Jongejan and Uilenberg, 1994; Mbassa et al., 2012). The management of ticks and tick borne diseasesin Sub-Saharan countries has relied on synthetic acaricides (Kategile and Mubi, 1993; Thullner et al., 2007, Fernandez Salas et al.,2012). However synthetic acaricidespose serious problems of ticks' resistance upon subsequent use (Perez Cogollo et al., 2010), others are residue and contamination in livestock production, in the environment and unaffordable prices for majority of poor pastoralists in developing countries. All these have made livestock industry to lag behind in tropical and Sub tropical countries (Jongejan and Uilenberg, 2004).

In East African countries the expenses incurred due to tick borne diseases from ECF was estimated to reach US$ 168 million (Mukhebi et al., 1992; Kivaria, 2007) and the studies have shown that death caused by TBD of more than 1.3 million cattle in Tanzania had the direct losses reached to US$ 364 million (Kivaria, 2006), moreover literatures documented that TBD accounted for 71.4% of annual cattle mortality in Tanzania in the year 1981-1993 with *Rhipicephalus appendiculatus* being a species that transmit a number one killer disease, the ECF (Kivaria, 2006; Babo Martin et al 2010). The *Amblyomma variegatum* also increase cattle mortality by causing heartwater and dermatophilosis which result into depreciation of value of hides and skin, low milk production, weight loss and death at acute stage (Norval et al., 1992).

The inaccessibility of synthetic acaricides at affordable prices, and theircontraindications to life of non targeted organismshas prompted the use of biopesticides which are ecofriendly (Gupta and Dikshit, 2010). Most of poor farmers in developing countries use plant concortions for the management of ticks (Zimmerman et al., 1984; Minja., 1994; Mwangi et al., 1995; Minja, 1999; Katosh et al; Kaaya, 2000; Chenyambuga et al., 2010; Habeeb, 2010; Swai et al., 2005), however only few plant species has been scientifically evaluated for acaricidal activities. One of such

plants is *Commiphora swynnertonii* which is used to control ticks infestation and repel insects of economic importance by poor Maasai pastoralist communities in East Africa (Kaoneka et al., 2007). This paper is therefore reporting acaricidal activity of *Commiphora swynnertonii* stem bark petroleum ether, ethyl acetate and methanolic extracts against *Amblyomma variegatum* and *Rhipicephalus appendiculatus*.

2. MATERIAL AND METHOD

2.1 Chemicals and reagents

Methanol (absolute) was bought from Fluka Chemie GmbH (Sigma-Aldrich®, Zwijndrecht, Netherlands) and dimethyl sulfoxide (DMSO) was purchased from RFCL Limited, Hayana, India. Petroleum ether and ethyl acetate were purchased from Loba Chemie Pvt Ltd, Mumbai, India). Paranex was brought from Farmbase limited, Tanzania.

2.2 Tick collection

Ticks were collected from local cattle in Simanjiro district and identified by Mr. Ibrahim Hamisi from the Department of Entomology, Tropical Pesticides Research Institute (TPRI), Arusha, Tanzania.

2.3 Plant material collection

Stem bark of *Commiphoraswynnertonii* was collected from Manyire Village inMeru district, Arusha, Tanzania. Identification of plant was done by Mr. Haji Selemani a botanist from Department of Botany, University of Dar es Salaamand a voucher specimen number CS 6872 is deposited at Nelson Mandela African Institution of Science and Technology.

2.4 Plant Material Processing and extraction

The collected stem bark was cleaned by running tap water to remove soil, cut into small pieces and dried under the shade at room temperature range of 30-32^{0}C. The dried material (1000 g) was pulverized and sequentially extracted using petroleum ether, ethyl acetate and methanol.

Extracts were filtered using filter funnel with Whatman no 1 filter paper to get rid of the solid particles and solvents were removed by vacuum using Rota evaporator. The concentrates were kept in labeled air tight bottle and stored at 4^0C until use.

2.5 Acaricidal activity assay

The acaricidal activity of *Commiphora swynnertonii* (Burtt) stem bark petroleum ether, ethyl acetate and methanolic extracts were assayed against adult *Rhipicephalus appendiculatus* and *Amblyomma variegatum*using contact method developed. Adult *Rhipicephalus appendiculatus* and *Amblyomma variegatum* were isolated from naturally infected cows. Five ticks were dipped in the solution of the tested extracts prepared at the concentration of 100, 90, 80, 70 and 60 mg/mL initially dissolved in dimethyl sulfoxide (DMSO) and diluted in water. The same solvent without the tested extracts was used as untreated control. Thesolution of paranex (0.5 mg/L), a standard acaricide, was used as positive drug control. All plates were incubated under laboratory conditions in BOD incubator at 27 – 28 ^{0}C and 80 - 85% relative humidity for seven days.After 6 hours, each tick was observed and all the motionless ticks were stimulated with a needle. Lack of reactions and persistent immobility indicated their death. The ticks were counted after every 24 hours for seven consecutive days.

2.6 Data analysis

The Fig P computer software was used to obtain regression equation, and from it LC_{50}, LC_{16}, and LC_{84} were calculated. The 95% Confidence Interval was then calculated using method reported by Lichfield & Wilcoxon, (1949). The LC_{50} less than 100 was considered to be toxic and above 100 to be non-toxic.

3. RESULTS

The *Commiphora swynnertonii* stem bark extracts were analysed for acaricidal activity against*Amblyomma variegatum* and *Rhipicephalus appendiculatus*using contacted method. The mean percentage mortalities were calculated using the completely dead ticks excluding the paralysed ticks (Fernandez Salas et al., 2012; Annan et al., 2011). The results revealed that, the

acaricidal activity of *Commiphora swynnertonii* was concentration and time dependent, results are detailed in Table 1.In which themaximum hours of observation was 156.Petroleum ether extractexhibited higher acaricidal activity with mean mortality of 87% (LC_{50}=71.6812 mg/mL) against*Rhipicephalus appendiculatus* and 100% (LC_{50} = 72.3126 mg/mL) against *Amblyomma variegatum*. However the acaricidal activity of ethyl acetate and methanolic extracts against both *Rhipicephalus appendiculatus* and*Amblyomma variegatum*were remarkable as depicted in Table 1. Ethyl acetate exhibited acaricidal activity with LC_{50} value of 77.0241 and 74.7064 mg/mL against *Amblyomma variegatum* and *Rhipicephalus appendiculatus* respectively after 156 hours. Methanolic extracts had LC_{50} values of 81.1246 and 75.5676 mg/mL against *Amblyomma variegatum* and *Rhipicephalus appendiculatus* respectively after 156 hours. Paranex which contains alphacypermethrin active ingredient was used as a positive control, 0.5 mg/L of Paranex was tested and caused 100% paralysis of tick species after 6 hours and after 96 hours, 100% mortality was observed. Negative control DMSO and water had the mortality of 0% to maximum hours of treatment.

Similar study was conducted by (Kaoneka and Mollel 2011) in which hexane/ethyl acetate (1:9) stem bark extract of *Commiphora swynnertonii* was evaluated for acaricidal activity against two weeks nymph of *Rhipicephalus appendiculatus* and the percentage mortality of 71% was reported. This report is therefore supporting that *Commiphora swynnertonii* extracts possess acaricidal activity.

4. DISCUSSION

Results of the current study have clearly shown that *Commiphora swynnertonii* stem petroleum ether, ethyl acetate and methanolic extracts exhibit varying acaricidal activity in a dose dependent manner. It is therefore supporting the use of this plant in the management of ticks as practiced by poor Maasai pastoralist community in Tanzania. Petroleum ether extracts ranked the highest in inducing death to *Rhipicephalus appendiculatus* and *Amblyomma variegatum*. Ethyl acetate ranked second and methanolic extract was the least. It is therefore clear that non polar secondary metabolites in *C. swynnertonii* possess high acaricidal activity, followed by moderate polar and polar secondary metabolites.

Table 1: LC_{50} and percentage mortality values of *Commiphora swynnertonii* extracts evaluated against *Amblyomma variegatum* and *Rhipicephalus appendiculatus*

Time	*Amblyomma variegatum*			*Rhipicephalus appendiculatus*		
	CSSM	**CSSE**	**CSSP**	**CSSM**	**CSSE**	**CSSP**
24h	LC_{50}= 138.4956 mg/mL R^2= 0.9423 95%CI=115.4803-66.0977 Y=137.73logX-244.94 100%mortality=319.4958	LC_{50}=125.6910 mg/mL R2=0.9155 95%CI=106.7439-148.0011 Y=152.36logX-269.85 100%mortality=267.5927	LC_{50}=117.4643 mg/mL R2=0.9862 95%CI=103.4380-133.3924 Y=220.01logX-405.4 100% mortality=198.2295	LC_{50}=192.1836 mg/mL R^2=0.9991 95%CI=137.9737-267.6925 Y=97.534logX-172.74 100%mortality=625.6885	LC_{50}=199.0913 mg/mL R^2=0.9677 95%CI=152.5136-259.8937 Y=93.93logX-165.95 100%mortality=678.2093	LC_{50}=179.1835 mg/mL R^2=0.8811 95%CI=141.2005-348.8702 Y=117.49logX-214.74 100%mortality=477.3822
48h	LC_{50}= 138.4956 mg/mL R^2=0.9423 95%CI=115.4803-66.0977 Y=137.73log X-244.94 100%mortality=319.4958	LC_{50}=119.9994 mg/mL R^2=0.8574 95%CI=100.4599-140.9212 Y=140.82logX-242.79 100%mortality=271.7955	LC_{50}=117.4643 mg/mL R^2=0.9862 95%CI=103.4380-133.3924 Y=220.01logX-405.4 100%mortality=198.2295	LC_{50}= 239.9328 mg/mL R^2=0.9556 95%CI=169.9240-338.7851 Y=81.144logX-143.13 100%mortality=991.4669	LC_{50}=140.0962 mg/mL R^2=0.8978 95%CI=113.5136-173.1589 Y=132.08logX-233.5 100%mortality=334.9537	LC_{50}=259.4284 mg/mL R^2=0.8913 95%CI=185.9968-361.8507 Y=75.248logX-131.65 100%mortality=1198.0836
72h	LC_{50}=108.8587 mg/mL R^2=0.9576 95%CI=94.1765-169.7107 Y= 172.8logX- 301.97 100%mortality=156.7811	LC_{50}=101.8650 mg/mL R^2=0.935 95%CI=88.8564-116.7780 Y=183.17logX-317.81 100%mortality=190.9834	LC_{50}=94.1401 mg/mL R^2=0.9495 95%CI=83.9412-105.5781 Y=244logX-431.68 100%mortality=151.0137	LC_{50}=194.4964 mg/mL R^2=0.9575 95%CI=143.6670-263.3092 Y=82.655logX-139.19 100%mortality=783.1335	LC_{50}=149.4340 mg/mL R^2=0.9845 95%CI=115.4733-193.3825 Y=108.57logX-186.08 100%mortality=431.5012	LC_{50}=143.1316 mg/mL R^2=0.9881 95%CI=115.0853-178.0127 Y=114.81logX-197.5 100%mortality=390.1554
96h	LC_{50}=96.1447mg/mL R^2=0.8710 95%CI=85.3708-108.2781 Y=235.44logX-416.86 100%=156.7811	LC_{50}=89.2915 mg/mL R^2=0.9764 95%CI=78.3052-101.8190 Y=190.69logX-322.66 100%mortality=164.6178	LC_{50}=89.2666 mg/mL R^2=0.9632 95%CI=81.9786-97.2021 Y=328.53logX-590.86 100%mortality=126.7309	LC_{50}=118.9109 mg/mL R^2=0.836 95%CI=97.8529-144.5005 Y=128.42logX-216.5 100%mortality=291.4535	LC_{50}=141.5591 mg/mL R^2=0.9267 95%CI=111.7983-179.2421 Y=106.07logX-178.15 100%mortality=419.1069	LC_{50}=124.3900 mg/mL R^2=0.9924 95%CI=100.3873-154.1316 Y=116.79logX-194.65 100%mortality=333.3529
108h	LC_{50}=92.2749 mg/mL R^2=0.9258 95%CI=80.2320-106.1253 Y=179logX- 301.75 100%mortality=175.5550	LC_{50}=83.6226 mg/mL R^2=0.9511 95%CI=74.4503-93.9249 Y=215.51logX-364.28 100%mortality=142.6696	LC_{50}=80.6260 mg/mL R^2=0.9806 95%CI=75.5703-86.0198 Y=431.65logX-772.93 100%mortality=105.2712	LC_{50}=105.4130 mg/mL R^2=0.9485 95%CI=85.3201-130.2377 Y=118.37logX-189.45 100%mortality=278.8037	LC_{50}=99.7855 mg/mL R^2=0.9246 95%CI=82.3788-120.8701 Y=139.44logX-228.75 100%mortality=227.8478	LC_{50}=106.0336 mg/mL R^2=0.851 95%CI=89.5478-125.5543 Y=148.17logX-250.11 100%mortality=230.6184
132h	LC_{50}=82.0430 mg/mL R^2=0.9914 95%CI=72.4633-92.8890 Y=217.78logX-366.84 100%mortality=139.1974	LC_{50}=82.9624 mg/mL R^2=0,9705 95%CI=72.6656-94.7181 Y=188.86logX-312.4 100%mortality=152.6258	LC_{50}=78.0707 mg/mL R^2=0.958 95%CI=73.8746-82.5051 Y=452.88logX-807.07 100%mortality=100.6686	LC_{50}=94.4310 mg/mL R^2=0.9951 95%CI=79.1144-112.7128 Y=141.45logX-229.38 100%mortality=213.1064	LC_{50}=85.1850 mg/mL R^2=0.8947 95%CI=72.9760-99.4364 Y=161.84logX-262.41 100%mortality=173.5043	LC_{50}=83.1124 mg/mL R^2=0.883 95%CI=72.8672-94.7980 Y=190.33logX-315.37 100%mortality=152.1834
156h	LC_{50}=81.1248 mg/mL R^2=0.9909 95%CI=72.8164-90.3811 Y=231.6logX-392.16 100%mortality=133.3654	LC_{50}=77.0241 mg/mL R^2=0.9626 95%CI=70.4188-84.2489 Y=278.99logX-476.35 100%mortality=116.3709	LC_{50}=72.3126 mg/mL R^2=0.9912 95%CI=67.4935-77.4757 Y=362.75logX-624.43 100%mortality=99.3231	LC_{50}=75.5676 mg/mL R^2=0.9732 95%CI=67.0996-85.1042 Y=210.58logX-345.54 100%mortality=130.5495	LC_{50}=74.7064 mg/mL R^2=0.9536 95%CI=68.1876-108.9083 Y=274.08logX-463.45 100%mortality=113.7068	LC_{50}=71.6812 mg/mL R^2=0.9327 95%CI=64.8757-79.2005 Y=250.84logX-415.4 100%mortality=113.4217

Previous phytochemical studies on *C. swynnertonii* revealed the presence of flavonoids, alkaloids, tannins, glycosides, steroids, saponins and terpenoids (Hanus et al., 2005). It has however reported that furanosesquiterpenes is the main active component of essential oil of *Commiphora myrrh* (Fatope et al., 2003). The acaricidal activity displayed might be attributed by the presence of furanosesquiterpenes. Tick repellency has been established to involve both olfaction and tactile chemoreception (Sonenshine, 1991). It yet not known on whether the two mechanism were involved in the present investigation.

Preparation of plant extracts from *Commiphora* species for management of ticks varied from one community to another. For instance, the Borana people of Ethiopia use *Commiphora erythraea* bark together with tobacco leaves altogether soaked in camel urine to control different species of ticks by applying to sites where ticks have attached (Zorloni, 2008). In Tanzania and Kenya, the Maasai pastoralist use sap of *Commiphora* species to control ticks infestation and repel insects. The Somali people use mixtures of gum resin from *Commiphora incise* and camel urine to detach ticks from animal skin, this application leaves an aroma on animals' skin and prevents ticks to reattach for duration of a week (Zorloni, 2008). It is therefore hypothesized that camel urine is used as readily available solvent.

Commiphora species are also utilized to repel insects of economic importance. For example, *Commiphora molmol* was found to be successfully in killing larvae of *Culexpipiens* and *Aedescaspius* (Habeeb, 2010). Watt and Breyer-Brandwijk, (1962) reported the use of *Commiphora* species for termite control in Africa.

5. CONCLUSION

The validity of Ethnovetenary application of *Commiphora swynnertonii*by local communities in Africa for tick control has been substantiated in this study. Further studies are needed to characterize secondary metabolites responsible for the reported acaricidal activity.

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