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Evaluation of acaricidal efficacy of *Synadenium glaucescens* (Euphorbiaceae) against boophilus species

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***Synadenium glaucescens* is a traditional medicinal plant used by some communities in Tanzania for the management of various diseases in animals and human including the use for control of ticks in cattle. The aim of this study was to investigate the 'acaricidal effect' of extracts from this plant *on Boophilus decoloratus and B. microplus*. The methodology involved the use of larval and adult immersion tests. Results indicated low larvicidal (corrected mortality 37.5%) and adulticidal (corrected mortality 33.33%, LC_{50} 666.91) activities respectively for methanol and ethanol extracts from leaves. Other extracts of this plant showed a non-significant activity of mortality. Thus, it is not recommended for field trials, rather additional research is needed to determine its potentials especially using fresh plant material**

Key words: *Synadenium glaucescens,* Acaricidal activity, ticks, Tanzania.

INTRODUCTION

Records indicated that the number of people relying on agriculture has gone down as from 2001 to 2010, yet still it is the only sector that provides a livelihood for the majority of the communities than any other industry in the world (Upton, 2004; World Bank, 2008; Cervantes-Godoy and Dewbre, 2010). In the agricultural sector, livestock keeping is one among important activities that is practiced by many poor communities in developing world (Randolph et al., 2007). In 2004, Upton reported that livestock keeping provided over half of the value of global agricultural output and one-third being in developing countries. Literature indicates that the number of animals is further experiencing a remarkable increase especially in developing world (Randolph et al., 2007; Thornton, 2010). Despite this amazing increase, livestock keeping is constrained by diseases transmitted by ectoparasites

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(Njoroge et al., 2006). The harmful effects of ectoparasites on the productivity of livestock are well documented (Bagavan et al., 2009, Gazim et al., 2011). Ticks and tick-borne diseases are important causes of losses to the livestock industry, in particular, the production of cattle and small ruminants in tropical and subtropical areas. The diseases are associated with a reduction in productivity, fertility and in some instances may result in the death of an animal (Bagavan et al., 2009; Gazim et al., 2011). A worldwide loss due to diseases transmitted by ticks and the costs of tick control is very high (Minjauw and McLeod, 2003). The economic importance of ticks is principally due to the ability to transmit a wide spectrum of pathogenic microorganisms, such as protozoa, rickettsiae, spirochaetes, and viruses.

In Africa, tick-borne protozoan diseases (e.g. theileriosis and babesiosis) and rickettsial diseases *e.g.* anaplasmosis and heartwater (cowdriosis) are the main health and management problems of domestic ruminants. Tick-borne diseases that are reported to affect livestock productivity in the East African Region include East Coast Fever, anaplasmosis, babesiosis and cowdriosis (McCosker et al., 1993; Kagaruki et al., 1996). In Tanzania, tick-borne diseases contribute to over 72% of the annual cattle mortality (Mtei and Msami, 1996; Kivaria, 2007). Ticks from the genus Boophilus are important due to their ability to transmit pathogens in cattle such as *Anaplasma marginale*, *Babesia bigemina*, *Brucella ovis*, *Babesia trautmanni* and *Borrelia theileri*.

Control of ticks aims at either eradication or prevention and has for a long time depended much on chemical control mainly synthetic chemicals. Main methods of applications include regular dipping of animals and sprays. Despite these novel efforts of control means, ticks control experiences many challenges, which include a rampant development of resistance against common control chemicals such as synthetic pyrethroids, organo-phosphates, and amitraz. The building and maintenance of dipping tanks or sprays and the purchasing of acaricides for tick control and therapeutic agents hike farmer's production costs.

This situation is pressing for concerted efforts to search for novel effective and eco-friendly anti-tick natural products. Natural sources especially plants are believed to be arsenals of such control agents and due to their versatile application; they are currently the main target. A study from Korea for example with a detailed analysis of ethnoveterinary plants revealed 143 medicinal plants in use for treatment of cattle diseases (Song and Kim, 2010). While some laboratory tests results report moderate toxic effects of herbal plants on adult ticks and larvae (Bagavan et al., 2009). Some plants reveal significant activity against economically important tick species including species resistant to acaricides (Borges et al., 2003; Sunil et al., 2013; Ghosh et al., 2013; Nawaz et al., 2015).

This study was therefore conceived to assess the activity of crude plant extracts from *S. glaucescens* against cattle ticks of the genus *Boophilus.* This plant has been reported to possess various pharmacological and insecticidal activities especially on its use as anti-ticks and in the post-harvest grain storage by local communities. However, there are no scientific reports regarding its acaricidal potentials against ticks. Nonetheless, other species of this genus have indicated good pesticidal activities against various ectoparasites (Bagavan et al., 2009; Hassan et al., 2012), thus building a base for investigating this plant species.

MATERIALS AND METHODS

Plant materials

Plant materials (leaves and root barks) of *S. glaucescens* Pax were harvested from Mufindi District in Tanzania during May and August 2012. The World Health Organization (WHO, 2003) guideline on Good Agricultural and Collection Practices (GACP) for medicinal plants was used. Thus, roots were dried at room temperature while some minor modifications were considered for leaves in which drying was effected in place with half day shade and half day sun because leaves of this plant contain a large amount of latex (Nyigo et al., 2015). The dried plant materials were pulverized and then subjected to extraction using solvents with different polarities sequentially in ascending order starting with hexane, dichloro-methane, ethyl acetate, methanol and ultimately water. After filtration, the extracts were dried *in vacuum* and in a freeze dryer to obtain different organic and water extracts, respectively (Table 1).

Ticks collection for adulticidal testing

Tests of plant extracts against adult ticks were conducted at the Faculty of Veterinary Medicine, Department of Veterinary Medicines and Public Health of the Sokoine University of Agriculture (SUA). Engorged adult ticks (*Boophilus decoloratus)* were collected from naturally infested cattle pastured on local freelance grazing from different areas of Morogoro and Coast regions in Tanzania. During collection, the researchers first enquired information on the application of acaricides to ensure that none has been applied 45 days before tick collection (Rosado-Aguilar et al., 2010, Gazim et al., 2011). Ticks were then washed with water and dried with a paper towel and were subjected into different groups for testing and control.

Adult ticks for larva production

Test of extracts against larvae was conducted at the University of Free State, South Africa. Fully engorged female ticks *B. microplus* and *B. decoloratus* were received from Clinvet International on 7 July, 2014. They were washed with tap water, dried and distributed into 5 conical flasks containing 20 females each. The flasks were incubated at 26 ±2°C at a Relative Humidity of >70% for oviposition and hatching, and the hatch date was determined to be the 26th of August 2014. Testing was performed between 17 and 25 days post hatching.

Sample preparation

Required weights of extract were prepared and dissolved using appropriate solvents. For organic extracts, the decision of solvent to

Table 1. Types of extracts of S. *glaucescens* and their codes**.**

Codes	Plant part	Extract type
RDCM	Root	Dichloromethane (DCM) extract of root prepared by extracting plant with DCM, after the plant materials extracted by Hexane
Rwater	Root	Water extract of the root after sequential extraction with Hexane, DCM, EtOAc, MeOH; and plant residue extracted with water (H_2O)
LDCM	Leaves	DCM extract of the leaves prepared by extracting plant with DCM, after the plant materials having been extracted by Hexane
LMeOH	Leaves	MeoH extract of leaves prepared after sequential extraction with DCM, EtOAc, and plant residue extracted with MeoH
Lwater	Leaves	Water extract of the leaves after sequentially extracted with hexane, DCM, ethyl acetate and MeoH
LEtoH	Leaf	Ethanol Extract; fresh ground dried leaves extracted with ethanol
REtoH	Root	Ethanol Extract; fresh ground root barks extracted with ethanol

use was reached after trials between DMSO and Tween 80. Since the solubility of extracts in DMSO was very low (Figure 1a), Tween 80 was chosen to be the dissolution solvent for organic extracts due its relatively better solubility (Figure 1b). Aqueous extracts were dissolved in distilled water while organic extracts were dissolved in 2% tween in distilled water. With aqueous extracts, the required amount of distilled water was measured and directly poured in the sample while for organic extract samples, the process involved first dissolving an extract in a known amount of tween 80 and diluted with distilled water to make the required volume of a solvent and in both cases, dissolutions were assisted by warming in a water bath. The controls composed of two solvents; 2% tween 80 in distilled water for organic extracts and 100% distilled water for aqueous extracts.

Larval immersion test (LIT)

Larvae obtained from the engorged female ticks of *B. microplus,* and *B. decoloratus* were rested unfed for 16 to 25 days after hatchability (Gazim et al., 2011). Approximately 200 larvae were placed between two round Whatman no 1 filter papers (diameter 120 mm) to form a larvae sandwich, placed in a pie plate. Ten milliliters of 1% solution from plant extracts was then poured over the larvae sandwich to expose them to the solution. Each run also included a positive control (300 ppm -Field concentration of Chlorfenvinphos- Supadip 30% m/v) and a negative control (diluent). After 30 min, excess solution was drained from the filter paper sandwich, then approximately 100 larvae were transferred to a clean filter paper (Whatman no 1, diameter 250 mm) envelop which was crimped closed as well as taped with masking tape over the crimped area to ensure that larvae cannot escape.

The envelopes were then placed in an incubator at a temperature of 26 ± 2°C and RH ≥70% for 72 h. After 72 h each envelope was opened and turned over to allow dead larvae to fall onto a clean filter paper circle (Whatman no 1, diameter 250 mm). Live larvae still clinging to the filter paper envelope were counted by squashing each larva counted onto the filter paper envelope. Then the filter paper containing dead larvae was inspected for any possible live larvae, which were also counted as live and picked up with a masking tape strip. The remaining larvae were then considered dead. Both counts were documented on a datasheet and transferred to a spreadsheet. Efficacy of extract to kill the larvae was determined against a negative control (diluent) by calculating corrected mortality Abbott's formula (Abbott, 1925).

Adulticidal tests through adult immersion test

The adult immersion tests (AIT) as described by Drummond et al. (1976) and Holdsworth et al. (2006) was adopted with some modification for acaricidal activity tests of crude extracts of plant materials from *S. glauscence* against *B. decoloratus* adult ticks. Ticks were grouped into four groups each with 12 engorged female ticks, three treated with different concentrations (triplicates) and one negative control. Both treatment and control groups were place in perforated cloth specially made to be able to hold the ticks while allowing them to be in contact with solvents (Figure 2). The ticks were then immersed for five minutes in 20 ml of the diluted crude extract with tween 80 and the control group immersed in tween with distilled water (Rosado-Aguilar et al., 2010) and distilled water alone. The ticks were then transferred into Petri dishes and observed for mortality for a maximum of three days at the condition of temperature and humidity described previously. The criteria used to diagnose dead ticks included the lack of movement of legs and change of cuticle color *(*Pirali-Kheirabadi and Teixeira da Silva, 2011). Efficacy of extract to kill the adult ticks was determined against negative controls, that is, distilled water for aqueous extracts and 2% tween 80 in distilled water for organic extracts by calculating Corrected mortalities

Definition of test scores for crude extracts

Definition of test scores was adopted from those reported by Rosado-Aguilar et al. (2010) as follows. Activity of crude plant extracts were classified in mean % of mortality of adult ticks and larvae at 24, 48 and 72 h as; high mortality (86-100%); relatively high mortality (71-85%); moderate mortality (56-70%); low mortality 31-55%; and non-significant activity of mortality (0-30%) (Rosado-Aguilar et al., 2010).

Statistical analysis

All data were recorded in an excel sheet and used it to perform descriptive statistics such as arithmetic means of triplicate tests and percentage mortalities of adult ticks and larvae in test and control groups. Efficacy of extract to kill the adult ticks and larvae for all extracts concentrations was calculated using Abbott's formula (Abbott, 1925).

Table 2. Corrected Mortalities (%) of ticks from Root extracts of *S. glaucescens*.

Extract type	Corrected %age Mortalities in different concentrations (mg/ml)								
	24 h			48 h			72 h		
	100	200	300	100	200	300	100	200	300
REtoH	2.86	8.57	9.57	3.06	8.57	12.11	9.57	13.3	16.56
RDCM	5.56	5.56	8.57	5.56	8.57	12.11	8.57	9.97	16.56
Rwater	2.78	5.56	5.56	2.78	2.78	5.56	2.78	5.56	5.56
LEtoH	9.16	9.16	16.7	16.7	16.67	25	25	33.3	33.33
LDCM	0	0	8.33	8.33	16.67	18.21	16.67	18.2	18.21
Lwater	0	0	0	0	0	0	9.05	9.05	16.67

$$\text{Corrected mortality} = \frac{(\text{Mortality in test bottles [\%]} - \text{Mortality in control bottle [\%]})}{(100\% - \text{mortality in control bottle [\%]})} \times 100$$

The corrected mortality results of adult ticks were then used to calculate lethal concentrations LC_{50} and LC_{90} for each extract using a graph pad Software version 5.0.

RESULTS

Adulticidal tests

Table 2 shows corrected percentage mortalities of adult ticks against different dried extracts of root barks and leaves of *S. glaucescens*. The minimum and highest mortalities in the last day of observation were 2.78 and 33.33%, respectively. These activities are regarded low especially when the highest mortality recorded below 50%, which appeared on the third day of the observation. However, among the extracts, the ethanol extracts from leaves was the most active (33.33%) while water extracts showed the least activity (2.78%).

Figure 3 shows the trend of mortality from day one to the third day of observation. It is evident that despite the low activity of extracts yet the tendency showed that the percentage mortality slightly increased with number of days and with increase in concentrations

Table 3 shows the lethal concentrations LC_{50} and LC_{90} of the different extracts. The high values are an indication of less effectiveness of the extracts. After 72 h, the LC_{50} of almost all of extracts are in terms of thousands except for LEtoH (666.91). This further indicates that the activities of the extracts were low including the most active amongst them.

Larvicidal activities

The larvicidal activity was tested only using two extracts. Table 4 shows the larvicidal activity of methanol and water extracts from the leaves of *S. glaucescens*. Similar results are observed in the larvicidal test as indicated in the adulticidal tests. Despite their higher susceptibility than adults (Williams et al., 2015), yet the activity of the extract against larvae was low with the highest and least mortality being 37.5 and 3.2% respectively (Table 4) with *B. decoloratus* larvae exhibiting higher resistance as compared to *B. microplus*.

DISCUSSION

Synadenium glaucescens is known for many traditional uses including use as pesticides agent in post harvests storage. Apart from traditional utilization, no any systematic study on acaricidal activity of the crude extracts from this plant had previously been reported. The existing reports are on pesticidal activities of other species in the genus (Afonso-Cardoso et al., 2011, Hassan et al., 2012). Thus, the evaluation of this plant on its effect in ticks is being reported for the first time and was based on these traditional values of the plant species and the existing pesticidal information in the genus. The study doses in this study are high and appear different from many studies that have been done on an acaricidal activity of various plant extracts (Bagavan et al., 2009; Rosado-Aguilar et al., 2010).

This is because during trials for an establishment of concentrations, the lower doses (25 and 50 mg/ml) could not perform well thus, necessitating trials of higher concentrations. Despite high test concentrations, yet extracts showed to exhibit very low activities on the adult ticks at 24, 48 and 72 h (Table 2). This is also indicated by high values of lethal concentrations (Table 3), which imply that the extracts exhibits low acaricidal effects. Therefore, most of the extracts have been grouped to bear non-significant activities while only one extract (LEtoH) exhibit low activity on adult ticks. Though only two extracts were tested for larvae efficacy, similar results have been observed where one extract exhibited low activity and the other exhibiting non-significant activity. This low activity against larvae further justifies the low effectiveness of the extracts as acaricide because larvae have relatively high susceptibility as compared to

Table 3. Lethal concentrations of Adult ticks after immersion in Root extract of *S. glaucescens.*

Extract type	LC_{50} and LC_{90} of Corrected %age Mortalities(CM) for Root and Leaf					
	24 h		48 h		72 h	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}
REtoH	1481.67	2673.92	1130.09	2014.07	1264.26	2418.66
RDCM	3086.16	5741.96	1459.64	2681.02	1158.7	2159.95
Rwater	3463.79	6141.49	3530.46	6408.15	3463.79	6341.49
LEtoH	1220.95	2286.2	933.57	1893.93	666.91	1627.29
LDCM	-	-	920.58	1730.3	4395.24	9590.04
Lwater	-	-	-	-	1208.14	2258.01

Table 4. Corrected percentage mortalities of larvae against leaf extracts of methanol and water.

Extract type	Conc (%)	Total	Alive	Dead	Total	Alive	Dead	Mortality	CM
	Larva species: ***B. microplus***								
LMeOH	1	139	76	63	127	64	63	47.4	37.5
LWater	1	137	104	33	127	87	40	27.7	14.1
	Larva species: *B. decoloratus*								
LMeOH	1	54	41	13	95	86	9	14.8	5.1
LWater	1	104	92	12	119	102	17	13	3.2

Conc = Concentration; CM = Corrected mortality.

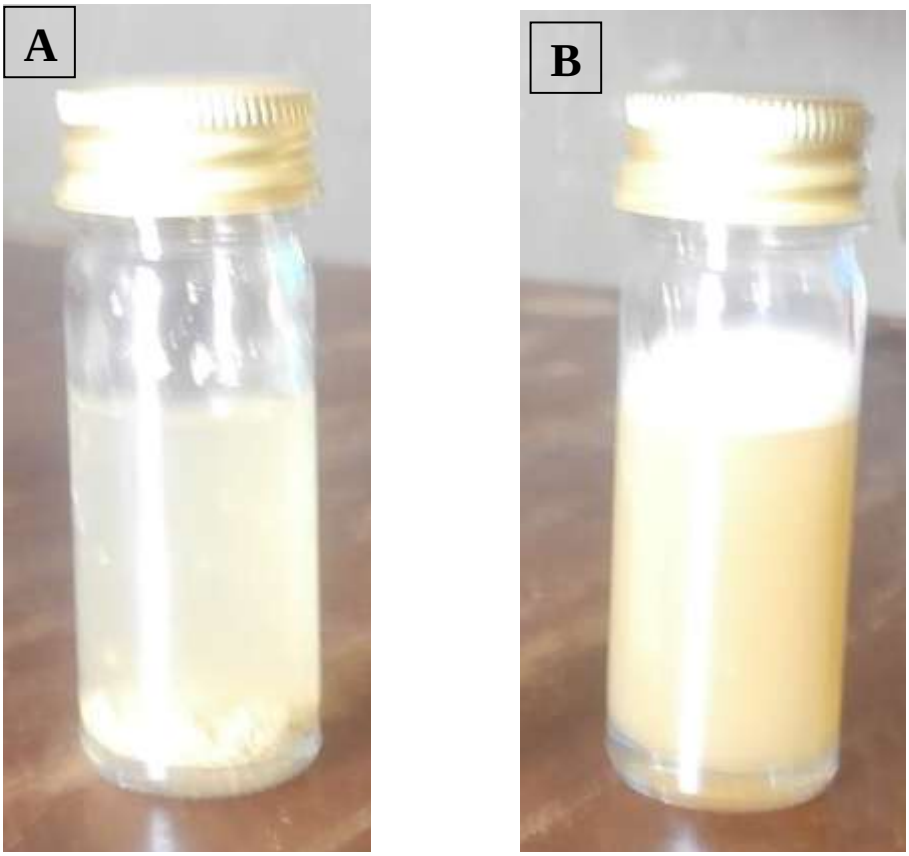

Figure 1. Extract dissolves in DMSO (a) and Tween 80 (b).

Figure 2. Adult tick immersion in a test and control solvents.

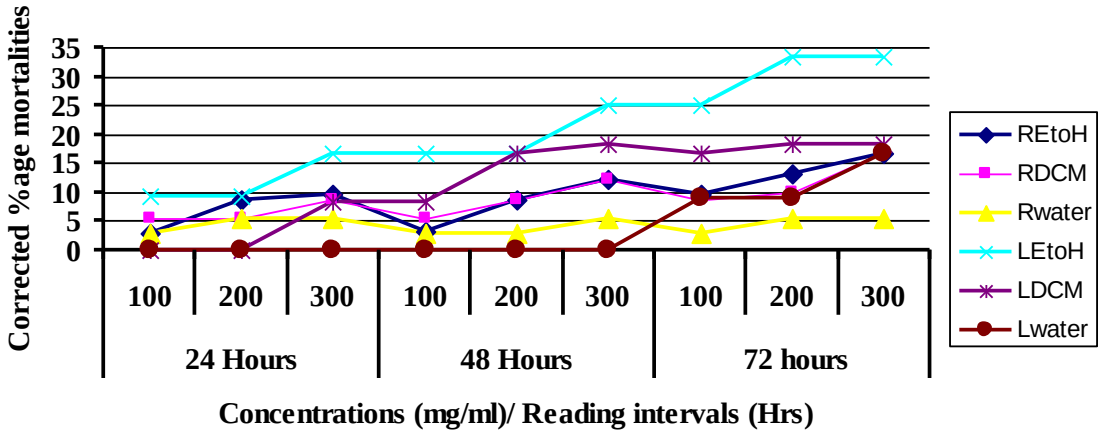

Figure 3. Mortality trends of adult ticks in the observation intervals.

adult ticks (Williams et al., 2015). These results are quite different from researchers' expectations and the claimed traditional efficacy on post-harvest storage protections. The reason for this difference is not well understood. However, it could probably be associated with conditions at which the test materials were used. In the traditional utilization, it is common that people use the fresh plant materials, but in this case, plant materials were dried for the purpose of standardization. Some changes may have happened on the constituents during processing that resulted from operational conditions such as temperature and pH (Durairaj et al., 2009). Since the current results were observed within 72 h, the duration of observation could also have affected the results especially if the

product has a slow onset of acaricidal actions (Holdsworth et al., 2006). Maybe longer time observations, which have also been the case for some studies could have a different result from the current observation (Holdsworth et al., 2006; Righi et al., 2013). None of the tested extracts could kill even 50% of the test subjects despite the high dosages used. Thus, none of the plant extracts is considered effective against tested ticks species. We, therefore, suggest further research on the plant by using fresh plant materials especially leaves as the fresh leaf latex has also shown to have activities on pest (Afonso-Cardoso et al., 2011).

Conclusion

Since the activity of extracts in adults and larvae were less than 50%, the extracts are concluded to exhibit low to non-significant activity against ticks under the conditions of the test described. Thus, it is not recommended for field trials, rather additional research is needed to determine its potential using fresh plant material especially those with latex.

Conflict of Interests

The authors have not declared any conflict of interests.

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